



**Etude d'une collection de pommes de terre (*Solanum tuberosum* spp *tuberosum* L.) native de Chiloé (Chili) :  
Conservation in situ, Diversité morphologique et  
génétique, Comportement vis-à-vis de *Phytophtora infestans***

Jaime Hernán Solano Solis

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**Etude d’une collection de pommes de terre (*Solanum tuberosum* spp *tuberosum* L.) native de Chiloé (Chili):  
Conservation *in situ*, Diversité morphologique et  
génétique, Comportement vis-à-vis de *Phytophtora infestans***

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## **Dédicace.**

Pour ma belle famille. Ma femme Jacqueline et mes enfants Mauricio Hernán et Daniela Constanza.

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-Mme Florence ESNAULT, Ingénieur de Recherche, INRA, Examinateur.

-M. Philippe BRABANT, Professeur d' AgroParisTech.

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## ABSTRACT

The overall objective was to assess the genetic diversity of a collection of native potato varieties originating from the island of Chiloe, to characterize the resistance of these varieties to late blight (*Phytophthora infestans*) and to inquire about the status of *in-situ* conservation. The following specific topics was developed: a) conservation *in-situ* of native Solanum within the great isle of Chiloe and its impact on diversity, b) evaluation of the morphological diversity of the native potatoes that we have collected c) assessment of genetic diversity of the collection by the mean of microsatellite and AFLP markers, d) characterization of field and *in-vitro* resistances to late blight. Based on the results of the surveys, we can conclude that *in-situ* maintenance of native potato diversity i is not well preserved, due to strong social and economic changes on the island of Chiloe. There is a clear process of replacing native varieties for commercial cultivars in response to market conditions. Native varieties are present in 80.5% of the farms of Chiloe, but the diversity of varieties has been significantly reduced. The results morphological diversity showed the formation of groups under the popular name and local attributes assigned by the farmers themselves. Molecular evaluation of the collection (SSRs and AFLP) reveals a high degree of genetic diversity. Both markers were consistent in classifying *Solanum fernandezianum* as the more distant genotype compared to all the others. The SSRs allowed the estimation of the polymorphic information content for seven loci which values ranged between 0.63 to 0.89. Both types of markers, did not provide the same groupings among the accessions. The SSR-based study showed a low differentiation between native potatoes and improved cultivated varieties. The analysis of diversity based on the AFLP was not inconsistent with this result because despite containing only one cultivar (Desiree), the cultivar was grouped with the native varieties. In relation to resistance to late blight, most accessions of native potato fall into the ranks moderately resistant to moderately susceptible.

## RÉSUMÉ

L'objectif global était d'évaluer la diversité génétique d'une collection de variétés de pommes de terre indigènes originaires de l'île de Chiloé, pour caractériser la résistance de ces variétés au mildiou (*Phytophthora infestans*) et pour en savoir davantage sur l'état de conservation *in-situ*. Les sujets suivants a été élaboré: a) la conservation in situ de *Solanum* indigènes à l'île la grande de Chiloé et son impact sur la diversité, b) l'évaluation de la diversité morphologique des pommes de terre indigènes que nous avons recueillis c) l'évaluation de la diversité génétique par marqueurs microsatellites et AFLP, d) la caractérisation du terrain et des résistances *in-vitro* au mildiou. Sur la base des résultats des enquêtes, nous pouvons conclure que le maintien in situ de la diversité de pommes de terre indigènes n'est pas bien conservé, en raison des forts changes sociaux et économiques sur l'île de Chiloé. Il y a un processus clair de remplacer les variétés indigènes pour les cultivars commerciaux en réponse aux conditions du marché. Les variétés natives sont présents dans 80,5% des fermes de Chiloé, mais la diversité a été considérablement réduite. Les résultats de la diversité morphologique ont montré la formation de groupes sous le nom populaire et des attributs locaux affectés par les agriculteurs eux-mêmes. L'évaluation moléculaire de la collection (SSRs et AFLP) révèle un haut degré de diversité génétique. Les deux marqueurs ont été uniformes dans la classification *Solanum fernandezianum* comme le génotype plus éloigné. La SSR a permis l'estimation de la teneur des informations polymorphes pour sept loci dont les valeurs se situaient entre 0,63 à 0,89. Les deux types de marqueurs, n'a pas fourni les mêmes groupes parmi les accessions. L'étude SSR basé a montré une faible différenciation entre les pommes de terre indigènes et l'amélioration des variétés cultivées. L'analyse de la diversité sur la base des AFLP n'était pas incompatible avec ce résultat parce que, malgré contenant un seul cultivar (Désirée), le cultivar a été regroupée avec les variétés indigènes. En ce qui concerne la résistance au mildiou, la plupart des accessions sont dans les rangs modérément résistante à modérément sensible.

## RESUMEN

El objetivo de este estudio fue evaluar la diversidad genética de una colección de variedades de papas nativas provenientes de la isla de Chiloé, caracterizar la resistencia de estas variedades a tizón tardío (*Phytophthora infestans*) y evaluar el estado de conservación *in-situ*. Se han desarrollado los siguientes temas específicos: a) conservación *in-situ* de Solanum nativos dentro de la Isla Grande de Chiloé y su impacto en la diversidad, b) evaluación de la diversidad morfológica de las variedades nativas que se han colectado c) evaluación de la diversidad genética por medio de marcadores microsatélites y AFLP, d) caracterización de campo e *in-vitro* de la resistencias a tizón tardío. Basándose en los resultados de las encuestas, se concluye que el mantenimiento *in-situ* de la diversidad de papas nativas no es bien conservada, debido a fuertes cambios sociales y económicos en la isla de Chiloé. Hay un claro proceso de sustitución de las variedades nativas por cultivares comerciales en respuesta a las condiciones del mercado. Las variedades nativas están presentes en el 80,5% de las granjas de Chiloé, pero la diversidad se ha reducido significativamente. Los resultados mostraron diversidad morfológica con formación de grupos concordantes con el nombre popular y los atributos locales asignados por los propios agricultores. La evaluación molecular de la colección (SSRs y AFLP) revela un alto grado de diversidad genética. Ambos marcadores fueron consistentes en la clasificación de *Solanum fernandezianum* como el genotipo más distante. El SSR permitió la estimación del contenido de información polimórfica de siete loci con valores que oscilaron entre 0,63 y 0,89. Ambos tipos de marcadores, no proporcionó las mismas agrupaciones entre las accesiones. El estudio basado en SSR mostró una baja diferenciación entre las papas nativas y mejoradas variedades cultivadas. El análisis de la diversidad sobre la base de la AFLP no fue discordante con este resultado porque a pesar de que sólo contiene un cultivar (Desiree), el cultivar se agrupó con las variedades nativas. En relación con la resistencia al tizón tardío, la mayoría de las accesiones de papas nativas se clasificaron entre moderadamente resistente y moderadamente susceptible.

## AVANT PROPOS

Ces travaux de thèse s'inscrivent dans le cadre général de la diversité génétique de la « pomme de terre native » au Chili, centre de diversification secondaire de la pomme de terre. Nous emploierons dans la suite du document le terme de « pomme de terre native » pour désigner les variétés locales traditionnelles chiliennes. Ces variétés sont *a priori*, et jusqu'à preuve du contraire, non-introgressées par la variabilité génétique de variétés améliorées européennes ou nord américaines, elles sont issues de la diversité des variétés existant avant l'arrivée des européens. Le travail porte sur la caractérisation, l'utilisation et la valorisation des pommes de terre natives et sur les possibilités de conservation « *ex situ* » et « *in situ* ». La dernière partie du document traite de la résistance au mildiou (*Phytophthora infestans*) des pommes de terre natives. L'objectif général est donc d'évaluer la diversité génétique d'une collection de variétés de pommes de terre natives de l'île de Chiloé (cette collection a été constituée par nos soins entre 2000 et 2007), de caractériser ces variétés vis-à-vis de la résistance au mildiou (*Phytophthora infestans*), d'envisager les conditions de leur conservation *in situ* et de leur utilisation éventuelle dans les programmes d'amélioration génétique.

Chili est considéré comme un sous-centre d'origine de la pomme de terre cultivée. Dans ce contexte, les pommes de terre indigènes de Chiloé sont caractérisées par une riche variété de formes, tailles, couleurs et caractéristiques phénologiques. Ce riche patrimoine génétique doit être décrite et individualisée dans le but d'être conservé et utilisé. Il ya une concentration remarquable de formes cultivées et sauvages de pommes de terre dans le nombre plus grand sud du Chili avec les variétés indigènes d'être situé dans l'île de Chiloé, et encore conservés dans Ils sont les champs des petits agriculteurs. Les caractéristiques distinctives de Chiloé, les conditions et son isolement naturelle, a permis la prolifération d'avoir un grand nombre de variétés locales, différentes propices à la culture et les qualités à différents moments dans le calendrier agricole, ainsi que d'un certain nombre de différentes formes de préparation et consommation. La province de Chiloé, est considérée comme un des centres de biodiversité des pommes de terre natives. Jusqu'à présent ces pommes de terre ont été conservées *in situ* par les petits paysans qui les vendent sur les marchés locaux et les utilisent pour leur consommation personnelle. Ces ressources constituent un matériel phyto-génétique original, issues directement d'une domestication ancestrale, elles constituent un patrimoine génétique et culturel d'importance pour les générations futures. Néanmoins, le nombre d'agriculteurs qui cultivent la pomme de terre native a diminué considérablement ces dernières années.

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## **CHAPITRE I. Introduction Generale.**

### **1.1. The complex of species of potatoes.**

Potatoes were domesticated in the Andes of southern Peru about 10.000 years ago (Simmonds, 1995). This author suggest, the wild species concerned is/are not known with certainty but, on systematic grounds, several candidates in the series Tuberosa have been proposed; of these *S. brevicaule*, *S. leptophyes*, *S. canasense*, *S. soukupii* and *S. sparsipilum* are most frequently mentioned. Since it hybridizes easily with wild relatives and cultivated diploids, several wild forms could have been implicated, either before or after the primary domestication.

Potatoes had a monophyletic origin from wild species of the *Solanum brevicaule* complex in Peru (Spooner et al., 2005; Spooner and Hetterscheid, 2006). Landrace potato cultivars are highly diverse, containing diploids ( $2n = 2 x = 24$ ), triploids ( $2n = 3 x = 36$ ), tetraploids ( $2n = 4 x = 48$ ), and pentaploids ( $2n = 5 x = 60$ ) (Huamán and Spooner, 2002). The taxonomy of cultivated potatoes is controversial; from one to twenty species recognized (Huamán and Spooner, 2002), but all form a common gene pool (Spooner et al., 2005). The tetraploid cytotypes are the highest yielding; (they are the sole cytotype of modern cultivars) and their ancestral forms are widely distributed in two regions in South America: the high Andes from Venezuela to Argentina (Andean landraces) and to the south in the lowlands of southcentral Chile in Chiloe Island, including the immediately adjacent Chilean lowlands and other islands to the Chonos Archipelago (Chilean landraces) (Hawkes, 1994, Huamán and Spooner, 2002).

Despite these altitudinal differences, their tuberization response is a factor of daylength, not altitudes. As a result, the Andean landraces tuberize poorly in the hight latitudes of Europe, but the Chilean landraces tuberize well there (Glendinning, 1983 cited Ames and Spooner, 2008).

Grun (1990), indicates that in the first stages of potato evolution in the northern Andes, diploid cultivated species of the *Solanum stenotomum* complex were selected, in all probability, from wild progenitors in the *S. brevicaule* complex. Tetraploid *Solanum tuberosum* ssp. *andigena* arose by fusion of unreduced gametes of a parent in the *S. stenotomum* complex with those of an unidentified wild species having actinomorphic calyces. Unreduced male gametes of several diploid species fertilized eggs of ssp. *andigena* leading to extensive introgression. *Solanum tuberosum* ssp. *tuberosum* probably originated from a cross between ssp. *andigena* as staminate parent and an unidentified wild species which contributed to cytoplasmic sterility factors encoded in mitochondria and/or plastids

having a distinctive type of DNA. Derivatives of this hybridization, which may have occurred in northwestern Argentina evolved to ssp. *tuberosum* in southern Chile and southern Argentina.

Studies of cytbotaxonomic of Simmonds (1995) suggest the tuber-bearing Solanums are one relatively small group of a very large genus. About 170 species are included, of which fewer than ten are relevant to the evolution of the crop. The basic chromosome number is  $x = 12$ . Among the wild forms rather less than half occurs in north and central America (to about  $40^{\circ}\text{N}$ ); they include diploids, allotetraploids and allohexaploids ( $2n = 24, 48, 72$ ) and have, at most, marginal bearing on cultivar history, a few having been used in potato breeding. The majority of species are South American (to about  $45^{\circ}\text{S}$ ) and most of these are diploids. It is from a few of these diploids ( $2n=24$ ) that the cultivars derive, though one of the rare tetraploids (*Solanum acaule*) has also contributed.

A conspicuous biological feature of the wild potatoes is the contrast between very variable, outbred diploids and the less variable inbred polyploids; the former have a gametophytic S-allele incompatibility system and are highly intolerant of inbreeding; the polyploids are self-compatible and often self pollinated.

Huamán and Spooner (2002) examined morphological support for the classification of potato landraces, and on the basis of poor morphological support and ongoing dynamic of hybridization, they recognized a single species, *Solanum tuberosum* L., with eight cultivar groups:

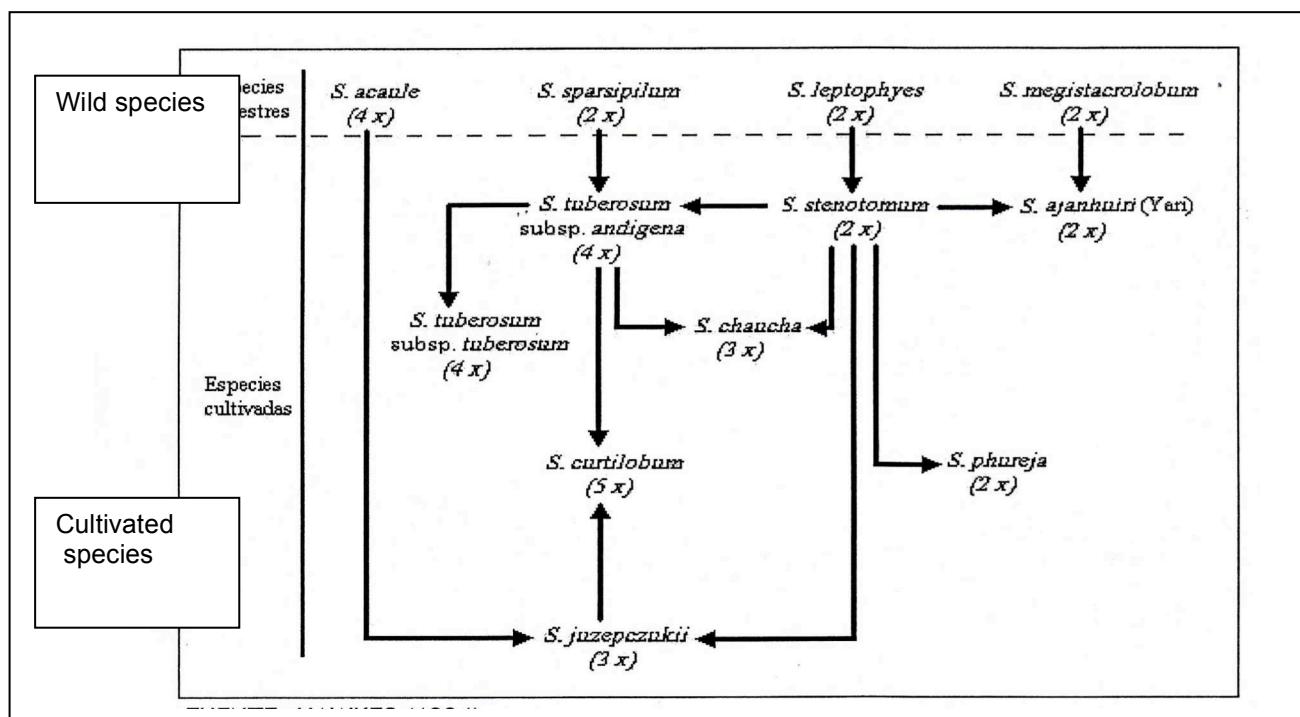
- Ajanhuirí Group (diploid,  $2n=2x=24$ ),
- Andigenum Group (tetraploid,  $(2n=4x=48)$ ),
- Chaucha Group (triploid,  $2n=3x=36$ ),
- Chilotanum Group (tetraploid,  $2n=4x=48$ ),
- Curtilobum Group (pentaploid,  $2n=5x=60$ ),
- Juzepczukii Group (triploid,  $2n=3x=36$ ),
- Phureja Group (diploid,  $2n=2x=24$ ) and
- Stenotonom Group (diploid,  $2n=2x=24$ ).

Cultivar groups are taxonomic categories of cultivated plants based on agro-morphological traits. These categories are used by the International Code of Nomenclature of Cultivated Plants, but imply no phylogenetic differences between the groups. Species and subspecies, in contrast, are treated by the International Code of Botanical Nomenclature and generally assume phylogenetic differences

All species in the Andes from western Venezuela to northern Argentina, except the Chilotanum Group (=*S. tuberosum* subsp. *tuberosum*), which occurs in lowland south-central

Chile of Chiloe Island, the Chonos Archipelago immediately to the south (where they occur as ruderal plants growing on beach), and the adjacent low elevation mainland. Remnant landrace populations outside of South America were all introduced in post-Columbian times.

The general outcome of the first phases of potato evolution was the diffusion through highland South America of a great complex of diploid, autotriploid and autotetraploid potatoes with a centre of variability in Peru-Bolivia. This was first revealed by the pioneering Russian expeditions in the 1920s. Secundary outcomes were the establishment in Chile, from migrant group Andigena, of group of tetraploids adapted to high latitudes; and the occurrence in the central Andes of a few allotriploids (*S. juzepczukii*,  $2n=3x=36$ ) and allohexaploids (*S. curtilobum*,  $2n=5x=60$ ) derived from hybridization with the wild Andean tetraploid, *Solanum acaule* (Figure 1).

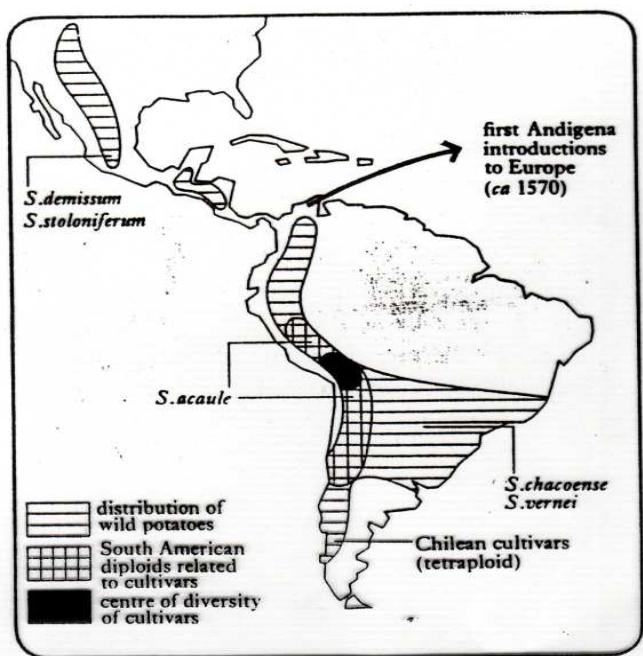


**Figure 1.** Evolutionary relationships of cultivated potatoes and their ploidy levels

**Source:** Hawkes (1990).

## 1.2.- Domestication.

Simmond (1995), indicated the area of domestication may be assumed to be where the wild and cultivated diploids are still present and most variable, the high plateau of Bolivia-Perú, in the region of Lake Titicaca (Figure 2). The diploids exist, but have been largely superseded by cultivated tetraploids and a few triploids. The tetraploids are variously referred to as *Solanum andigena*, *S. tuberosum* subsp. *andigena* or Group Andigena.



**Figure 2.** Distribution of the tuber – bearing species of *Solanum*.

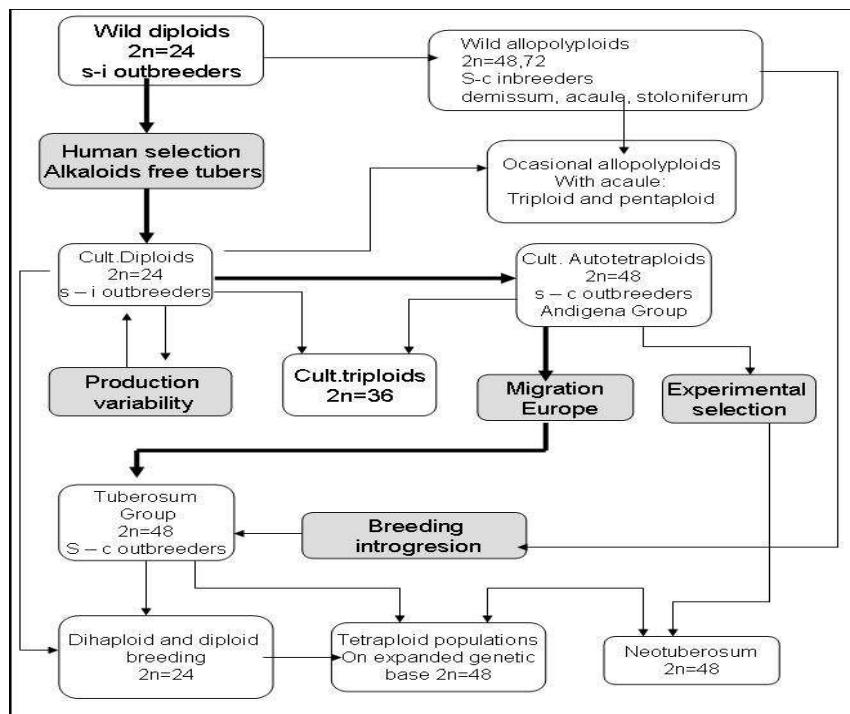
**Source.** Simmonds, (1995).

Montaldo (1984) suggested that the region of Lake Titicaca would be the center of origin of the cultivated potato because there exists a large number of species, as well as cultivated varieties, there would have been born more primitive agriculture based on cultivation of potatoes and other root crops.

Greenpeace (2000), notes that the potato (*Solanum tuberosum* L.) originated in the Andes region of central Peru, because that is where the greatest number of species of the genus *Solanum* are established.

On the other hand, Montaldo (1984), mentioned as the center of origin for the tuberous potatoes (*Solanum tuberosum* L.) the region of Chiloé, Chile, and for Andean cultivated potatoes (*Solanum andigenum* L.) Ecuador, Peru and Bolivia. Chilean populations of subsp. *tuberosum* are speculative Juzepczuk and Bukasov in 1929, proposed that is originated from indigenous tetraploid cultivated species *S. fonckii* Phil. Ex Reich., *S. leptostigma* and *S. molinae* (Hawkes (1990), cited by Raker and Spooner (2002))..

Hawkes (1990; 1994) mentioned that the first domesticated potato Solanum species belonging to the *Solanum stenotomum*, which was derived from *S. leptophyllum* (Figure 3). In turn, *S. stenotomum* probably crossed with *S. sparsipilum*, a wild species to produce diploid *S. andigena*, forerunner of the current tetraploid potato (spp *andigena* and ssp. *tuberosum*).



**Figure 3.** Evolution of the cultivated potatoes, *Solanum tuberosum*.

**Source.** Simmonds, (1995).

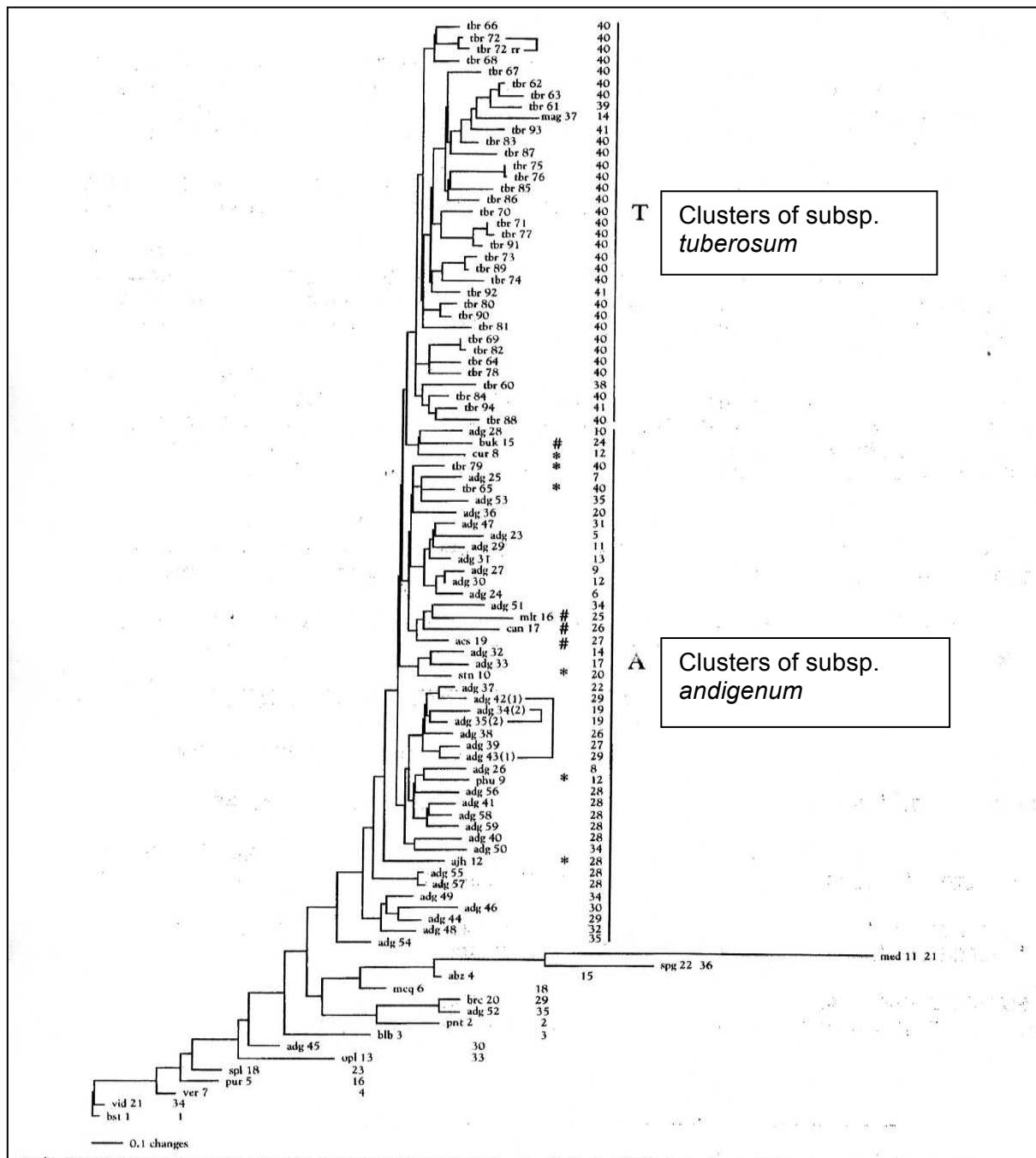
Within the species *S. tuberosum* L. ( $2n = 48$ ) are included all tetraploid cultivars, the Andean and Chilean tetraploid cultivated potatoes have been treated as two separate species, *S. andigenum* Juz. and Bukasov and *S. tuberosum* L.; or as subspecies of *S. tuberosum*; or as two cultivar groups within *S. tuberosum* (Huamán and Spooner, 2002). The Chilean populations of potatoes and modern cultivars are classified collectively under the name *S. tuberosum* ssp. *tuberosum* due to their similarities and adaptation morphologic flowering and tuberization under long-day conditions (Huamán and Spooner, 2002).

Simmond (1995) suggested wild potato tubers are all bitter to the taste and contain potentially toxic amounts of various steroidal alkaloids. The first step in the evolution of the crop must have been the recognition and selection at the gathering stage of clones that were less bitter than usual. Wild tuber are still gathered and eaten in various places in South and Central America. The first step was therefore the emergence of alkaloid free diploids which could safely be eaten in quantity. The cultivated diploids developed a great deal of new

variability in foliage characters and in tuber shapes and colours. They have been classified into a number of species (with *S. stenotomum* as the most important) but they are all readily intercrossed as “cultivated diploids”.

Dudds, 1965, cited in Simmond, 1995, indicates the cultivated diploids retain the self-incompatibility out breeding habits of their putative ancestors. On the other hand, potato domestication from these wild species involved selection for underground characters of shorter stolons, larger tuber, colored and variously shaped tubers, and the reduction of bitter tuber glycoalkaloids; above-ground characters of wild and cultivated species are similar but with cultivated types exhibit high vigor and extensive segregation for flower and foliage traits (Spooner *et al.*, 2005).

Raker and Spooner (2002) studied differences between two subspecies of *S. tuberosum* using the mapped potato microsatellite loci. The microsatellites separated subsp. *andigenum* from subsp. *Tuberosum* but the separation between the two sub-species appeared not very sharp. Some *non-andigenum* cultivated species and wild relatives fell within the *andigenum* group. The microsatellite data place *S. maglia* with subsp. *tuberosum*, which could be interpreted to support this species as its progenitor, but this is considered unlikely, because *Solanum maglia* is restricted to coastal Chile (Figure 4).

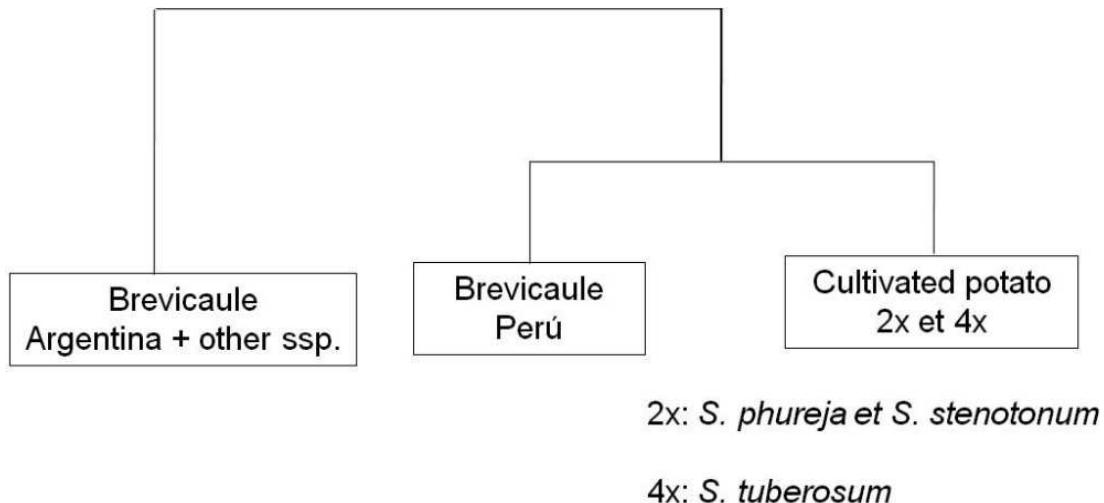


**Figure 4.** Neighbor-joining tree generated from microsatellite data analyzed with Nei's similarity coefficient. The vertical lines T and A referred to clusters of subsp. *tuberosum* and subsp. *andigenum*, respectively. (\*) and (#) highlight non *andigenum* cultivated and wild species respectively.

**Source.** Raker and Spooner (2002)

Different authors have suggested complex hybrid or multiple origins of the cultivars from both northern and southern members of the *S. brevicaule* complex (Huamán and Spooner, 2002; Grun, 1990; Van den Berg *et al.*, 1998; Hosaka, 1995).

Spooner *et al.*, (2005) support a monophyletic origin of the landrace cultivars from the northern component of this complex in Peru, rather than from multiple independent origins from various northern and southern members. A summary diagram is presented in Figure 5.



**Figura 5.** Origin of the cultivated potato: results diagrammed according Spooner *et al.*, PNAS, 2005. Based on AFLP data of 362 accessions.

**Source:** Spooner *et al.*, (2005).

### **1.3.- Origin of European potato varieties**

Different authors consider the European potato originated in the Andean region of central and northern South America (Salaman, 1937, Hawkes 1944; Salaman, 1946, cited by Castronovo, 1949), while others consider the south of Chile as the true "center of origin for european potatoes" (Bukasov and Lechnovitz, 1935; Bukasov, 1939; Bukasov, 1941, De Candolle, 1886, cited by Castronovo, 1949).

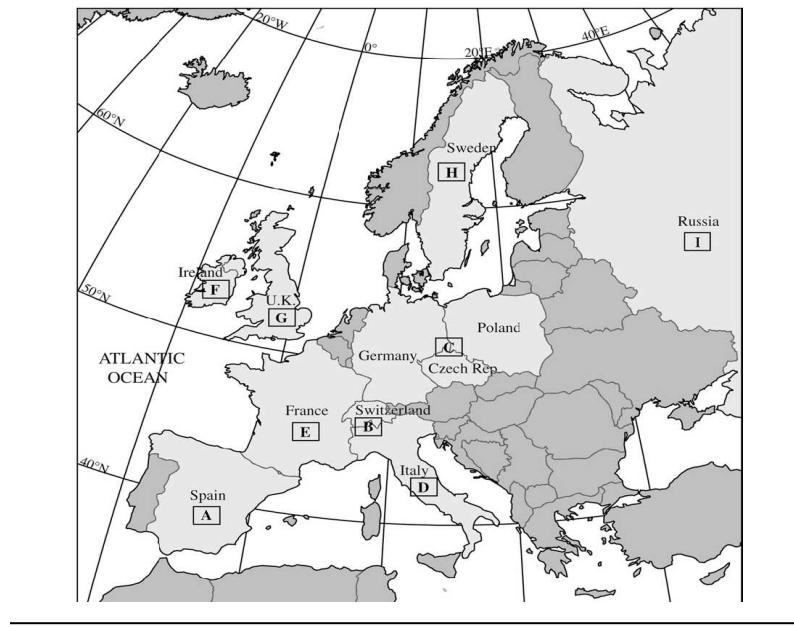
Hawkes (1994) mentioned that the potato was introduced to Europe twice, one to Spain in 1570, and would correspond to the type Andigena potatoes from the northern Andes. A second introduction would have happened around 1590 in Great Britain, which is believed to have been independent of the first Spanish introductions (Simmond, 1995).

The first record of cultivated potato outside South America was in the Canary Islands in 1567 (Hawkes and Francisco-Ortega, 1993; Ríos *et al.*, 2007), and shortly thereafter in continental Spain in 1573 (Hawkes, 1990; Hawkes and Francisco-Ortega, 1992). The first botanical description was made by Bauhin in 1596 although the origin of the plant was not known (Hawkes, 1990). The potato then spread throughout Europe and worldwide and is here referred to as the "European" potato. It was slow to be adopted as a major food crop until about 100 years later, and in some European countries it was rejected as an acceptable food well into the late 1700s. For example, in eastern France although its acceptance was rapid as early as 1600, many public demonstrations were necessary to show that potatoes were a safe food before widespread acceptance throughout the country. In 1771 a high prize was offered by the French Academy of Besançon for the discovery of a new food that would replace cereals in case of famine. Antoine-Augustin Parmentier promoted potatoes, and Louis XVI gave him land to plant them as his prize. At the beginning of the 19th century, the French National Society of Agriculture documented a collection of about 120 potato varieties. In Italy and Germany potatoes were cultivated in small gardens by 1601. A famine in Prussia effectively stimulated potato cultivation; however, in some regions of Italy acceptance of potato was slow. For example, in Naples, a boat load of potatoes was rejected by the residents in 1770 during a famine. In Sweden, by 1764 a royal edict was issued to encourage potato cultivation. In 1850, Nicholas I of Russia forced people to plant potatoes, establishing its cultivation there. In England, potatoes started to gain importance as a crop by 1662 when The Royal Society recommended planting them to prevent famine, and by 1830 potatoes were well established as a field crop. In Scotland, potatoes were mostly grown in gardens before 1760, and in Ireland potatoes started to be considered as a field crop in 1640 thanks to English immigrants (Ames and Spooner, 2008) (Figure 6).

In the 1570's ssp. *andigena* was first imported to Europe and spread from there to become a major crop with worldwide distribution. In the 1840's it was essentially eliminated by late blight, *Phytophthora infestans*. *Solanum tuberosum* ssp. *tuberosum* was introduced from Chile into North America and Europe in the late 1800's, and in turn achieved a worldwide distribution, filling the vacated agricultural niche of ssp. *andigena*.

The potatoes of the central Andes were adapted to the prevailing short days of those latitudes; they tuberize very late or not at all in the long days of a north temperate summer. Andean potatoes are therefore ill-adapted to Europe and, indeed, it needed nearly 200 years before the crop began to have any significant agricultural impact in its new home. By the late eighteenth century, clones adapted to long days had emerged. This was no doubt accomplished simply by selecting for earliness and size of tubers among seedlings raised from open-pollinated berries but there must have been a considerable amount of natural selection. That Andigena has the genetic capacity to respond to such selection has been demonstrated experimentally. Thus the potato is one of a considerable list of crops in which a day-length bottleneck had to be passed before adaptation to a new environment was possible (Simmond, 1995).

Potato blight (due to *Phytophthora infestans*) was a significant episode from the 1840s. Besides the tremendous social and historical impact of the disease it imposed a major new selective factor on the potatoes of the time. Some resistance became available in the 1870s but the episode must be presumed still further to have narrowed the genetic base. The modern potatoes which emerged around the end of the nineteenth century were all founded genetically upon the original Andigena introductions supplemented by (probably few) later accessions (such as Rough Purple Chile); wild species had no part in the story (Simmond, 1995).



- A: 1567: Potato first documented in Europe in the canary Island. 1573. First record of potato used for human consumption in continental Spain.
- B: 1596: First botanical description of the potato by Gaspar Bauhin.
- C: 1601: Potatoes were cultivated in Prussia. 1771. A famine stimulated potato cultivation.
- D: 1601: Potatoes were cultivated in a few gardens. 1770. residents of Naples refused to eat potatoes during a famine.
- E: 1600: Potato cultivation established in eastern France. 1749. Potato considered "exotic". 1761. Public demonstrations that potatoes were a safe food. 1771. Parameter effectively promoted potatoes as a safe food. 1814. A collection of 120 potatoes varieties were gathered by the National Society of Agriculture.
- F: 1640: potato documented as a field crop.
- G: 1662: potato became an object of importance, and the Royal Society recommended planting potatoes to prevent famine. 1760. Potatoes gained wider acceptance as a field crop in Scotland. 1830. Potatoes commonly cultivated in England.
- H: 1764: A royal edict issued to encourage potato cultivation
- I: 1850: Nicholas I forced people to cultivate potatoes

**Figure 6.** Important dates and places that document the introduction and acceptance of potato outsider of its original home in South America.

**Source:** Ames and Spooner (2008).

Ames and Spooner (2008) mentioned the origin of the European potato has been the subject of long controversy. Juzepczuk and Bukasov (Russian investigators) were first proposed that the European potato was introduced from tetraploid landraces from Chile. On the other hand, British investigators suggested that it came from the Andes and persisted until the potato late blight epidemics beginning in the UK in 1845, after which it was replaced with Chilean germplasm through introductions and breeding efforts. Potato landraces from the high Andes and from lowland Chile can be distinguished, although sometimes with difficulty, by the following five traits:

- 1) cytoplasmic sterility factors: hybrids of Chilean landraces as females with Andean landraces as males have male sterility, but the reciprocal cross is fertile (Grun, 1979 );
- 2) morphology, with the Chilean landraces having wider leaflets held more outward from the plant and other minor morphological differences (Huamán and Spooner, 2002 );
- 3) the Chilean landraces tuberize under long days, the Andean landraces under short days (Glendinning, 1975);
- 4) a suite of microsatellite markers (Spooner *et al.*, 2004 );
- 5) a 241-bp deletion in the *trn V-UAC/ ndh C* intergenic region of the plastid DNA molecule, which is absent in 94% (or 95%) of the Andean tetraploid landraces and present in 86% (or 81%) of the tetraploid Chilean landraces, depending on the studies of Hosaka (2004) or Spooner *et al.*, (2007). This plastid deletion is associated with specific mitochondrial DNA types not found in Andean germplasm (Kawagoe and Kikuta, 1991; Hosaka, 1995; Lössl *et al.*, 1999).

The Chilean origin hypothesis was proposed because of similarities of Chilean landraces to modern European cultivars with respect to morphology and tuberization under long days (traits 2 and 3).

Alternatively, the Andean origin hypothesis suggests that these two traits in European potato evolved rapidly from Andean landraces to a Chilean-type potato through selection following import to Europe. It has been proposed that the late blight epidemics beginning in the United Kingdom in 1845 killed the Andean forms that later were replaced by introductions and breeding with the Chilean landraces. The Andean origin hypothesis has been generally accepted over the last 60 years (Glendinning, 1975; Hawkes, 1990; Hawkes and Francisco-Ortega, 1992). It was supported, in part, by the identity of putative long-remnant landrace introductions of potato in India and in the Canary Islands (Hawkes and Francisco-Ortega, 1992) as Andean potatoes. However, the Indian landraces (Figure 7) were shown to

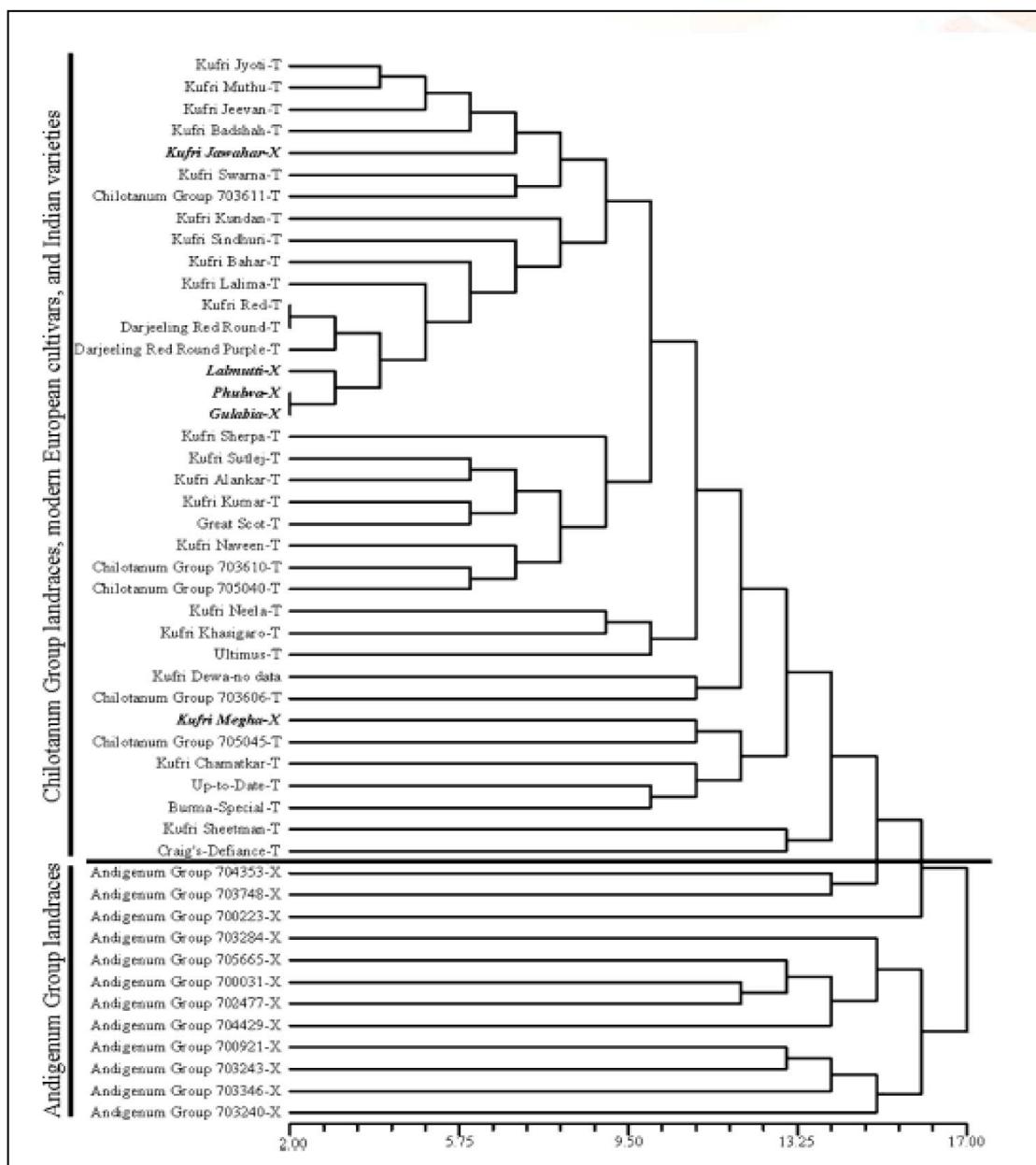
be of Chilean origin (Spooner *et al.*, 2004) and the Canary Island landraces as of Andean and Chilean origins (Ríos *et al.*, 2007) (Table 1).

Regarding the controversy of origin of potatoes, work led by Ríos *et al.*, (2007) with nuclear microsatellite and chloroplast DNA analyses of 19 Canary Island landraces, 14 Andean landraces, 11 Chilean landraces, and two wild potato species as outgroups, and with chloroplast DNA data of 150 landraces from South America. The molecular results document a wide variation of Andean and Chilean-type cultivars on the Canary Islands and possible hybrids between the two types. These results support a hypothesis that there were multiple introductions of Andean and Chilean germplasm to the Canary Islands and that the early European potato was selected from Chilean introductions long before the late blight epidemics of the 1840s (Figure 8).

**Table 1.** Key dates bearing on the history of potato (*Solanum tuberosum* L.) in the Canary Islands.

Date	Event
1532	Pizarro discovered Peru
1551	Valdivia mentioned the growth potatoes in Valdivia (160 km north of Chiloé Island, Chile) by Araucanian Indians (Salaman, 1949).
1552	First mention of potato from Peru (López de Gómora, 1552).
1559	Discovery of Chiloé Island, Chile
1567	Potato was first documented from the canary Islands (Grand canary Island) for consumption (Lobo-Cabrera, 1988); Hawkes and Francisco-Ortega, 1993). Hawkes and Francisco-Ortega (1993) speculated that potato was brought to the Canary Islands as early as 1562.
1573	First mention of potato consumption in continental Spain (Hamilton, 1934, as quoted by Salaman, 1937; Hawkes and Francisco-Ortega, 1992). Hawkes and Francisco-Ortega (1992) speculated that these potatoes were cultivated in continental Spain.
1574	Second record of potato from the Canary Island (Tenerife Island) as shipment to Rouen France (Hawkes and Francisco-Ortega, 1993).
1587	Potatoes were recorded as crated for shipment from Chile to Europe.
1622	Firs record of potatoes from the Canary Islands (Tenerife) from a known place (Peru) [Viera y Clavijo, 1866 (but written in 1799 from historical archives from the Catholic Church; Bandini, 1816]. Both references report that the Canary Island growers used true seed.
1681	First record of potatoes from the Canary Islands used as payment of tithe to the Catholic Church (Macías, 1986).
1776	Potatoes were documented as the second most important crop for the Canary Islands (After wine grapes); Macías, 1986). Today, potato is the third most important crop after grapes and bananas (Servicio de Estadística, 2004).
1797	Doyle (1797) described three different potato groups from the Canary Islands that were distinguished by harvest dates (July, December, May).
1800	Jose de Betheencourt and Castro reported that the poor people of Tenerife preferred to eat potatoes more than grains (Rodríguez, 1992).
Aprox 1800	First record of seed potato (tuber-stock for planting) imported from Europe (Holland) to the Canary Islands (Sánchez-Manzano, 1984; Régulo, 1973).
1868	Alvarez-Rixo (1868) described 20 cultivars of Canary Island potatoes; most his names are still in use there.
1955	Zubeldia <i>et al.</i> , (1955) identified, based on morphological and ploidy data, canary Island potatoes as landraces of <i>S. tuberosum</i> spp. <i>andigenum</i> (tetraploid), spp. <i>tuberosum</i> (tetraploid), and <i>S. mamilliferum</i> (triploid). Hawes and Francisco-Ortega (1993) later identified <i>S. mamilliferum</i> as <i>S. chaucha</i> .

**Source:** Ríos *et al.*, (2007).



**Figure 7.** Neighbor joining tree of potato (*Solanum tuberosum* L.) based on SSRs and chloroplastic DNA markers of 32 cultivars from India, 12 landraces of *Andigena* Group and 5 landraces of *Chilotanum* Group.

**Source:** Spooner *et al.*, 2004.

Salaman (1937), Salaman and Hawkes (1949), Hosaka and Hanneman (1988), Grun (1990), and Hawkes (1990) cited by Ríos *et al.*, (2007), proposed what the Andean introduction hypothesis includes:

- (i) A convergent rapid selection of European potato to the morphology and daylength adaptation shown by members of the Chilotanum Group, and
- (ii) That the late blight epidemics beginning in 1845 in the UK and later spreading world-wide replaced most existing European cultivars by Chilean germplasm or hybrids with this germplasm.

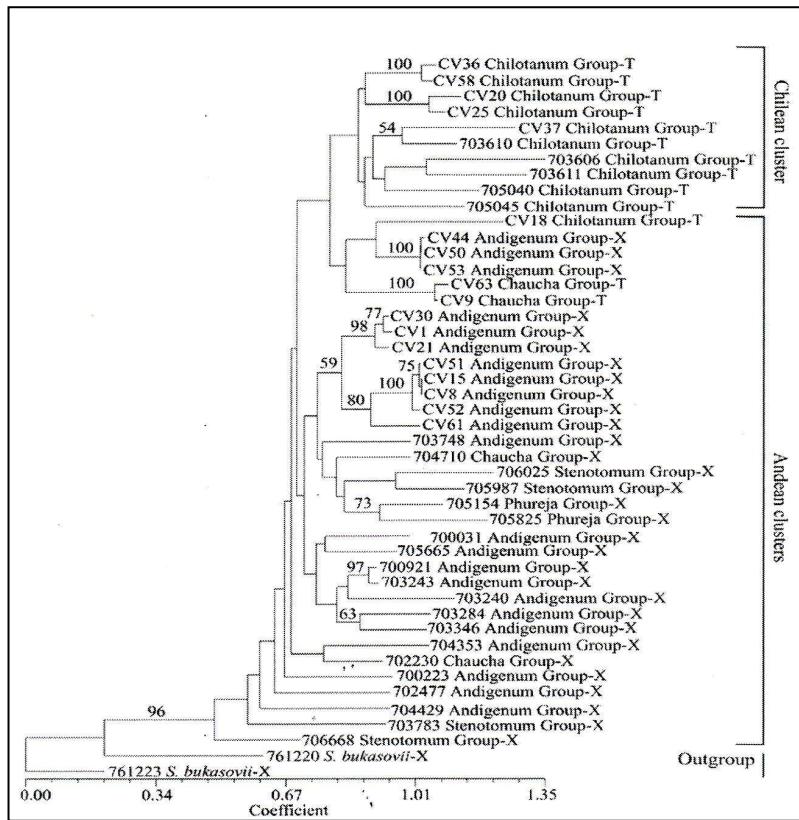
These researchers collectively suggested the following:

1. *Solanum tuberosum* in Chile arose from Andean landraces, either directly, or through a cross with an unidentified wild species. The cytoplasmatic types of Chilean landraces and modern potatoes were identical and that both differed from Andean landraces by a unilateral incompatibility when Chilean germplasm is used as a female (but not as male).
2. Chloroplast DNA deletion, a likely wild species contributor to a Chilean tuberosum as the Bolivian and Argentinean species progenitor *S. tarjense* Hawkes.
3. The first European potatoes were introduced from the Andes, with the first record in the Canary Islands in 1567. Putative late harvest dates (“putative” because the data are for late purchases, not late harvests) of early potatoes in Spain implied Andean introductions, as would be expected from short-day adapted Andigenum Group.
4. Early herbarium specimens of potato in Europe had the narrow-leaved phenotype thought to distinguish the Andigenum Group from the Chilotanum Group.
5. The trip from Chile to Europe took longer than from Peru (or Colombia) to Europe, and tubers from Chile would have less chance to survive.
6. Artificial selection of Andigenum Group produced some Chilotanum Group-like clones (“neo-tuberosum”) with higher flowering, shorter stolons, greater yield, earlier tuberization, reduction of cytosterility, and greater late blight resistance, suggesting the possibility for rapid selection of Andean to Chilean types. Putative early Andean introductions in Europe rapidly evolved into a wider leaf morphotype with long-day adaptation, a parallel event to long-day selection in Chile.
7. The fungal disease late blight (*Phytophthora infestans* (Mont.) de Bary) in Europe killed most of these evolved Andean types in the 1840s, but the modern potato was rapidly mass selected and bred for blight resistance with subsp. *tuberosum*, purchased in Panama (as cultivar Rough Purple Chile) but believed to have come from Chile.

Spooner and Hetterscheid (2005) suggested a different hypothesis that early introductions of potato came from both the Andes and from Chile, with the Chilean introductions rapidly being selected as the European potato, long before the late blight epidemics of the 1840s. They argued the following:

1. The leaf shape data was insufficient to identify early herbarium species as Andean rather Chilean.
2. The argument that Chilean tubers would not have survived the long trip from the Andes to Europe ignored the simple possibility of transport of true seeds, of potted plants, or even well-preserved tubers. Potatoes were documented as an items of ship's stores from Chile, and there are records as early as 1587 of potatoes crated for shipment in storehouses .
3. The vast majority (>99%) of extant advanced potatoes have T-type DNA typical of most Chilean germplasm. This includes a clone released before the 1840s.
4. Chilotanum Group clones are not known for late blight resistance and would have been poor breeding stock to combat this disease.
5. A similar argument that putative remnant populations of Andean potatoes from India supported Andean introductions was discounted by Spooner *et al.*, in 2005, who showed, with microsatellite and cp DNA evidence, that these potatoes were Chilean, not Andean.
6. Juzepczuk and Bukasov's in 1929 argument that Chilean landraces were preadapted to the long day of Europe are compelling, and early introductions from Chile would rapidly be selected over Andean clones. Although neo-tuberosum clones showed the possibility to select for long-day adaptation from Andigenum clones, Chilean introductions would not require such intentional selection.

Regarding the controversy of origin of potatoes, work led by Rios *et al.*, (2007) with nuclear microsatellite and chloroplast DNA analyses of 19 Canary Island landraces, 14 Andean landraces, 11 Chilean landraces, and two wild potato species as outgroups, and with chloroplast DNA data of 150 landraces from South America. The molecular results document a wide variation of Andean and Chilean-type cultivars on the Canary Islands and possible hybrids between the two types. These results support a hypothesis that there were multiple introductions of Andean and Chilean germplasm to the Canary Islands and that the early european potato was selected from Chilean introductions long before the late blight epidemics of the 1840s (Figure 8).



**Figure 8.** Neighbor-joining tree based on SSRs and chloroplastic markers of potato (*Solanum tuberosum* L.) landraces and cultivar groups from the Canary Island (CV accessions) and from South America

Chilean cluster:	CV 36 Palmera lagarteada;	Tenerife Island, Anaga.
	CV 58 Peluca colorada;	Tenerife Island, Fasnia.
	CV 20 Peluca negra;	Tenerife Island, Buena vista.
	CV 25 Peluca rorasa;	Tenerife Island, La matanza.
	CV 37 Brasileña or Grasileña;	Tenerife Island, Anaga.
	703610 Papa cacho;	Chile, Chiloé, Chonos Archipelago.
	703606 Papa chonca;	Chile, Chiloé, Chonos Archipelago.
	703611 Papa colorada;	Chile, Chiloé, Chonos Archipelago.
	705040 Unknown;	Chile, Chiloé, Chonos Archipelago.
	705045 Estrella;	Chile, Chiloé, Chonos Archipelago.

**Source:** Adapted of Ríos *et al.*, (2007).

#### 1.4.- Selection in the nineteenth and twentieth century.

The differences between ssp. *andigena* and ssp. *tuberosum* in South America are sufficient that the two could reasonably be considered as two subspecies. Since the 1960's the two taxa have been hybridized often in breeding programs. Neo – *tuberosum*, a northern-adapted strain of ssp. *andigena*, has been selected to mimic ssp. *tuberosum* (Ghislain *et al.*, 2009). Substitution back-cross products have been produced that have the chromosomal genes of ssp. *tuberosum* combined with cytoplasmic factors of Andean species. These

breeding activities are blurring the distinctions between the two subspecies throughout much of the world, though they remain distinct in their native areas in South America.

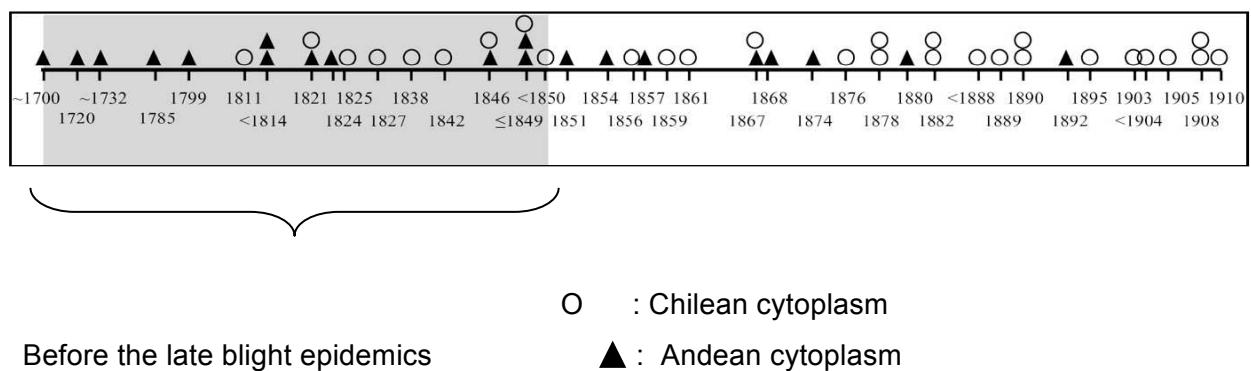
Ames and Spooner (2008), report efforts were put into creating “Neo-Tuberosum” populations through artificial selection of long-day adaptation from Andean potatoes. The goal was based on the unquestioned assumption that the European potato was solely of Andean origin and was mass selected for long daylength adaptation like the Chilean potato, but in the process of selection, many desirable characters for general adaptation, such as resistance to potato late blight, were lost. However, these authors, document that European potato germplasm was derived from high latitude Chilean forms of *Solanum tuberosum* in Europe long before the potato blight epidemics. Molecular examinations of the plastid *trn V-UAC/ ndh C* intergenic spacer region provide the first direct evidence to bear upon the long-held controversy of the extra-Andean origin of this major food plant and completely change our understanding of the history of the potato outside of South America. Neo-Tuberosum potato adapted to long-day tuberization and a syndrome of related morphological and physiological trait, developed by intercrossing and selection of short day adapted potatoes of the *Solanum tuberosum* Andigenum Group, native from the Andes.

Ames and Spooner (2008), present the first direct evidence of the introduction of potato to Europe from either the high Andes or lowland Chile, through an examination of the plastid 241-bp deletion marker from historical (1700 – 1910) herbarium specimens. Through a plastid DNA deletion marker from historical herbarium specimens, they report that the Andean potato predominated in the 1700s, but the Chilean potato was introduced into Europe as early as 1811 and became predominant long before the late blight epidemics in the UK. The results provide the first direct evidence of these events and change the history of introduction of the European potato. They shed new light on the value of past breeding efforts to recreate the European potato from Andean forms and highlight the value of herbarium specimens in investigating origins of crop plants.

Finally, Ames and Spooner (2008), supports original introductions from the Andes, but refutes the idea that the late blight epidemics beginning in Europe in 1845 stimulated introductions of Chilean germplasm as breeding stock to fight this disease and eliminated the Andean potato, which persisted up until 1892. Chilean potatoes became predominant by at least 1811, 34 yr before the late blight epidemics (Figure 9). These authors are against the idea that Chilean potatoes were the germplasm sources after the late blight epidemics. First, Chilean potatoes are not noted as sources of late blight resistance. Second, plastids are not transferred in pollen in the Solanaceae, so only crosses of Chilean potatoes as

female with Andean potatoes as male would produce the over 99% of extant modern varieties having Chilean-type plastid DNA. However, this cross is hindered by the unilateral incompatibility of Chilean and Andean potatoes described (Grun, 1979; Jansky and Peloquin, 2006).

In this context, Hosaka *et al.*, (1988), indicated common potato *Solanum tuberosum* ssp. *tuberosum* has a unique chloroplast DNA (so called T type ctDNA). This author suggests that T type ctDNA of the common potato and of Chilean *tuberousm* originated from W type ctDNA (*S. chacoense*).

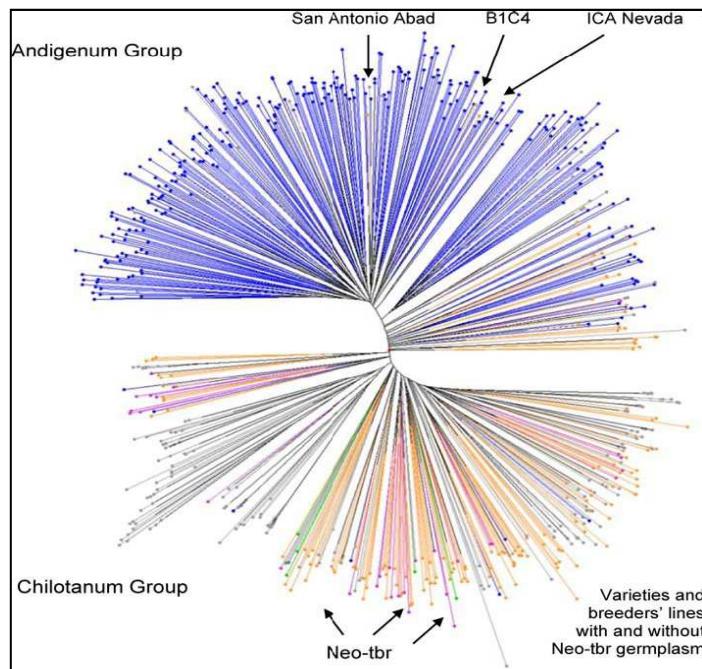


**Figure 9.** Chronological summary of the 241 bp deletion in the trnV-UAC/ndhC intergenic spacer region of the plastid DNA of 49 herbarium specimens of *Solanum tuberosum* collected in Europe from the early 1700s to 1910, distinguishing germplasm originating from the high Andes or from lowland Chile.

**Source:** Ames and Spooner (2008).

On the other hand, Ghislain, *et al.*, (2009) mentioned that Neo-Tuberous theory, has been universally accepted for almost 40 years, and has had tremendous impact in planning some breeding programs and supporting phylogenetic conclusions in cultivated potato. These authors utilized microsatellite and plastid DNA marker data, suggested that Neo-Tuberous germplasm is closely related to Chilotanum Group landraces from lowland south-central Chile rather than to Andigenum Group germplasm (Figure 10). These results question the long-standing Neo-Tuberous derived theory and have implications in breeding programs and phylogenetic reconstructions of potato. In addition, these authors suggested that Neo-Tuberous and Andigenum Group germplasm did not serve to broaden the overall genetic diversity of advanced potato varieties, but rather that Neo-Tuberous lines and lines not using this

germplasm are statistically identical with regard to genetic diversity as assessed by microsatellite.



**Figure 10.** Dissimilarity tree using Neighbor-joining cluster analysis of (1) Six “pure” Accessions of neo-Tuberosum (Neo-tbr) that represent some of the original populations developed by Plaisted from Simmond materials, (2) 33 varieties or breeders’ lines developed with Neo-Tuberosum in their pedigree, (3) 154 varieties or breeders’ lines without Neo-Tuberosum in their pedigree chosen from the CIP genebank selected to cover a wide range of modern germplasm of cultivated potato around the world, (4) 305 of Andigenum landraces, (5) 190 Chilotanum landraces. Also are indicated the three breeding clones serving as controls of Andigenum-based breeding.

**Source:** Ghislain (2009).

The first interspecific crosses in potatoes were tried about 1850 but it was not until the XX<sup>th</sup> century that wild species were extensively used in potato breeding. Essentially, the procedure has been to cross cultivars by species having desirable characters (virtually always disease resistance) and backcross to cultivars. By this means breeding stocks have become somewhat introgressed by: *S. demissum* (race-specific hypersensitivity to blight); *S. stoloniferum*; *S. chacoense*; *S. acaule* (resistances to viruses); *S. multidissectum*; *S. spegazzinii*; *S. kurtzianum*; *S. oplocense* and *S. vernei* (resistances to eelworms). In the late 1960s, only four varieties out of 30 bore any genetic contribution from wild species (all from *S. demissum*) and the leading varieties were all pure Tuberosum (Simmond, 1995). More recent studies revealed that Chilean material has contributed to the improvement of

the potato in Europe from selections made before the late blight epidemic in the early 1840s (Ríos et. al., 2007).

### **1.5.- Conclusion.**

The taxonomy of Solanum related to *S. tuberosum* and assumptions about the origin of grown potatoes are constantly evolving since the 1920s under the influence of different researchers. One point of consensus is the status of autotetraploid of *S. tuberosum*:  $2n = 4x = 48$  chromosomes. The diversity of information does not accurately reflect the genetic distances and the potential for genetic exchange.

Spooner et al., (2005), indicate that the tuber-bearing wild relative potatoes, are given in Section Petota. Under Petota about 20 species are classified as complex brevicaulis. The complex brevicaulis is itself split into two sub-groups of different geographical origins: central Peru and northern Argentina.

The taxonomy of the complex brevicaulis is evolving. The complex is polyphyletic brevicaulis. The number of species within the complex is now about 20, but Spooner et al., (2005) suggests a possible reduction to a single species. The origin of *S.tuberosum* is a single origin from central Peru brevicaulis, which implies that *S. tuberosum* cultivated is considered monophyletic by Spooner et al. (2005).

On the other hand, the hypothesis of an origin of andigenum European potatoes was favored for 40 years, from 1960 to 2000, following the work of Simmonds on Neo-tuberosum. Recent research has confirmed that the genetic material of Chile contributed to the improvement of the potato in Europe and its spread throughout the world outside of northern South America, and that selection from before the outbreak of late blight in 1845. Studies based on the diversity of molecular markers showed that most varieties of potatoes worldwide (outside the region of origin) have genes of *S. tuberosum* ssp *chilotanum*. This gives the potatoes native of Chile the status of original genetic resource. The use of this material needs to perform the duties of collection, preservation (in-situ and ex situ), characterization and evaluation to determine their characteristics.

### **1.6.- Objectifs du travail de thèse**

Ces travaux de thèse s'inscrivent dans le cadre général de la diversité génétique de la « pomme de terre native » au Chili, centre de diversification secondaire de la pomme de terre. Nous emploierons dans la suite du document le terme de « pomme de terre native » pour désigner les variétés locales traditionnelles chiliennes. Ces variétés sont *a priori*, et

jusqu'à preuve du contraire, non-introgressées par la variabilité génétique de variétés améliorées européennes ou nord américaines, elles sont issues de la diversité des variétés existant avant l'arrivée des européens. Le travail porte sur la caractérisation, l'utilisation et la valorisation des pommes de terre natives et sur les possibilités de conservation « *ex situ* » et « *in situ* ». La dernière partie du document traite de la résistance au mildiou (*Phytophthora infestans*) des pommes de terre natives.

L'objectif général est donc d'évaluer la diversité génétique d'une collection de variétés de pommes de terre natives de l'île de Chiloé (cette collection a été constituée par nos soins entre 2000 et 2007, Tableau II), de caractériser ces variétés vis-à-vis de la résistance au mildiou (*Phytophthora infestans*), d'envisager les conditions de leur conservation *in situ* et de leur utilisation éventuelle dans les programmes d'amélioration génétique.

Les différents chapitres aborderont les thèmes suivants :

- Conservation *in situ* dans l'île grande de Chiloé de *Solanum* natifs du Chili et son impact sur la diversité.
- Evaluation de la diversité morphologique de la collection de pommes de terre (*Solanum tuberosum* L.) natives du Chili.
- Evaluation de la diversité génétique de la collection par les marqueurs microsatellites (SSRs).
- Evaluation de la diversité génétique de la collection par les marqueurs AFLP.
- Caractérisation de la résistance au champ des accessions natives de pomme de terre aux infections naturelles de mildiou (*Phytophthora infestans*) du sud du Chili.
- Evaluation de la résistance au mildiou (*Phytophthora infestans*) dans les accessions des *Solanum* natives, sur un test de folioles détachés.

L'annexe1 est constituée du questionnaire utilisé pour réaliser l'enquête auprès des paysans de Chiloé.

L'annexe2 est un article sur une expérience de conservation *ex-situ* de *Solanum fernandezianum* (Solanaceae).

L'annexe3 est constituée du descriptif (texte et illustrations photographiques) des trente variétés locales collectées par nos soins à Chiloé. Le descriptif est basé sur ceux de l'IBPGR 1977 et de l'UPOV.

La collecte de pommes de terre native de Chiloé a été formé depuis l'année 2000, après que l'auteur de ce travail en 1998 pour suivre le cours sur «la collecte, la caractérisation et la conservation des ressources génétiques», publié au Chili Institut de recherche agricole (INIA) et le Conseil international des ressources phytogénétiques (IBPGR; International

Board for Plant Genetic Resources), avec le soutien financier de l'Agence de coopération Internationale (JICA, Japan International Cooperation Agency). Suite à cela, entre 2000 et 2007 il ya eu plusieurs expéditions de collecte dans le Sud du Chili, qui ont inclus principalement les communautés d'Ancud, Chonchi, Castro, Quemchi, Queilen, Quinchao Curaco de Velez, Quellon, Dalcahue et Puqueldon dans l'archipel de Chiloé. En outre, un niveau continental, le matériel a été recueilli dans la municipalité de Los Muermos.

Por otra parte, durante, el año 2002, se realizó una expedición de colecta a la Isla de Robinson Crusoe en el Archipiélago de Juan Fernández, con el fin de colectar material diploide de papa. Par ailleurs, au cours de l'année 2002, une collection d'expédition de Robinson Crusoe dans l'archipel de Juan Fernandez, afin de recueillir les pommes de terre diploïdes matériel.

**Tableau 2.** Collecte de pommes de terre native évalué.

Accessions	Nom	Región	Origine	Spèce	Entrée	Matériels
UCT-11Mgb	Meca gato blanca	de los Lagos	Isla grande,	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2000	Tubercule
UCT-14MgRe	Redonda	de los Lagos	Isla grande,	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2000	Tubercule
UCT-17Br	Bruja	de los Lagos	Isla Quinchao.	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2000	Tubercule
UCT- 6Gc	Guadacho colorado	de los Lagos	Chonchi.	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2000	Tubercule
UCT-24Tn	Tonta	de los Lagos	Castro.	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2000	Tubercule
UCT-22Cm	Clavela morada	de los Lagos	Castro.	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2000	Tubercule
UCT-25Gñ	Guicoña	de los Lagos	Quellón	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2000	Tubercule
UCT- 7Ca	Camota	de los Lagos	Isla grande,	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2000	Tubercule
UCT-18Mn	Michuñe negro	de los Lagos	Isla grande,	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2000	Tubercule
UCT-26Ach	Azul chañihue	de los Lagos	Isla grande,	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2000	Tubercule
UCT-27Mu	Murta	de los Lagos	Quellón.	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2000	Tubercule
UCT-28MiR	Michuñe rojo	de los Lagos	Isla grande,	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2000	Tubercule
UCT-29Mol	Molejona	de los Lagos	Isla grande,	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2000	Tubercule
UCT- 3Cl	Clavela	de los Lagos	Los Muermos	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2000	Tubercule
UCT- 1Ma	Michuñe azul	de los Lagos	Isla grande,	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2000	Tubercule
UCT-16At	Azul table	de los Lagos	Isla grande,	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2000	Tubercule
UCT-30Ño	Ñocha	de los Lagos	Isla grande,	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2000	Tubercule
UCT-19Aq	Azul de quento	de los Lagos	Castro.	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2000	Tubercule
UCT- 2Lv	Lengua	de los Lagos	Castro.	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2000	Tubercule
UTC-31Ob	Ojitos blanco	de los Lagos	Ancud.	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2003	Tubercule
UCT-32Ci	Cielito	de los Lagos	Castro.	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2000	Tubercule
UCT-20Ro	Rosada	de los Lagos	Isla grande,	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2000	Tubercule
UCT-33Cab	Cabrita	de los Lagos	Isla grande,	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2003	Tubercule
UCT-15MgRo	Meca gato rojo	de los Lagos	Isla grande,	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2000	Tubercule
UCT-21Ac	Azul cristalina	de los Lagos	Isla grande,	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2000	Tubercule
UCT-34Cor	Cordillera	de los Lagos	Castro.	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2003	Tubercule
UCT-35AzC	Azul caucheque	de los Lagos	Castro.	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2000	Tubercule
UCT- 8Gb	Guadacho blanco	de los Lagos	Ancud.	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2000	Tubercule
UCT- 9MgM	Meca gato morada	de los Lagos	Ancud.	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2000	Tubercule
UCT-10MgL	Meca gato morada larga	de los Lagos	Los muermos,	<i>Solanum tuberosum</i> spp. <i>tuberosum</i>	2000	Tubercule
UCT-23Sf		de Valparaíso	Island Juan Fernandez. Plazoleta Yunque	<i>Solanum</i> <i>fernandezianum</i>	2002	Botanical seed

Puis dans le tableau 3, présente la liste des évaluations sur les accéssions de pommes de terre native évalué

**Tableau 3.** Les évaluations du matériel de pommes de terre native.

Accession	Name	Origine	Les evaluations							
			Morpho (2007)	Morpho (2009)	SSR (2010)	AFLP (2006)	Mildiou Terrain (2006/7)	Mildiou Terrain (2008/9)	Mildiou Terrain (2009/10)	Mildiou Folioles detaches (2010)
UCT-11Mgb	Meca gato blanca	Isla grande,	Qui	Qui	Qui	Qui	Qui	Qui	Qui	
UCT-14MgRe	Redonda	Isla grande,	Qui	Qui	Qui	Qui	Qui	Qui	Qui	
UCT-17Br	Bruja	Isla Quinchao.		Qui	Qui	Qui	Qui	Qui	Qui	
UCT- 6Gc	Guadacho colorado	Chonchi.	Qui	Qui	Qui	Qui	Qui	Qui	Qui	Qui
UCT-24Tn	Tonta	Castro.		Qui	Qui		Qui	Qui	Qui	
UCT-22Cm	Clavela morada	Castro.		Qui	Qui	Qui	Qui	Qui	Qui	
UCT-25Gr	Guicoña	Quellón		Qui	Qui		Qui	Qui	Qui	
UCT- 7Ca	Camota	Isla grande,	Qui	Qui	Qui	Qui	Qui	Qui	Qui	
UCT-18Mn	Michuñe negro	Isla grande,		Qui	Qui	Qui		Qui	Qui	Qui
UCT-26Ach	Azul chañihue	Isla grande,		Qui	Qui			Qui	Qui	Qui
UCT-27Mu	Murta	Quellón.		Qui	Qui			Qui	Qui	
UCT-28MiR	Michuñe rojo	Isla grande,		Qui	Qui			Qui	Qui	
UCT-29Mol	Molejona	Isla grande,		Qui	Qui			Qui	Qui	
UCT- 3Cl	Clavela	Los Muermos	Qui	Qui	Qui	Qui	Qui	Qui	Qui	Qui
UCT- 1Ma	Michuñe azul	Isla grande,	Qui	Qui	Qui	Qui	Qui	Qui	Qui	Qui
UCT-16At	Azul table	Isla grande,	Qui	Qui	Qui	Qui	Qui	Qui	Qui	
UCT-30Ño	Ñocha	Isla grande,		Qui	Qui		Qui	Qui	Qui	Qui
UCT-19Aq	Azul de quento	Castro.		Qui	Qui	Qui		Qui	Qui	
UCT- 2Lv	Lengua	Castro.	Qui	Qui	Qui	Qui		Qui	Qui	
UTC-31Ob	Ojitos blanco	Ancud.	Qui	Qui	Qui	Qui		Qui	Qui	
UCT-32Ci	Cielito	Castro.		Qui	Qui			Qui	Qui	
UCT-20Ro	Rosada	Isla grande,	Qui	Qui	Qui	Qui	Qui	Qui	Qui	
UCT-33Cab	Cabrita	Isla grande,		Qui	Qui			Qui	Qui	
UCT- 15MgRo	Meca gato rojo	Isla grande,	Qui	Qui	Qui	Qui	Qui	Qui	Qui	Qui
UCT-21Ac	Azul cristalina	Isla grande,	Qui	Qui	Qui	Qui	Qui	Qui	Qui	
UCT-34Cor	Cordillera	Castro.		Qui	Qui			Qui	Qui	Qui
UCT-35AzC	Azul caucheque	Castro.		Qui	Qui			Qui	Qui	Qui
UCT- 8Gb	Guadacho blanco	Ancud.	Qui	Qui	Qui	Qui	Qui	Qui	Qui	
UCT- 9MgM	Meca gato morada	Ancud.	Qui	Qui	Qui	Qui	Qui	Qui	Qui	Qui
UCT-10MgL	Meca gato morada larga	Los muermos,	Qui	Qui	Qui	Qui	Qui	Qui	Qui	

Puis dans le tableau 4, présente la liste des évaluations sur les cultivars commerciaux inclus dans l'étude, qui ont généralement été considérées comme des témoins.

**Tableau 4.** Les évaluations du matériel de pommes de terre commercial.

Nom	Origine	Année de sortie	Parental	Les évaluations					
				AFLP	SSR	Mildiou Terrain 2006/7	Mildiou Terrain 2008/9	Mildiou Terrain 2009/10	Mildiou folioles détachés
Desirée	Introduit ZPC – Holanda	1962	Urgenta x Despeche	Qui	Qui	Qui	Qui	Qui	
Karú	Chili	2002	Yagana x Fanfare		Qui		Qui	Qui	Qui
Shepody	Introduit Canada	1980	Bake-King x F58050		Qui				
Baraka	Introduit Allemagne		SVP 50-358 x Avenir		Qui				
Híbrida	Hybride LT8 xTS-9 Chili	----	LT-8 x TS-9		Qui				
Rosara	Introduit Solana – Alemania	1990	Secura x 2605 77		Qui				
Yagana	Chili	1983	Hydra x 904/61		Qui				
Pukará	Chili	1993	Cleopatra x Yagana		Qui				
Rodeo	Introduit				Qui				
Craig's Royal	Introduit		Différentielle						Qui

## **1.7 REFERENCES.**

- Ames, M. And D. Spooner. 2008. DNA from herbarium specimens settles a controversy about origins of the European potato. *American Journal of Botany* 95(2):252-257.
- Bradshaw, J. E., G.J. Bryan and G. Ramsay. 2006. genetic Resources (Including wild and cultivated Solanum Species) and progress in their utilization in potato breeding. *Potato Research*. 49:49-65.
- Castronovo, A. 1949. Papas chilotas. Ministerio de Agricultura y Ganadería. Instituto de Fitotécnica. Buenos Aires. Argentina. 245 p.
- Ghislain, M., J. Núñez., M. del R. Herrera and D. M. Spooner. 2009. The single Andigenum origin of Neo-Tuberosum potato materials is not supported by microsatellite and plastid marker analyses. *Theor. Appl. Genet.* 118:963-969.
- Glendinning, D. 1975. Chilean potatoes: an appraisal. *Potato Research*. 18: 306-307.
- Greenpeace. 2000. Centros de diversidad. La riqueza biológica de los cultivos tradicionales, herencia mundial amenazada por la contaminación genética. México. 65 p.
- Grun, P. 1990. The evolution of cultivated potatoes. *Economic Botany*. Vol 44, N°3, pp. 39-55.
- Hawkes, J. G., 1990. The potato. Evolution, biodiversity and genetic resources. Belhaven Press. London, UK. 178p.
- Hawkes, J. G., and J. F. Ortega. 1992. The potato in Spain during the late 16th century. *Economic Botany* 46: 86-97.
- Hawkes, J. G., and J. F. Ortega. 1993. The early history of the potato in Europe. *Euphytica* 70:1-7.
- Hawkes, J. 1994. Origins of cultivated potatoes and species relationships. In: Bradshaw, J. and Mackay, G. (eds). *Potato Genetics*. Wallingford, U.K, CAB International. pp:3-39.
- Hosaka, K. , de Zoeten G.A. and R.E. Hanneman, Jr. 1988. Cultivated potato chloroplast DNA differs from the wild type by one deletion - Evidence and implications. *Theor. Appl. Genet.* 75:741-745.
- Hosaka, K. 1995. Succesive domestication and evolution of the Andean potatoes as revealed by chloroplast DNA restriction endonuclease analysis. *Theor. Appl. Genet.* 90:356-363.
- Huamán, Z., and D.M. Spooner. 2002. Reclassification of landrace population of cultivated potatoes (Solanum sect. Petota). *American Journal of Botany*. 89:947-965.
- Jansky, S. H. and S. J. Peloquin. 2006. Advantages of wild diploid Solanum species over cultivated diploid relatives in potato breeding programs. *Genetic Resources and Crop Evolution*. 53:669-674.

- Kawagoe, Y., and Kikuta. 1991. Chloroplast DNA evolution in potato (*Solanum tuberosum* L.). *Theoretical and Applied Genetics* 81:13-20.
- Lössl, A., N. Adler., R. Horn., U. Frei and G. Wenzel. 1999. Chondriome-type characterization of potato: mt  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ,  $\epsilon$  and novel plastid-mitochondrial configurations in somatic hybrids. *Theor. Appl. Genet.* 1999:981-10.
- Montaldo, A. 1984. Cultivo y mejoramiento de la papa. Instituto Interamericano de Cooperación para la Agricultura. San José, Costa Rica. 676 p.
- Raker, C. y Spooner, D., 2002. Chilean tetraploid cultivated potato, *Solanum tuberosum*, is distinct from the Andean populations: microsatellite data. *Crop Science*. 42:1451-1458.
- Rios D., M. Ghislain., F. Rodriguez. And D. Spooner. 2007. What is the origin of the European Potato?. Evidence from Canary Island landraces. *Crop Science*, Vol.47, May-June 2007. pp 1271-1280.
- Ruiz de Galarreta, J.L., J. Pascualena., F. J. Legorburu., L. barandalla and E. Ritter. 2006. The History of potato (*Solanum tuberosum* L.) Research in Spain. *Potato research* 49:19-25.
- Simmonds, N. W. 1995. Potatoes – *Solanum tuberosum* (Solanaceae). In: J. Smartt and N. W: Simmonds (eds.), Evolution of crop plants, 466-471. Longman Scientific and Technical, Essex, UK.
- Spooner, D. M., and W. L .A. Hetterscheid. 2005. Origins, evolution, and group classification of cultivated potatoes. P.285-307. In T. J. Motley et al. (ed.) Darwin's harvest: New approaches to the origins, evolution and conservation of crops. Columbia Univ. Press, New York.
- Spooner. D. M., Mc. Lean K, G. Ramsay., R., Waugh, G.J. Bryan. .2005. A single domestication for potato based on multilocus amplified fragment length polymorphism genotyping. *Proceedings of the National Academy Sciences of the United States of America*. 102:14694–14699.
- Spooner. D. M., J. Nuñez., F. Rodriguez, P.S Nair, and M. Ghislain. 2004. Nuclear and chloroplast DNA reassessment of the origin of Indian potato varieties and its implications for the origin of the early European potato. *Theor.Appl.Genet.* 110:1020-1026.
- Spooner, D. M., J., Nuñez., G, Trujillo., M. Herrera., F. Guzman and M. Ghislain. 2007. Extensive simple sequence repeat genotyping of potato landraces supports a major reevaluation of their gene pool structure and classification. *Proc. Natl. Acad. Sci.* 104 (49):19398-10403.

Van den Berg, R. G., Miller, J.T., Ugarte, M.L., Kardolus, J. P., Nienhuis, J. and D. M. Spooner. 1998. Collapse of morphological species in the wild potato *Solanum brevicaule* complex (sect. Petota). American Journal of Botany. 85, 92-109.

**Chapitre II Préambule extrait du chapitre XIII du voyage d'un naturaliste de Darwin :**

En préambule et avant d'aborder le chapitre II, nous avons souhaité reproduire quelques extraits du chapitre XIII du voyage d'un naturaliste de Darwin<sup>1</sup>. Ces extraits sont empruntés à l'ouvrage disponible en téléchargement à l'adresse suivante :

[http://classiques.uqac.ca/classiques/darwin\\_charles\\_robert/voyage\\_naturaliste\\_around\\_the\\_world/voyage\\_naturaliste.html](http://classiques.uqac.ca/classiques/darwin_charles_robert/voyage_naturaliste_around_the_world/voyage_naturaliste.html)

Ce texte rédigé par un aristocrate britannique du XIXème siècle contient vraisemblablement quelques biais d'appréciation et aussi des formulations un peu romancées. Il est dans ce sens intéressant de souligner les remarques sur le climat ou celles sur le niveau de civilisation des indigènes comparé à celui des colons. Mais au-delà de son appartenance à l'empire britannique, très dominant dans le monde à l'époque, Darwin était un naturaliste et un observateur éclairé et la relation qu'il fait de son passage à Chiloé et dans l'archipel de Chonos nous a paru pouvoir constituer une mise en perspective assez juste du milieu naturel et des conditions de vie qui ont régné à Chiloé jusqu'au milieu du XXème siècle.

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<sup>1</sup> Charles-Robert Darwin (1809-1882), [Voyage d'un naturaliste autour du monde fait à bord du navire le Beagle de 1831 à 1836](#). Traduit de l'anglais par M. Edmond Barbier. Paris: C. Reinwald et Cie, Libraires-Éditeurs. 1875, 552 pages, 14 figures. Une édition numérique réalisée par M. [Jean-Marc Simonet](#), professeur retraité de l'enseignement, Université de Paris XI-Orsay.

## Chapitre XIII.

### Chiloé et les îles Chonos.

10 novembre 1834. — Le *Beagle* quitte Valparaiso et se dirige vers le sud pour relever les côtes de la partie méridionale du Chili, celles de l'île de Chiloé et visiter ces îles nombreuses connues sous le nom d'*archipel Chonos*, en poussant jusque vers la péninsule de Tres Montes. Le 21, nous jetons l'ancre dans la baie de San Carlos, capitale de Chiloé.

Cette île a environ 90 milles (145 kilomètres) de longueur sur une largeur d'un peu moins de 30 milles (48 kilomètres). Elle est entrecoupée de collines, mais non pas de montagnes, et recouverte absolument d'une immense forêt, excepté là où on a défriché quelques champs autour de huttes couvertes en chaume. A une certaine distance, on croirait revoir la Terre de Feu, mais, vus de plus près, les bois sont incomparablement plus beaux. Un grand nombre d'arbres toujours verts, des plantes au caractère tropical, remplacent ici les sombres et tristes hêtres des côtes méridionales. En hiver le climat est détestable ; il ne fait pas, d'ailleurs, beaucoup plus beau en été. Je crois qu'il y a, dans les régions tempérées, peu de parties du monde où il tombe autant de pluie. Le vent y souffle toujours en tempête, le ciel est toujours couvert ; une semaine entière de beau temps est presque un miracle. Il est même difficile d'apercevoir la Cordillère ; pendant tout le temps qu'a duré notre premier séjour, nous n'avons aperçu qu'une seule fois le volcan d'Osorno et c'était avant le lever du soleil ; à mesure que le soleil s'élevait, la montagne disparaissait graduellement dans les profondeurs brumeuses du ciel, et ce lent effacement ne manqua pas de nous intéresser vivement.

A en juger par leur teint et par leur petite taille, les habitants semblent avoir trois quarts de sang indien dans les veines. Ce sont des gens humbles, tranquilles, industriels. Bien que le sol fertile provenant de la décomposition des roches volcaniques soutienne une luxuriante végétation, le climat n'est cependant pas favorable aux produits qui ont besoin de soleil pour arriver à maturité. Il y a peu de pâturages pour les grands quadrupèdes ; en conséquence, les principaux aliments sont les cochons, les pommes de terre et le poisson. Les habitants portent tous d'épais vêtements de laine que chaque famille tisse elle-même et qu'on teint en bleu avec de l'indigo. Toutefois tous les arts sont encore à l'état le plus grossier et, pour en avoir la preuve, on n'a qu'à examiner leur singulier mode de labourage, leur mode de tissage, leur manière de mouler le grain ou la construction de leurs bateaux. Les forêts sont si impénétrables, que la terre n'est cultivée nulle part, sauf près de la côte et sur les îlots voisins. Aux endroits mêmes où existent des sentiers on peut à peine les traverser, tant le

sol est marécageux ; aussi les habitants, comme ceux de la Terre de Feu, circulent-ils principalement sur le bord de la mer ou dans leurs bateaux. Bien que les vivres soient en abondance, les habitants sont très-pauvres ; il n'y a pas de travail et, en conséquence, les pauvres ne peuvent se procurer l'argent nécessaire pour acheter le plus petit objet inutile ; en outre, l'argent monnayé fait défaut à tel point, que j'ai vu un homme porter sur son dos un sac de charbon qu'il allait donner en payement d'un menu objet et un autre échanger une planche contre une bouteille de vin. Chacun est donc obligé de se faire marchand pour revendre ce qu'il a reçu dans ces nombreux échanges.

/.../

*25 novembre.* — Il pleut à torrents ; nous côtoyons cependant l'île jusqu'à Huapi-Lenou. Toute cette partie orientale de Chiloé présente le même aspect : une plaine entrecoupée de vallées et divisée en petites îles ; le tout est recouvert par une impénétrable forêt vert noirâtre. Sur la côte, quelques champs défrichés entourent des huttes à toits fort élevés.

/.../

*26 novembre.* —

/.../ Nous débarquons dans l'après-midi et nous voyons une famille de pure race indienne. Le père ressemble beaucoup à York Minster ; on aurait pu prendre pour des Indiens des Pampas quelques jeunes garçons au teint bronzé. Tout ce que je vois me confirme de plus en plus la proche parenté des différentes tribus américaines, bien qu'elles aient toutes des langages différents. Cette famille savait à peine quelques mots d'espagnol. Il est fort agréable de voir que les indigènes en sont arrivés au même degré de civilisation que leurs vainqueurs de la race blanche, quelque infime d'ailleurs que soit ce degré de civilisation. Plus au sud, nous avons eu l'occasion de voir beaucoup d'Indiens de race pure, tous les habitants de quelques îlots ont même conservé leurs noms indiens. D'après le recensement de 1832, il y avait à Chiloé et dans ses dépendances quarante deux mille habitants, dont le plus grand nombre paraît être de sang mêlé.

/.../

Nous atteignons dans la soirée une charmante petite baie située au nord de l'île de Cauchahue. Les habitants se plaignent beaucoup ici du manque de terres.

/.../

Dans la plupart des pays on se débarrasse facilement des forêts en les brûlant ; mais à Chiloé le climat est si humide, les essences forestières de telle nature, qu'il faut absolument abattre les arbres. C'est là un obstacle sérieux à la prospérité de cette île. Au temps de la domination espagnole, les Indiens ne pouvaient pas posséder de terres ; une famille qui avait défriché le sol pouvait se voir expulsée et son terrain était saisi par le gouvernement. Les autorités du Chili accomplissent aujourd'hui un acte de justice en donnant une pièce de terre à chacun de ces pauvres Indiens.

/.../

7 janvier 1835. Après avoir relevé toute la côte, nous jetons l'ancre près de l'extrémité septentrionale de l'archipel des Chonos, dans le port de Low ; nous y restons une semaine. Ces îles, tout comme celle de Chiloé, se composent de couches stratifiées fort molles, et la végétation y est admirable. Les bois s'avancent jusque dans la mer.

/.../

Nous trouvons ici un groupe de cinq hommes de Caylen, « el fin del Cristiandad », qui, pour venir pêcher dans ces parages, se sont aventurés à traverser dans leur misérable canot l'immense bras de mer qui sépare Chonos de Chiloé. Très-probablement, ces îles se peupleront bientôt comme se sont peuplées celles qui avoisinent la côte de Chiloé.

La pomme de terre sauvage pousse abondamment dans ces îles dans le sol sablonneux plein de coquillages, sur le bord de la mer. Le plant le plus élevé que j'aie vu avait 4 pieds de haut. Les tubercules sont ordinairement petits ; j'en ai trouvé quelques-uns, cependant, de forme ovale, qui avaient 2 pouces de diamètre ; ils ressemblent sous tous les rapports aux pommes de terre anglaises et ont la même saveur ; mais quand on les fait bouillir, ils se réduisent beaucoup et ont un goût aqueux et insipide, mais sans amertume. Il n'y a pas à douter que la pomme de terre ne soit indigène dans ces îles. On la trouve, selon M. Low, jusque par 50 degrés de latitude sud, et les Indiens sauvages de ces régions lui donnent le nom d'*Aquinas* ; les Indiens de Chiloé lui donnent un nom différent. Le professeur Henslow, qui a examiné les spécimens desséchés que j'ai rapportés en Angleterre, soutient que ces pommes de terre sont identiques à celles décrites par M. Sabine<sup>2</sup>, de Valparaiso, mais qu'elles forment une variété que quelques botanistes considèrent comme spécifiquement distincte. Il est remarquable que la même plante se retrouve sur les montagnes stériles du Chili central, où il ne tombe pas une goutte d'eau pendant plus de six mois, et dans les forêts humides de ces îles méridionales.

/.../

4 février. —

/.../ Nous sommes tous enchantés de dire adieu à Chiloé ; ce serait cependant une île charmante, si des pluies continues n'y engendraient autant de tristesse. Il y a aussi quelque chose de fort attrayant dans la simplicité et l'humble politesse de ses pauvres habitants.

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<sup>2</sup> *Horticultural Transact.*, vol. V, p. 249. M. Caldbleugh a envoyé en Angleterre deux tubercules qui, cultivés avec soin, ont produit, dès la première année, de nombreuses pommes de terre et une grande quantité de feuilles. Voir l'intéressante discussion de Humboldt sur cette plante, laquelle, paraît-il, était inconnue au Mexique, *Polit. Essay on New Spain*, liv. IV, chap. ix.

## **CHAPITRE II. Conservation in situ de *Solanum* natifs du Chili et son impact sur la diversité.**

### **1.- INTRODUCTION**

#### **1.1. Les ressources génétiques.**

Les ressources Phylogénétiques constituent la base biologique de la sécurité alimentaire mondiale. Ces ressources sont formées par la riche diversité de matériel génétique constituée par les variétés traditionnelles, les variétés modernes, ainsi que les plantes sauvages ancêtres des variétés cultivées et d'autres espèces de plantes sauvages apparentées à l'espèce cultivée. Cette variabilité forme un dépôt d'adaptabilité génétique qui sert de garantie face au danger potentiel présenté par les changements de l'environnement et de l'économie. Pour cette raison, la conservation et l'utilisation soutenable des ressources phyto-génétiques sont fondamentales pour améliorer la productivité de l'agriculture et contribuer au développement des différentes nations et donc du développement mondial, visant à atténuer la pauvreté et la dénutrition (FAO, 1996).

La pomme de terre (*Solanum tuberosum* L.) est une des cultures alimentaires parmi les plus productives. Elle est largement cultivée dans le monde entier, son amélioration génétique est basée sur une source diverse de germoplasme (Huaman *et al.* 1997 ; Hijmans et Spooner, 2001). *S. tuberosum* est une espèce tétraploïde fortement hétérozygote, dont les variétés cultivées sont reproduites par multiplication végétative. Malgré tout, cette espèce a gardé une reproduction sexuée efficace et peut se croiser, moyennant quelques astuces techniques, avec la plupart de ses apparentés sauvages. Cette caractéristique ouvre des possibilités d'amélioration, en particulier pour les résistances aux stress biotiques et abiotiques (Spooner *et al.* 2005).

Le Chili est un pays très riche en Ressources Génétiques et est considéré comme un centre secondaire d'origine, ou du moins de diversification, de la pomme de terre cultivée (Spooner *et al.* 2005). Dans certaines régions du pays, du matériel génétique natif est toujours cultivé qui co-existe avec du matériel introduit. Dans le Sud du Chili, on trouve une notable concentration de formes de pomme de terre cultivées et sauvages, c'est le cas de l'île grande de Chiloé qui abrite un grand nombre de variétés natives. Actuellement c'est dans les îles de la partie orientale et dans la partie sud de l'île grande que l'on trouve les petits agriculteurs qui conservent encore ces variétés dans leurs potagers (Montaldo, 1984a; Montaldo 1984b). La province de Chiloé, est considérée comme un des centres de

biodiversité des pommes de terre natives. Jusqu'à présent ces pommes de terre ont été conservées *in situ* par les petits paysans qui les vendent sur les marchés locaux et les utilisent pour leur consommation personnelle (Figure 1, 2 et 3). Ces ressources constituent un matériel phyto-génétique original, issues directement d'une domestication ancestrale, elles constituent un patrimoine génétique et culturel d'importance pour les générations futures. Ce matériel a été étudié par différents auteurs (Mecias *et al.*, 1988 ; 1989 ; Spooner *et al.*, 2005 ; Rios *et al.*, 2007). Néanmoins, le nombre d'agriculteurs qui cultivent la pomme de terre native a diminué considérablement ces dernières années.



**Figure 1.** Conservation *in-situ* de pomme de terre; variétés natives et marché local



**Figure 2.** Variétés natives et marché local, Castro, Chiloé.



**Figure 3.** Le territoire de Chiloé. Plantation; paysage typique et récolte.

## 1.2. Agriculture domestique paysanne au Chili.

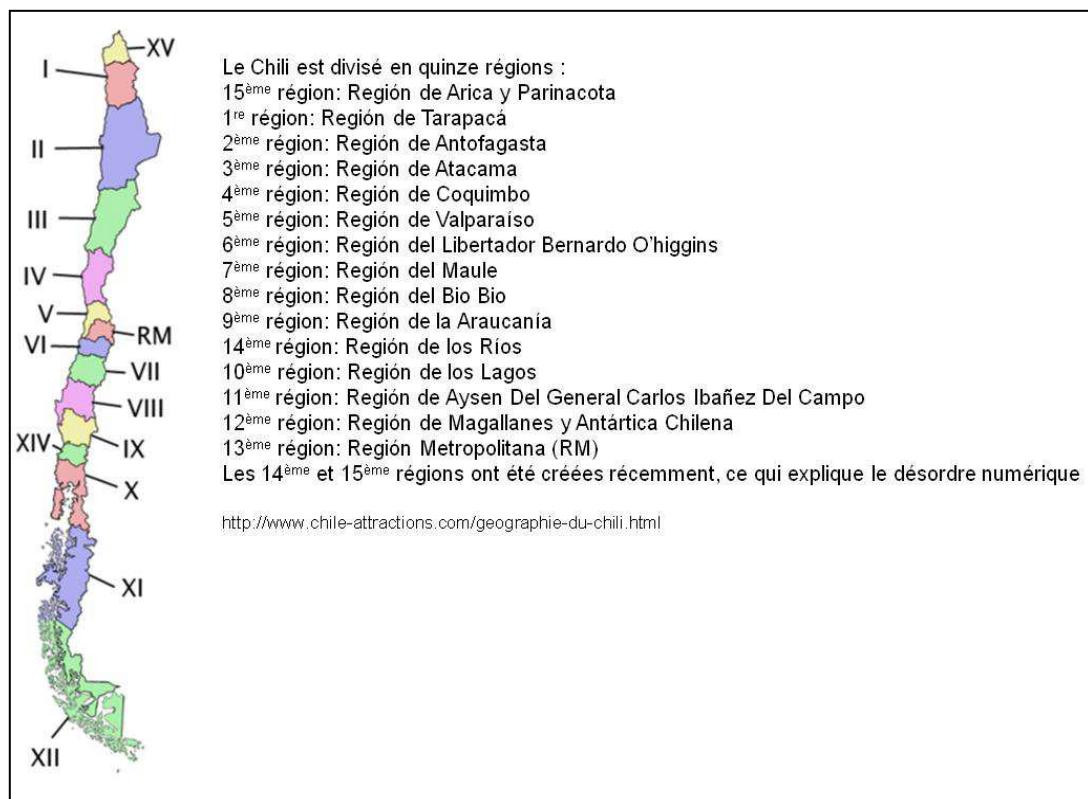
Le Chili est historiquement un pays agricole (FAO, 2007), son développement et son économie sont en partie basés sur la productivité et la richesse de ses sols associées à une diversité de climats favorables qui permettent de produire des aliments diversifiés et de qualité pour la consommation interne et pour l'exportation. Le pays présente près de deux cent quatre vingts milles (280.000) exploitations d'agriculteurs paysans, lesquelles se trouvent pour 95% d'entre elles entre la quatrième (région de Coquimbo, 29° de latitude sud) et la dixième région (region de Los Lagos, 44° de latitude sud), territoire qui s'étend sur environ 1650 km du nord au sud (Figure 4). L'archipel de Chiloé se situe dans la X<sup>ème</sup> région (capitale, Puerto Montt). De ce total, quatre vingts milles (80.000) correspondent à des

exploitations avec des surfaces équivalentes ou inférieures à un H.R.B. (l'abréviation H.R.B. correspond à "Hectaria Riego Basico" qui est la surface équivalente au potentiel de production d'un hectare de classe 1 dans la vallée du Rio Maipo, zone très fertile des environs de Santiago). Le concept de « paysan » est utilisé dans un sens très large, comprenant tous ceux qui travaillent avec leurs mains directement la terre suivant différents systèmes et structures, en opposition au patron ou à l'agriculteur plus capitalisé. Au Chili, le Ministère de l'Agriculture (1990) a défini comme « petit producteur agricole » celui qui exploite une surface inférieure à 12 H.R.B, dont les actifs ne dépassent pas l'équivalent à 3.500 CLF (Chilean Unidades de Fomento, le CLF est une unité de compte ajustée en permanence pour tenir compte de l'inflation, 1 CLF est équivalent à 32,85€ à la date du 04/03/2011, 3500CLF~115.000€), aux conditions que ses revenus proviennent principalement de l'exploitation agricole et qu'il travaille directement la terre, quel que soit son régime de tenant. De plus, on conçoit comme « paysan » la personne qui habite, travaille et vie habituellement de l'activité agricole réalisée de manière personnelle, quelle que soit la qualité juridique où elle se réalise, tant que les conditions économiques ne sont pas supérieures à celles d'un petit producteur agricole. Dans l'ensemble qui comprend les habitants de la campagne, on distingue des groupes définis comme le sont les indigènes et les fermiers. Dans le premier groupe, on trouve des groupes ethniques divers, lesquels ont des liaisons dans le domaine productif avec le secteur agricole (Ministère d'Agriculture, 1992; 2001).

Cox (1983), soutient que l'hétérogénéité de l'agriculture paysanne constitue une de ses caractéristiques les plus importantes, car elle est déterminée par les conditions agro-écologiques, la diversité des agro-écosystèmes, les potentialités et limitations productives du milieu et des équipements disponibles, les aspects techniques et sociaux. La FAO (1992) signale que les petits agriculteurs présentent une économie extrêmement décapitalisée, très souvent accompagnée de ressources productives limitées et de connaissances techniques inadéquates. D'autre part, Thorner (1979), Schejtman (1980), Boeninger *et al.* (1981) et Gómez (1982) convergent pour relever certains aspects marquants de l'agriculture paysanne, parmi lesquels on trouve :

- 1) l'existence d'unités économiques domestiques, généralement non rémunérées; particulièrement dans le cas des femmes et des jeunes.
- 2) une disponibilité limitée des moyens de production en terre et capital à quoi s'ajoute très peu de possibilités de crédit.
- 3) une quantité et une qualité de production et des réseaux de commercialisation inadéquats, qui conditionnent entre autres les relations de subordination avec les marchés.

- 4) un accès limité à des technologies appropriées à l'amélioration de la production paysanne.



**Figure 4:** Territoire du Chili et ses régions.

### 1.3. Contexte, ruralité et transformations de l'agriculture à Chiloé.

« *Dans l'archipel de Chiloé, la pomme de terre était parmi les biens de la nature le plus cultivé quand les espagnols ont assiégié le plateau de Quilquihue en 1567 pour fonder la ville de Castro* » (Cárdenas, 2003).

Cubillo (1995), signale que l'agriculture dans le système insulaire du Pacifique est pratiquement restreinte à la Grande île de Chiloé et les îles voisines du secteur oriental. Les conditions d'environnement sont propices pour la culture de la pomme de terre et les prairies naturelles, l'excès de pluie limite la culture du blé, qui est remplacée par celle de l'avoine, pour la production de grain. La prairie permet de développer des petites exploitations laitières et l'abondance de pommes de terre permet l'élevage des cochons.

Cardenas (2003), signale que durant le XX<sup>ème</sup> siècle la culture de la pomme de terre a été déterminante pour les activités à l'intérieur de l'archipel et que les habitants de Chiloé étaient les plus importants producteurs de ce secteur. La pomme de terre était destinée à la consommation humaine, à nourrir les cochons et les bovins en hiver. Les excédents, quand il y en avait, étaient vendus sur le marché ce qui constituait un revenu monétaire pour les paysans. Postérieurement, le « mildiou » qui a ruiné plusieurs fois les cultures de pommes de terre durant les années 50 et 60, a entraîné une grande crise économique et provoqué d'importantes migrations vers la Patagonie.

Le développement économique de Chiloé depuis les années 80 est essentiellement dû à l'installation d'entreprises de salmoniculture qui ont été déterminantes en ce qui concerne le changement des coutumes agricoles et plus généralement, socioculturelles de cette population. A ceci, s'ajoute le déplacement et l'augmentation des surfaces de cultures vers le Sud Chili; la Patagonie, le grand acheteur d'autrefois, produit aujourd'hui ses propres pommes de terre. Ces changements d'activités économiques ont eu pour effet d'éloigner de plus en plus de paysans de la culture des pommes de terre, culture qui est aujourd'hui majoritairement maintenue pour l'usage personnel. Vera (2003) et Salieres *et al.* (2005) rapportent que l'agriculture de Chiloé est actuellement soumise à une série d'influences qui tendent à la transformer après des siècles d'immobilité. A cause des conditions environnementales difficiles de l'archipel de Chiloé, l'agriculture est essentiellement vivrière, elle porte aujourd'hui encore la trace de ses origines, caractérisée par des espaces réduits pour l'agriculture et pour l'élevage, des activités dans le bois et la mer et orientée principalement vers l'autoconsommation. Dans les zones rurales, on observe actuellement de nouvelles liaisons entre activités agricoles traditionnelles et non-traditionnelles, avec l'incorporation d'activités non-agricoles, comme l'aquaculture, la pêche et les services associés.

La croissance de la salmoniculture à Chiloé, est une transformation productive dont les effets sur d'autres secteurs et les acteurs sociaux sont importants. Les principaux effets de l'activité aquacole sur les systèmes économiques sont :

- 1) passage de la force de travail des systèmes économiques (campagnards) vers l'activité des entreprises de salmoniculture et d'autres cultures ;
- 2) migrations interrégionales vers les villes intermédiaires, liées à de nouvelles opportunités de travail ;
- 3) travail salarié des femmes et de la jeunesse ;
- 4) changement dans la structure de la dépense familiale.

Salière et al. (2005) signalent qu'à Chiloé l'agriculture traditionnelle basée sur la logique de la production pour l'autoconsommation se situe géographiquement dans la partie centrale, les îles intérieures et les secteurs côtiers de l'archipel, alors qu'une agriculture plus spécialisée correspondant aux exploitations laitières se développe dans le quart nord-est de la grande île dans les communes d'Ancud, Quemchi et Chonchi. L'agriculture traditionnelle se trouve au cœur d'un processus profond de transformation, pouvant aboutir à la disparition ou à la spécialisation.

Différentes sources signalent la tendance à une réduction graduelle en ce qui concerne la taille des exploitations à Chiloé avec comme conséquence la subdivision des prairies et parallèlement, la surface destinée à la culture de la pomme de terre a été réduite. La tendance à la baisse observée est attestée par les derniers recensements agricoles au Chili, où la surface totale semée de pommes de terre à Chiloé a varié de 7.800 hectares en 1977 à seulement 3.306 hectares en 2007. Cette tendance à la réduction existe sur l'ensemble du pays, mais de façon moins marquée puisque sur la même période la surface cultivée en pommes de terre sur l'ensemble du pays sont passées de 86.000 ha à 55.000 ha (source <http://faostat.fao.org/>), pour un objectif de production nationale à peu près constant de 950.000 tonnes. Sur la même période la moyenne des surfaces de culture de pomme de terre par exploitation de Chiloé a été réduite de près de 50% en variant de 0,61 hectares à 0,35 hectares par exploitation. Salières et al. (2005) signalent que la disponibilité décroissante et le coût élevé de la main d'œuvre ainsi que la baisse du prix de la vente ont renforcé le processus de détachement de la production vers des marchés de produits agricoles, pour privilégier les productions d'autoconsommation.

Salieres et al. (2005), signalent que les produits d'autoconsommation ont un rôle central dans l'économie domestique, qui constitue une épargne non négligeable dans le budget alimentaire des familles. Ce sujet est essentiel pour que les paysans puissent conserver leur indépendance face aux employeurs extérieurs. Ainsi donc, il est important de stimuler une production efficiente à des prix minimums de pomme de terre, de légumes et d'animaux de basse-cour, avec une mise au point spéciale de ces produits. La persistance de l'activité agricole dans les petits domaines agricoles de Chiloé peut contribuer aussi à la subsistance d'une certaine identité paysanne (ou campagnarde) dans le secteur rural.

Le système productif traditionnel du paysan de Chiloé est en train de disparaître, les changements qui ont eu lieu durant les deux dernières décennies ont été très rapides. La hausse de la main d'œuvre et la compétitivité des aliments apportés du continent, ont été des facteurs très importants des bouleversements récents du système agricole traditionnel. Si

l'alternative laitière n'avait pas été ouverte, une grande crise des systèmes productifs de Chiloé aurait eu lieu sans alternative de revenus agricoles.

#### **1.4. Agriculture traditionnelle et maintien de la diversité génétique.**

Brush (1992), rapporte que la perte de la biodiversité dans les Andes du Pérou est liée à la modernisation de l'agriculture. Dans le système traditionnel par exemple à Ayacucho au Pérou, les semences de pomme de terre sont transmises par les parents à leurs enfants, qui plus tard produisent leur propre sélection et entretiennent des variétés. Il existe aussi des apports extérieurs aux variétés indigènes par le commerce, les dons et les rémunérations de service en nature sous forme de pommes de terre. Les femmes jouent un rôle essentiel dans l'identification et la sélection des variétés, en plus d'être fortement impliquées dans chaque étape de production telle que la sélection des semences, la production, la récolte, le stockage, la transformation et la cuisson. Un petit nombre de variétés « cosmopolites » sont cultivées un peu partout dans les Andes, dans les villages et les régions, mais de très nombreuses variétés ne sont cultivées que par quelques foyers ou quelques villages. Elles sont le fruit de sélections autochtones destinées à des usages culinaires (diversité des goûts et des textures) et à des débouchés diversifiés (autoconsommation, vente sur les marchés)

Des situations de disparition de variétés de pommes de terre natives ont été constatées dans certaines communautés du Pérou par Brush (1992), Ordinola *et al.*, (2009). Ces disparitions sont très souvent associées à l'économie du milieu rural qui montre une faible rentabilité, des revenus domestiques bas, une économie inadéquate d'échelle des divers processus productifs, la non coordination des marchés, la volatilité de leurs prix. Dans ce contexte, la production de la pomme de terre native est sévèrement touchée par des problèmes techniques et des risques climatiques qui aggravent la faible productivité et affectent la qualité de la récolte.

Dans ce contexte de changements rapides, le maintien de la culture des pommes de terre locales traditionnelles dans l'île de Chiloé est un réel défi. Il nécessite de trouver des débouchés rentables et donc d'identifier des marchés de niche qui seront suffisamment rémunérateurs, la pomme de terre traditionnelle n'étant absolument pas compétitive sur le marché de gros traditionnel qui n'offre aucun débouché pour la diversité que les récoltes colorées de Chiloé offrent.

## **1.5. Diversité et conservation de variétés natives de pomme de terre.**

Cubillos (1995), indique que la substitution d'anciennes variétés par des variétés modernes dans diverses cultures, est un phénomène intense et de caractère irréversible. Des exemples de ce phénomène peuvent se citer dans des cultures comme l'avoine, l'orge, la lentille, le melon, la pomme de terre, le blé, etc... Cet auteur signale que la richesse des ressources phyto-génétiques du Chili n'est pas bien conservée, ce qui rend urgent le fait de développer des plans de conservation *in-situ* et *ex-situ*. La richesse de diversité présente au Chili est assez exceptionnelle. Beaucoup d'espèces sauvages présentes à l'état naturel offrent un intérêt social ou économique, mais n'ont jamais été utilisés dans le développement ou l'amélioration de variétés cultivées. Il apparaît nécessaire de créer des répertoires nationaux de ressources génétiques, de réaliser des études ethnobotaniques, des estimations de variabilité génétique, d'estimer les tailles de populations à conserver et de proposer les conditions de la conservation et de l'utilisation des ressources phyto-génétiques.

En général les agriculteurs ne valorisent pas spécialement la diversité des ressources génétiques locales, étant disposés à les remplacer par d'autres dès que se produisent les conditions adéquates pour l'adoption d'une nouvelle variété ou culture. Un exemple de cela, c'est le cas de la pomme de terre à Chiloé et des cultures andines dans le nord chilien qui ont rapidement évolué en adoptant des techniques et des variétés issues de programmes de transfert de technologies réalisés par des agences de l'état et des organismes non gouvernementaux (Cubillos 1995).

Pour le Pérou, Ordinola *et al.* (2009), signalent que la plupart des pommes de terres natives sont inconnues, et de ce fait il n'est pas facile d'avoir une quantification juste de l'érosion de variabilité, mais force est de constater la diminution de leur production ainsi que le danger latent de disparition. D'autre part des scientifiques du Groupe Consultatif sur des Recherches Agricoles Internationales (CGIAR, 2007), signalent que des variétés sauvages de pommes de terre se trouvent en danger d'extinction. Ils ajoutent, que dans les prochaines 50 années, 12% des 108 espèces sauvages de pommes de terre étudiées pourraient s'éteindre, à cause des changements climatiques.

Gay (1974), en analysant l'histoire physique et politique du Chili, rapporte une série de variétés de pommes de terre natives présentes dans le pays, telles que Picunes, Pedanes Lingues, Niamen, Nanulues, Coluna, Caimoavidanes, Curavoane, Quethio poñi, Reina, Ilquilda, Voycañes, Amarilla, Latiga, Huapa, Choñas, Iiles, Rosas, Pitieu poñi, Cauchas,

Memichun, Soladado, Quehuembaca, Maoudi, Mechay, Pachaon, Vidoque. D'autre part, des observations ethno-linguistiques réalisées par Cárdenas (2003) citent plus de 300 noms pour les pommes de terre cultivées à Chiloé. Quelques uns sont : Alemana, Alerce, Americana colorada, Americana musca, Andina azul, Araucana, Ásperas, Australia, azul, Barcaza, Barones, Bastoneza, Blanca, Boliviana, Borrega, Bruja, Caballera, Cabeza amarrada, Cabeza de guagua, La cabra, Cabrito, Cachimba, Cacho, Caica, Camota, Camotilla, Cardenas, Canadense, Caribaja, Carichagua, Caro, Cari, Carica, Castilla temprana, Cauchao, Cracucha, Castilla Temprana, Centella, Chacahuana, Chagua, Chaitenera, Chamizada, Chanchán, Chaped, Chaucha, Chaulineca, Chanques, Chelinis, Cheuca, Chona, Cielo, Clavela, Coraila, Redonda, Rosada, Cordillera, Francesa negra, Frutilla, Guadacho, Guapa, Guicaña, Huevo, Lemuyana, Lengua de vaca, Mantequilla, Michuña, (meca de gato y cacho), Molejón, Murta, Natalina, Noventa días, Ñocha, Ojitos lindos, Oro, Riñón, Tonta, Soberana, Quila.

Enfin, Cárdenas (2003), signale que dans l'archipel de Chiloé, la pomme de terre était l'espèce domestiquée la plus présente quand les espagnols se sont établis sur le plateau de Quilquihue en 1567, pour fonder Castro.

Sur la base de ce qui précède, l'objectif de notre travail a été de décrire grâce à un travail d'enquête les facteurs actuels qui ont un impact sur la conservation et la diversité des pommes de terre natives à Chiloé. Nos objectifs spécifiques ont été : 1) décrire le contexte ou se développe l'activité agricole des paysans de Chiloé ; 2) décrire la gestion agronomique de la culture de la pomme de terre en général ; 3) décrire la démarche de la production utilisée pour la culture de variétés natives et évaluer son impact sur la conservation de ces variétés ; 4) décrire la démarche de la production utilisée dans la culture de variétés améliorées et 5) déterminer les principaux problèmes de maintien des pommes de terre natives et déterminer les risques de leur disparition.

## **II. MATÉRIEL ET MÉTHODES.**

### **2.1. Conception de l'échantillon et de l'enquête.**

Nous avons réalisé une enquête personnelle auprès de 108 paysans, représentés par des hommes et des femmes, âgés de 22 à 80 ans. Les exploitations considérées dans cette étude, appartiennent à l'agriculture domestique de la campagne de la Grande île de Chiloé, elles ont été choisies parmi la catégorie définie par le ministère de l'agriculture chilien

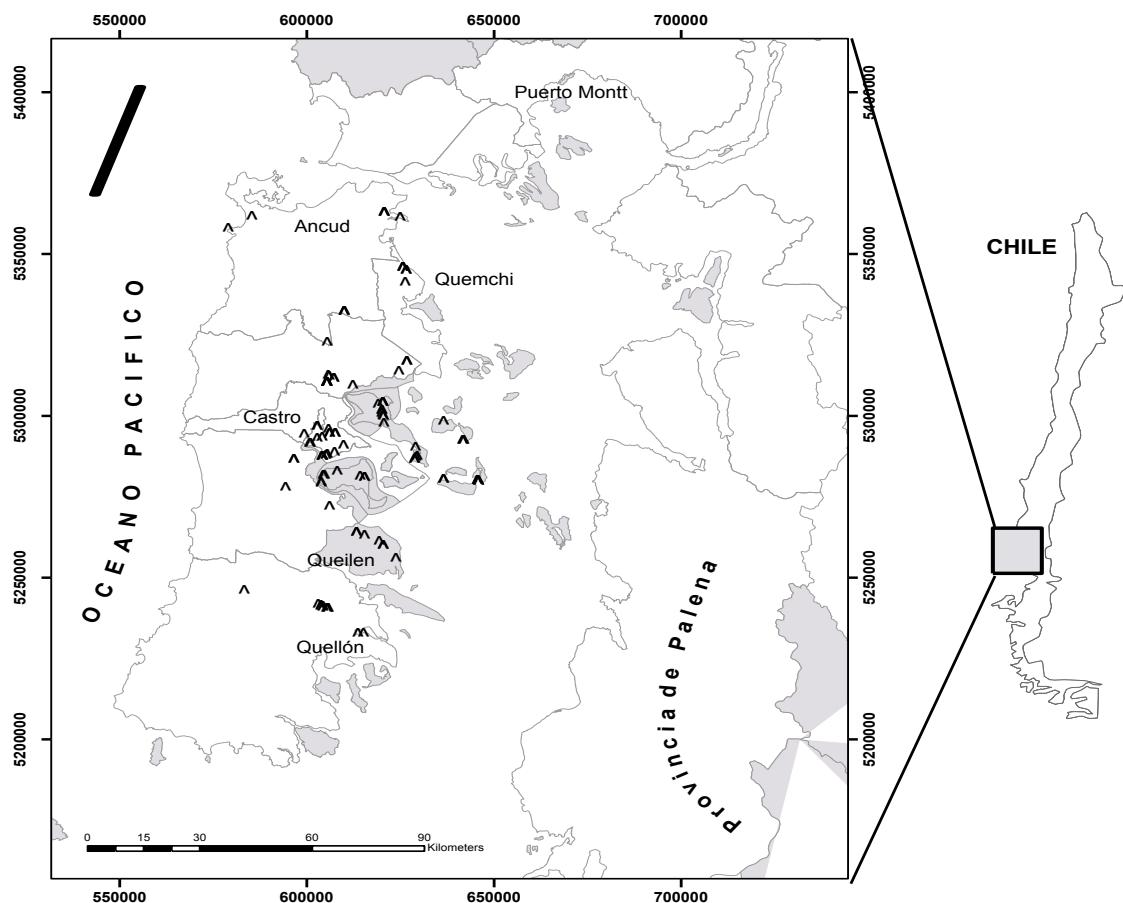
comme « Agricultura Familiare Campessina (AFC) ». Dans cette catégorie nous n'avons retenu que des exploitations répertoriées (source Institut National de la Statistique ; INE 2007) comme plantant des pommes de terre. Le nombre total d'exploitations qui cultivent de la pomme de terre à Chiloé est 9.422, pour une surface totale de 3.307 hectares. Il n'existe pas d'information officielle sur le nombre d'exploitations qui plantent des variétés natives au Chili. Par conséquent certaines des exploitations enquêtées (une minorité) ne cultivaient pas de pommes de terre natives. Les secteurs de l'île inclus dans cette étude ont été sélectionnés d'après l'information fournie par des institutions publiques de l'état et des organismes consultants privés présents dans le territoire insulaire, comme les bureaux de l'Institut National de Développement Agricole « Instituto Nacional de Desarrollo Agropecuario (INDAP) » et des Départements de Développement Communautaire des différentes mairies de la province de Chiloé. D'autres sources d'information ont été trouvées auprès des organismes consultants « Agraria » et « Agrochiloé ». Ces institutions ont fourni de l'information sur les communautés où se concentre la culture de la pomme de terre en général et en particulier celle de la pomme de terre native. En outre, elles ont fourni des noms de paysans qui se consacrent plus particulièrement à cette culture.

L'emplacement des exploitations a été geo-référencée par le système d'information géographique et d'analyse spatiale, en utilisant le programme Arc GIS version 9.2 (Figure 5). La distribution des personnes sondées par communes a été: Ancud 13; Chonchi 9; Castro 20; Quemchi 10 ; Queilen 7 ; Quinchao 22 ; Curaco de Velez 9 ; Quellon 3 ; Dalcahue 7 et Puqueldon 8. Le plus grand nombre de cas correspond aux communes de la province de Chiloé qui présentent la plus grande surface de pommes de terre, comme le sont les communes Castro, Ancud et Quinchao avec 459,9 ; 464,4 et 436,7 hectares respectivement (INE, 2007).

L'étude a un caractère descriptif et exploratoire, elle vise à donner des clés de compréhension globale des phénomènes dans le contexte actuel tel qu'il se présente et sous l'influence de la réalité dans laquelle il se déroule. Comme instrument, afin de recueillir l'information, nous avons élaboré un questionnaire (Annexe1) avec des questions ouvertes et fermées en rapport avec l'établissement de la culture de pommes de terre en général et en particulier des variétés natives. Nous avons interrogé les paysans sur les caractéristiques et le contexte socio-économique général dans lequel se déroule l'activité agricole et productive de Chiloé. Ensuite, les questions de l'enquête sont dirigées sur la gestion agronomique générale de la culture de pomme de terre et la gestion de la production de variétés de pommes de terre natives et améliorées. Finalement, nous avons recueilli les opinions des paysans sur l'état actuel et sur les perspectives de l'exploitation de pommes de

terre natives afin de déterminer les risques de disparition. Le questionnaire contient aussi des informations sur le statut des personnes sondées : genre, âge, zone de résidence, occupation, niveau d'études et possession de la terre, entre autres. Les sondages ont été appliqués dans les endroits de résidence des paysans, et ont eu lieu entre le mois de juillet 2007 et le mois de mars 2008. Les données numériques recueillies ont été analysés en fonction des éléments de la statistique descriptive, les moyennes ont été utilisées comme mesures des tendances centrales et les histogrammes de distribution (exprimés en pourcentages) pour visualiser la dispersion des données (Hair *et al.* 1999). Les données ont été recueillies à partir des réponses fournies par les agriculteurs. De ce fait beaucoup de données quantitative (surface des parcelles, poids récolté etc...) ne sont pas connues avec une totale précision ce qui conduit à de légères incohérences dans les tableaux de résultats. Par exemple, la déclaration de la surface moyenne cultivée en pomme de terre par chaque agriculteur n'était pas toujours égale à la somme des surfaces cultivées en pommes de terre améliorées et natives. Ceci étant, les ordres de grandeurs sont informatifs.

Afin de replacer nos résultats par rapport à des résultats déjà disponibles, nous les avons confrontés à ceux du V<sup>ème</sup>, VI<sup>ème</sup>, et VII<sup>ème</sup> Recensement National Agricole (1977; 1997; 2007). De la même façon, nous avons utilisé différentes publications officielles de l'Organisation des Nations Unies pour l'Alimentation et l'Agriculture (FAO).

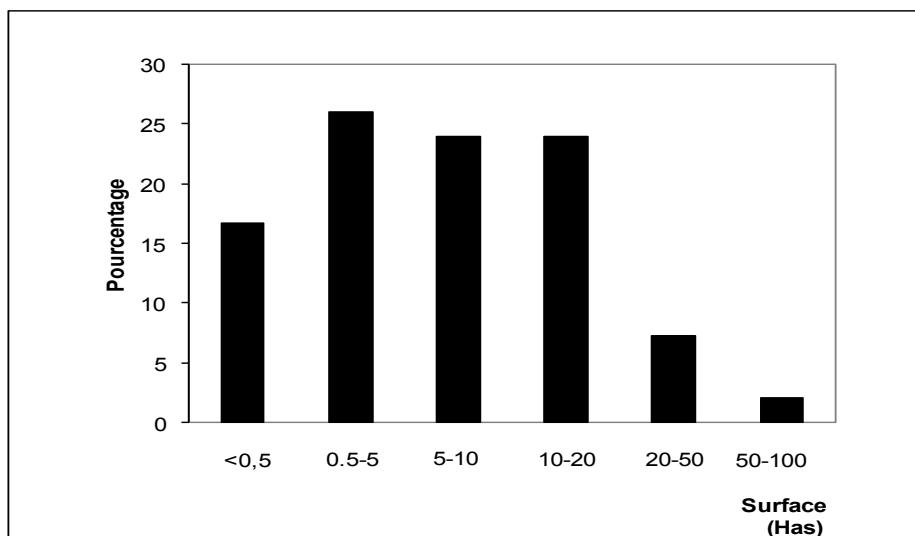


**Figure 5.** Emplacement territorial des exploitations enquêtées à Chiloé.

### III. RÉSULTATS DE L'ENQUETE AUPRES DE 108 EXPLOITANTS CULTIVANT LA POMME DE TERRE A CHILOE

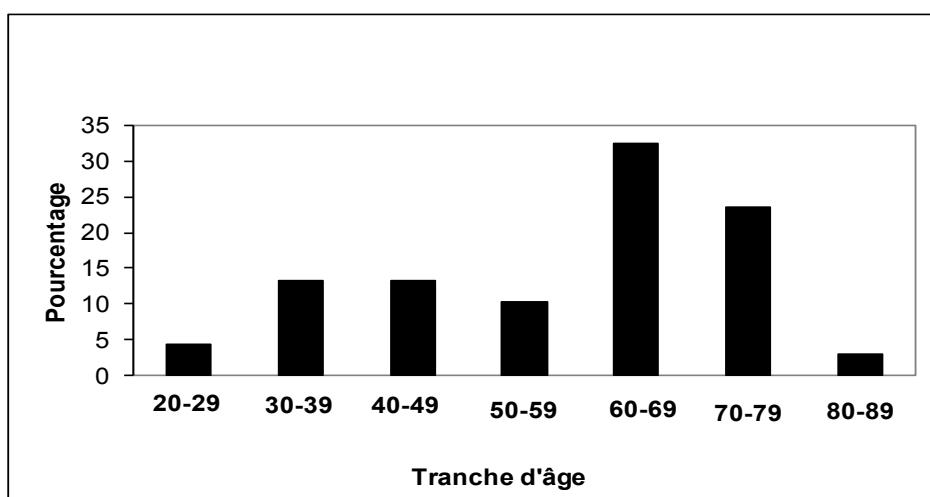
#### 3.1. L'agriculture domestique campagnarde (Agricultura Familiare Campessina : AFC) de Chiloé.

Les exploitations enquêtées, ont majoritairement des petites surfaces. La surface moyenne est de 9,92 ha, mais la distribution est dissymétriques (figure 6). 66% des exploitations ont une surface inférieure à 10 ha physiques (16,7 % des exploitations sont de grandeurs inférieures à 0,5 ha, 26% se trouve entre 0,5 et 5 ha et 24% entre 5 et 10 ha) et moins de 10% ont une surface supérieure à 20ha.



**Figure 6.** Surface des exploitations campagnardes à Chiloé.

La figure 7 représente les âges des paysans de l'AFC de Chiloé. En général, on apprécie le fait que ce sont les personnes d'âge avancé qui ont joué un rôle très important dans la conservation in situ des variétés natives de pommes de terre à Chiloé. Environ 60% des paysans qui cultivent des pommes de terre ont plus de 60 ans : 33% sont âgés de 60 à 69 ans, 23% se situent entre les âges de 70 et 79 ans et 3% ont plus de 80 ans. Seulement 20% des exploitants correspondent au groupe plus jeune avec des âges inférieurs à 40 ans.



**Figure 7.** Rang des âges des paysans de Chiloé.

En ce qui concerne l'emploi de la force de travail, 43 agriculteurs sur 102 qui ont répondu ont une activité secondaire, celle-ci est distribuée en une gamme d'occupations et/ou d'activités variées (pluri-activité) parmi lesquelles se distinguent toutes celles qui ont rapport avec le travail dans les entreprises de salmoniculture et d'aquaculture, les activités de pêche

artisanale, du tourisme et des activités diverses de main d'œuvre et de services dans les zones urbaines proches de l'exploitation (Tableau1). La grande majorité des paysans qui ont une activité secondaire n'en pratique qu'une seule en plus de leur activité agricole primaire.

**Tableau 1 : autres emplois dans les activités productives de Chiloé.**

Activités secondaires	La pêche artesanales	Salmoniculture (aquaculture)	La culture et la récolte des algues	Tissus	Forêts	Tourisme	Autres (services)	Total
Nombre de paysans	8	14	1	2	1	2	15	43
Proportion (%)	18,6	32,5	2,3	4,7	2,3	4,7	34,8	100

### **3.2 Gestion agronomique de la culture de la pomme de terre à Chiloé.**

#### 3.2.1. Surface affectée à la culture de la pomme de terre et gestion technologique de la culture.

Le tableau 2 représente la surface moyenne des exploitations étudiées. On observe que les paysans à Chiloé disposent d'exploitations de petite taille de seulement 9,92 hectares. En moyenne environ 5% de la surface totale a été consacrée à la culture de pomme de terre durant la saison 2007, ce qui équivaut à une surface inférieure 0,5 hectares par exploitation. La surface totale des champs destinés à la culture des variétés natives de pomme de terre est très petite car elle représente seulement le 2,2% de la surface totale avec une moyenne de 0,22 hectares par exploitation. Une tendance identique s'observe en ce qui concerne la surface destinée à la culture des variétés de pomme de terre améliorées, celle-ci en moyenne, ne dépassent pas les 0,43 hectares par exploitation.

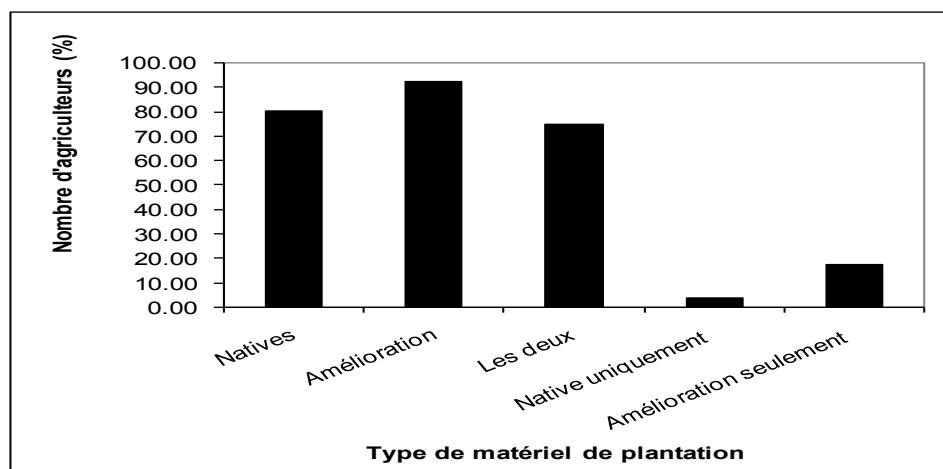
**Tableau 2. Surface des exploitations et surface destinée à la culture des pommes de terre.**

Surface moyenne par exploitation	Hectares
Grandeur d'exploitation	9,92
Surface plantée de variétés natives	0,22 <sup>(*)</sup>
Surface plantée de variétés améliorées	0,43 <sup>(*)</sup>

(\*) surfaces estimées à partir du nombre de sacs de pommes de terre plantées

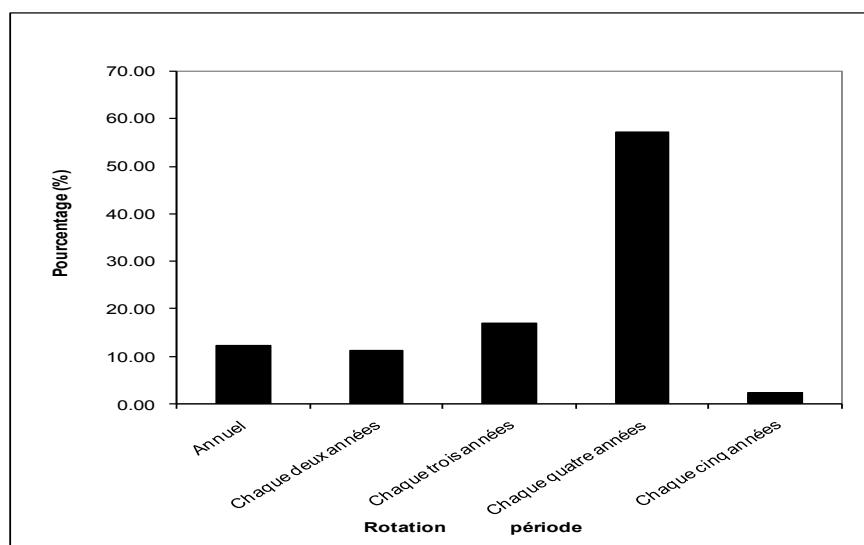
La figure 8, représente les différents types de variétés et leurs associations établies dans les exploitations des paysans de Chiloé. En général, on observe que les deux sortes de variétés améliorées et natives sont plantées cultivées au sein de la même exploitation. Les variétés

améliorées sont présentes dans le 92,5% des exploitations, alors que les variétés natives ne le sont que dans 80,5% des exploitations. Cependant, dans 75,0% des exploitations, les deux sortes de variétés sont présentes.



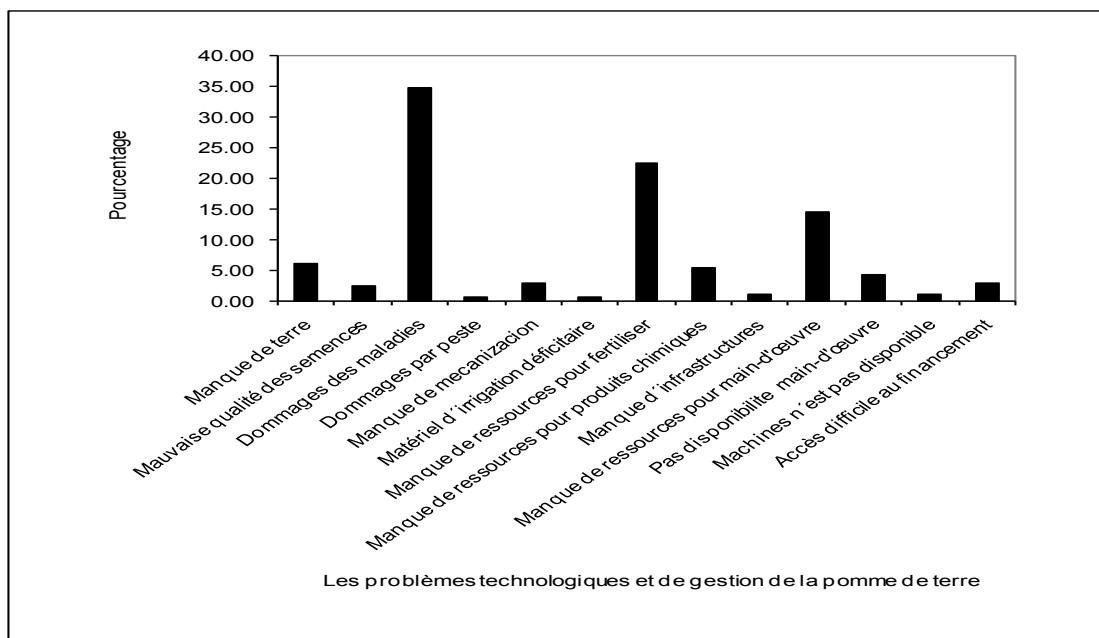
**Figure 8.** Sorte de variétés de pomme de terre plantée dans les exploitations paysannes de Chiloé.

La figure 9 représente la durée des rotations entre deux cultures de pomme de terre. La pratique paysanne la plus courante consiste à ne planter des pommes de terre sur la même parcelle qu'une fois tous les quatre ans. On constate toutefois une fréquence assez élevée de rotations plus courtes allant jusqu'à la plantation de pommes de terre chaque année, ou au moins deux années de suite, sur la même parcelle.



**Figure 9.** Fréquence de retour de la pomme de terre sur une même parcelle.

En rapport avec la gestion technologique de la culture de la pomme de terre, la figure 10 présente quel est actuellement le problème technologique le plus important de la culture de la pomme de terre, selon l'avis des paysans. Le questionnaire comportait 13 items, les paysans étaient invités à répondre de façon ouverte sans limiter leur nombre de réponses et sans les hiérarchiser. La plupart des paysans ont signalé deux ou trois problèmes, les fréquences présentées dans la figure 10 ont été calculées sur le nombre total de problèmes évoqués par les agriculteurs, la somme de ces fréquences est donc de 100%.



**Figure 10.** Problèmes associés à la gestion technologique de la culture de la pomme de terre.

Les trois principaux problèmes détectés sont les dégâts de maladies (35% des réponses), puis le manque de moyens pour fertiliser (22% des réponses) et le manque de ressources pour et de disponibilité en main-d'œuvre (14% + 4%). Enfin sont évoqués le manque de terre (6%) et de ressources pour l'achat de produits phytosanitaires (5%).

### 3.3. Gestion de production de variétés natives de pomme de terre.

#### 3.3.1. Source d'origine de la semence de matériel natif.

Le tableau 3 représente l'origine de la semence de variétés natives utilisées par les. En général, la tendance majoritaire est l'utilisation de semences auto-produites,, c'est-à-dire que la plantation se fait grâce aux tubercules produits l'année précédente par l'agriculteur lui-même. 71% des agriculteurs n'utilisent que de la semence auto-produite. A ceux-ci

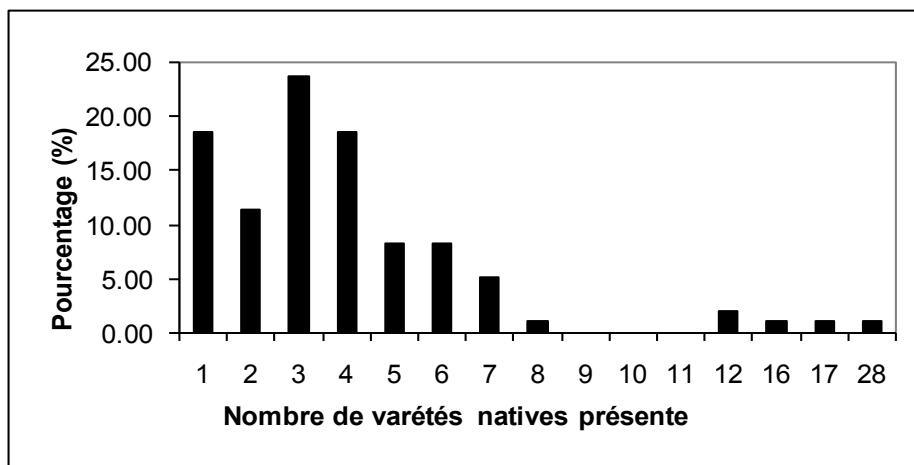
s'ajoutent ceux qui utilisent leurs semences auto-produites et d'autres sources d'approvisionnement, ce qui fait que seulement 18% des agriculteurs enquêtés déclarent ne pas utiliser de semences auto-produites. La deuxième source d'approvisionnement est l'acquisition auprès d'autres paysans de la communauté, presque 18% des agriculteurs y ont recours. Seulement 5,5% des agriculteurs achètent des semences provenant des programmes d'assistance technique qui existent dans le territoire. Parmi les paysans sondés, on a détecté au moins chez l'un d'eux, l'utilisation de semences botaniques pour établir la culture de pommes de terre natives.

**Tableau 3.** Origines de la semence de matériel natif.

Origine des semences	Ville	Communauté	Echange	Foires locales	L'assistance technique	Propres	Communauté et Propres	Communauté et échange	Communauté et échange et propres	Communauté et échange et assistance technique et propres	Nombre total d'agriculteurs ayant répondu
Nombre	1	6	1	1	4	64	8	3	1	1	90
Proportion (%)	1,1	6,7	1,1	1,1	4,4	71,1	8,9	3,3	1,1	1,1	100

### 3.3.2. Diversité de variétés natives en culture.

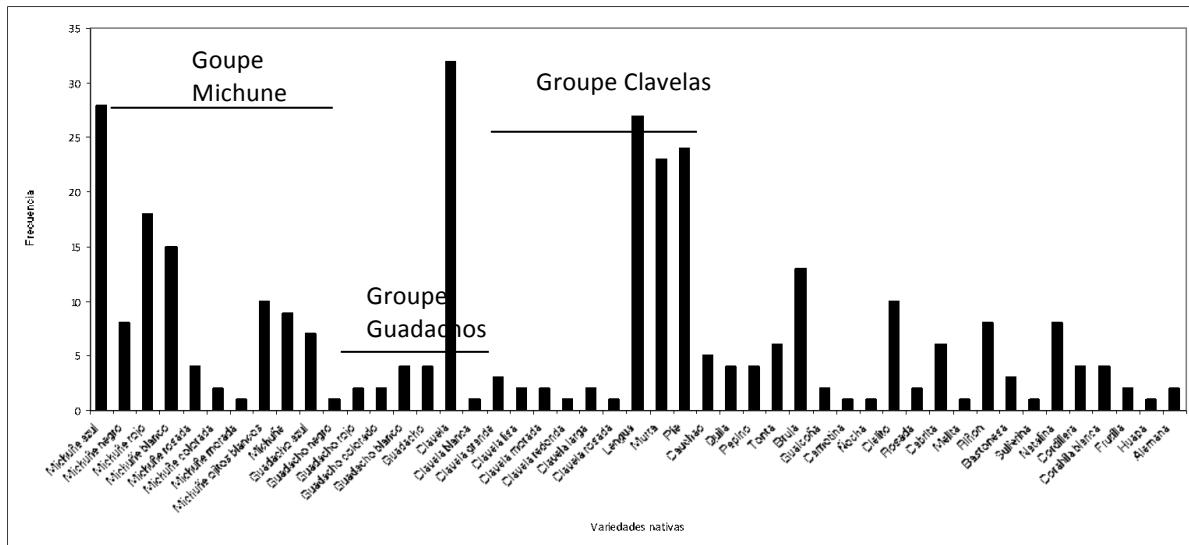
La figure 11 présente la diversité du nombre de variétés natives actuellement en culture dans les exploitations des paysans à Chiloé. La majorité des exploitants, environ les trois quarts, cultivent entre une et quatre variétés. Certains paysans en nombre limité sont intéressés par le maintien de la diversité de leur production de pomme de terre native. On remarque en effet en queue de distribution un groupe de 5 paysans qui cultivent au moins 12 variétés différentes et l'un d'entre eux en maintient 28. Cette situation est intéressante car elle identifie des agriculteurs qui sont intéressés par le maintien de diversité *in situ*, elle est aussi préoccupante car finalement très peu y participent spontanément.



**Figure 11.** Nombre moyen de variétés natives présentes dans les exploitations de Chiloé.

La figure 12 montre la grande disparité dans la fréquence d'utilisation des variétés natives. Les paysans regroupent certaines variétés sous des appellations génériques qui correspondent à certaines caractéristiques morphologiques des tubercules, sans pour autant que les variétés ainsi regroupées soient très similaires pour d'autres caractères tels que le feuillage, la précocité ou la sensibilité aux maladies. La désignation "Michuñe" correspond aux variétés avec des tubercules fusiformes et constraint au niveau des yeux. La désignation "Guadacho" correspond à la forme allongée (elongada). Les "Clavela" sont plutôt ovales et présentent deux couleurs de peau.

Sur les 47 variétés répertoriées chez les 108 paysans enquêtés, 5 sont cultivées par plus de 20 paysans, 10 par plus de 10 paysans et 18 par plus de 5 paysans. 29 variétés parmi les 47 sont donc actuellement cultivées par moins de 5 paysans et une majorité d'entre elles le sont par un nombre extrêmement restreint d'exploitants. On remarque en effet que 10 variétés ne sont trouvées que chez un seul paysan et 10 supplémentaires ne sont cultivées que par deux paysans. Ceci montre à quel point le maintien de diversité *in situ* est actuellement en situation précaire.



**Figure 12.** Diversité des variétés natives de pomme de terre dans les exploitations paysannes de Chiloé.

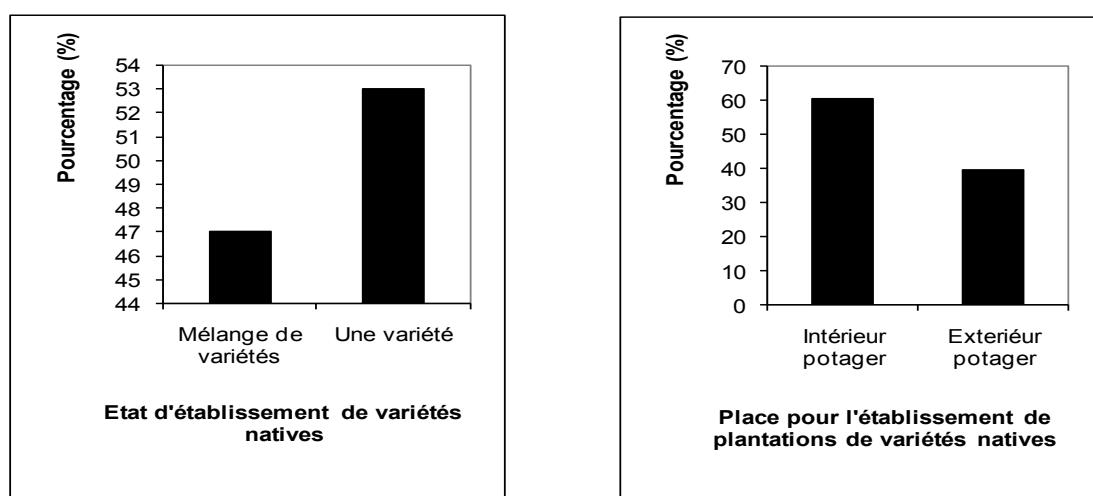
Du tableau 4 ci-dessous il ressort que pour quelques variétés pour lesquelles nous avons obtenu l'information auprès des agriculteurs, il existe une grande variabilité de comportement vis à vis de la résistance au mildiou (*Phytophtora infestans*), mais il n'apparaît pas que ce soit le facteur déterminant de la mise culture plus ou moins fréquente de la variété chez les différents agriculteurs. En effet, Murta qui est jugée très sensible est très utilisée et Pepino qui est jugée très résistante est peu utilisée. On déduit donc qu'à Chiloé coexiste du matériel résistant et sensible à la principale maladie de la culture.

**Tableau 4.** Opinion des paysans sur le comportement local en rapport au *mildiou* du matériel natif de pomme de terre.

Nom de la variété	Nombre de paysans qui la cultive	Opinion vis-à-vis de la résistance au mildiou
Michuñe azul	28	Très résistante
Michuñe negra	8	Résistante
Guadacho colorado	4	Moyennement sensible
Camota	1	De très à extrêmement sensible
Murta	23	Très sensible
Pie	24	Assez résistante
Pepino	4	Très résistante
Natalina	8	Très sensible
Quila	4	Résistante
<u>Frutilla</u>	2	Bien résistante

### 3.3.3. Modes de culture et surfaces des parcelles consacrées à la culture de pommes de terre natives.

Les pommes de terre peuvent être mises en culture soit dans des parcelles de l'exploitation soit à proximité de l'habitation dans le potager. Par ailleurs la culture peut être établie sous forme mono-clonale (une seule variété par parcelle) ou en mélange de plusieurs variétés au sein de la même parcelle. Les proportions des différents types de culture sont présentées dans la figure 13.



**Figure 13.** Modes de culture des pommes de terre natives dans les exploitations de Chiloé.

Dans 60% des exploitations paysannes les pommes de terre natives sont établies à l'intérieur du potager avec différents légumes. D'autre part, les résultats montrent que dans 47% des cas les variétés sont établies en mélanges. La vente peut se faire elle aussi en mélange (cf. fig. 1 et 2), mais les variétés sont en général suffisamment distinctes pour que l'agriculteur sache, si nécessaire, faire le tri après récolte.

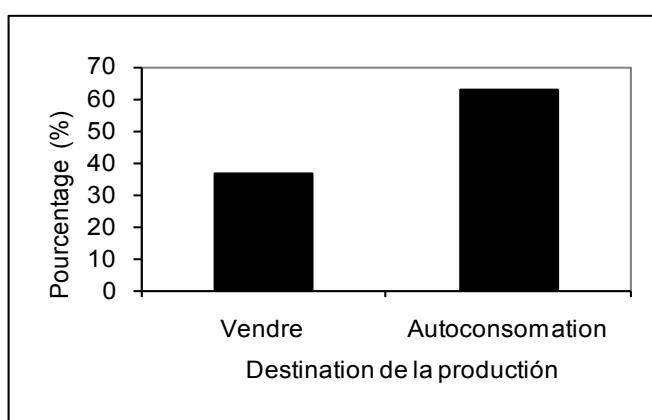
### 3.3.4. Rendement et débouchés de la production de pommes de terre natives.

Le tableau 5 présente la dose de semence, la moyenne de rendement et de l'indice de multiplication, calculé pour la culture de pommes de terre natives dans les exploitations appartenant à l'Agriculture Domestique Paysanne de Chiloé. Les valeurs reproduites sont des approximations que nous avons dû établir à partir des déclarations des paysans qui ont l'habitude de raisonner en nombre de sacs ; nombre de sacs semés et nombre de sacs récoltés. La quantité plantée et la quantité récoltée par hectare sont d'environ 1.300 kg/ha et 6.350 kg respectivement.

**Tableau 5.** Dose de semence, rendement et indice de multiplication pour la culture de variétés natives.

Paramètre	kg/ha
Dose de semence	1.300
Moyenne de rendement	6.350
Indice de multiplication	4,9

La figure 14 montre comment sont valorisées les pommes de terre natives. 37% des paysans valorisent tout ou partie de leurs pommes de terre au marché pendant que 63% les destinent à l'autoconsommation domestique.



**Figure 14.** Destination de la production de pommes de terre natives.

### **3.4. Gestion de la production de variétés améliorées de pommes de terre.**

#### **3.4.1. Sources d'origine de la semence de matériel amélioré.**

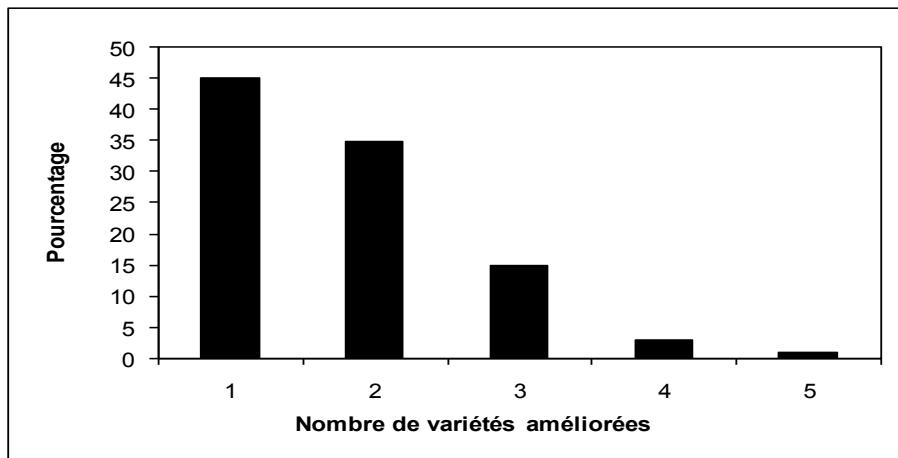
Du tableau 6 on déduit que les semences autoproduites sont la source la plus fréquente d'approvisionnement mais de façon moins nettement majoritaire que pour les variétés natives. 49% des agriculteurs n'utilisent que des semences auto-produites, auxquels il faut ajouter 17,5% d'agriculteurs qui utilisent des semences auto-produites et une autre source d'approvisionnement, au total 67% des agriculteurs utilisent des semences auto-produites. En ce qui concerne les semences certifiées fournies par les programmes d'assistance technique, 16% des agriculteurs déclarent n'utiliser que cette source d'approvisionnement et 15% déclarent s'approvisionner en semences certifiées mais pas uniquement. La proportion d'agriculteur utilisant des semences certifiées est donc de 31% pour les variétés améliorées. L'autre source fréquente (18,5%) d'approvisionnement est l'acquisition auprès d'autres agriculteurs de la communauté, 13% des agriculteurs déclarent que c'est leur unique source d'approvisionnement.

**Tableau 6.** Origine des semences de variétés améliorées.

Origine des semences	Ville	Communauté	Communauté et l'assistance technique	Communauté et propres	Echange	Echange et propres	Foires locales	L'assistance technique	L'assistance technique et propres	Propres	Nombre total d'agriculteurs ayant répondu
Nombre	1	11	2	3	0	1	1	14	11	42	86
Proportion (%)	1,2	12,8	2,3	3,5	0	1,2	1,2	16,2	12,8	48,8	100

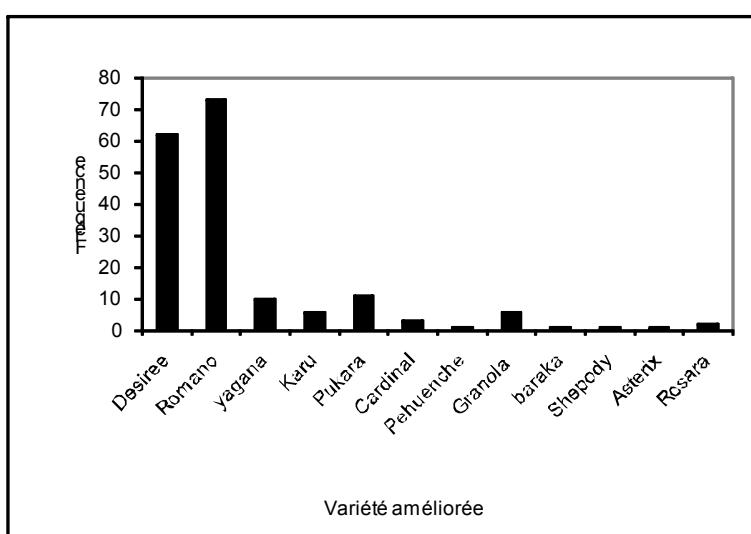
#### **3.4.2. Diversité des variétés améliorées en culture.**

La figure 15 présente le nombre moyen de variétés améliorées plantées par exploitation. On constate que ce nombre est très faible au sein de chaque exploitation, beaucoup plus faible que ce qui a été constaté pour les variétés natives. 45% des exploitations ne plantent qu'une variété et 35% n'en plantent que deux.



**Figure 15.** Nombre de variétés améliorées présentes dans les exploitations paysannes.

En rapport à la diversité du matériel amélioré actuellement en culture, la figure 16, montre la diversité présente dans l'AFC de Chiloé. Le nombre total de variétés améliorées en culture atteint la douzaine. « Romano » correspond à la variété plus fréquente plantée suivie de la variété « Désirée » qui a été introduite dans le pays en 1962.



**Figure 16.-** Diversité de variétés améliorées dans les exploitations paysannes de Chiloé.

### 3.4.3. Rendement et débouchés des variétés améliorées.

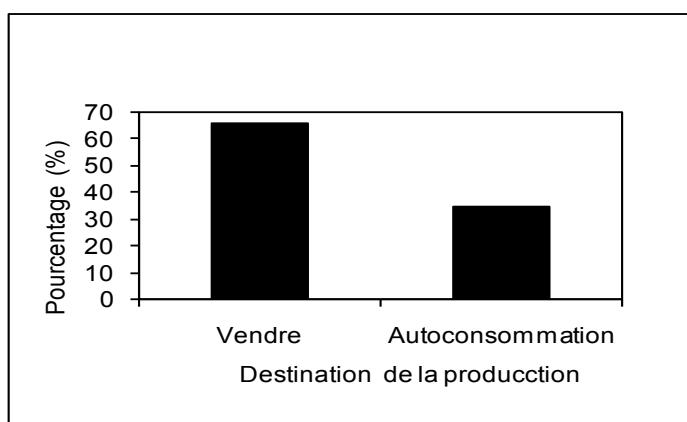
La dose de semence, le rendement et l'indice de multiplication moyen atteint pour la culture de variétés améliorées de pomme de terre dans les exploitations de Chiloé sont présentés dans le tableau 7. En général, il est possible d'observer que la dose de semence utilisée se trouve dans les rangs recommandés pour ces sortes de variétés dans le pays, atteignant en

moyenne 1.980 kg/ha. Par contre les rendements observés en ce qui concerne les variétés améliorées sont comparativement bas, inférieur au potentiel normalement décrit pour ces variétés, (Kalazich, 2008) ne dépassant pas les 13.500 kg/ha. En conséquence l'indice de multiplication obtenu est bas et atteint la valeur de 6,8.. Cette valeur est beaucoup plus petite que de l'appréciation qu'en ont les paysans, qui citent fréquemment un indice de 10.

**Tableau 7.** Dose de semence, rendement et indice de multiplication pour la culture de pommes de terre améliorées.

Paramètre	Kg/ha
Dose de semence	1.980
Moyenne de rendement	13.420
Indice de multiplication	6,8

La Figure 17 représente les débouchés actuels de la production de la culture de pommes de terre améliorées. Les variétés améliorées sont majoritairement destinés à la vente (60%), à l'opposé de ce qui avait été observé pour les variétés natives.



**Figure 17.** Débouchés de la production de variétés améliorées.

## **IV. DISCUSSION.**

### **4.1. Agriculture domestique paysanne (AFC) de Chiloé : Contexte et ruralité.**

#### 4.1.1. Grandeur des exploitations de l'agriculture paysanne (AFC) de Chiloé.

L'archipel de Chiloé est formé par un ensemble d'îles, la principale étant celle que l'on appelle la « Isla Grande » de Chiloé (la Grande Île de Chiloé) où se trouve la plus grande concentration de la population et les trois villes principales de l'archipel : Ancud, Castro et Quellón. Tout autour de la Grande île, se trouvent une série de petites îles essentiellement rurales dont l'accès aux centres urbains ne peut se faire que par la mer. Dû à leur isolement géographique, les habitants de l'archipel de Chiloé sont loin d'atteindre les niveaux d'éducation, santé, connaissance et technologie de leurs voisins du continent. L'inexistence de voies de communication viables entre les îles, la grande île et le continent, limite toute initiative de nouvelles entreprises productives et commerciales.

Dans le cas de l'AFC de Chiloé, prédominent les petites exploitations agricoles. Nos résultats coïncident avec les chiffres publiés par l'INE en 1997 qui indiquaient que l'agriculture de petits domaines et de subsistance qui se situent dans la couche de moins de 5,0 hectares physiques représentait 28,6% des exploitations physiques et 2,4% de la surface agricole total de Chiloé.

De la même façon, les valeurs obtenues sont en cohérence avec celles du VII Recensement National Agricole (INE, 2007) qui indiquait l'existence de 438 (3,1%), 2.956 (22,37%) et 2.889 (21,86%) exploitations avec une taille de moins de 1 hectare, entre 1 et 5 hectares et de 5 à 10 hectares respectivement. Dans le recensement de 2007, c'était donc près de 6.300 exploitations représentant approximativement 47,0% du total des exploitations existant à Chiloé, qui se concentraient dans la classe des exploitations de surface inférieure à 10 hectares. Dans notre enquête ce sont les deux tiers des exploitations qui ont une surface inférieure à 10ha. Cela s'explique par les critères que nous avons utilisés pour constituer l'échantillon : exploitation familiale paysanne cultivant la pomme de terre et en particulier la pomme de terre native. Ces critères ont de façon évidente enrichi notre échantillon en exploitations de petites tailles orientées vers l'agriculture vivrière et les débouchés locaux.

D'après Salieres et al. (2005) Chiloé souffre d'un phénomène de « mini – domaines » persistant car auparavant les enfants étaient nombreux et se divisaient entre eux le domaine

de leurs parents. Après une succession de divisions apparaissaient donc de très petits domaines de terre qu'on appelle des minis – domaines caractéristiques de l'île de Chiloé. La tradition de subdivision du patrimoine familial continue de nos jours, entraînant la fragmentation et rapetissement des parcelles. Vers 1977, la grandeur moyenne des exploitations était de 32,5 hectares (INE, 1977), en 1997, elle était estimée à 28,1 hectares (INE, 1997) pour finalement atteindre 27,0 hectares en 2007 (INE, 2007). Ce qui représente une réduction de 17% sur 30 ans. Cette augmentation du nombre d'exploitations de petite taille s'est accompagnée corrélativement de l'accroissement quantitatif de l'agriculture paysanne de subsistance. Un autre fait marquant que révèle notre enquête est la prédominance d'exploitants âgés, 60% ont plus de 60 ans et 25% ont plus de 70 ans.

#### 4.1.2. Activités productives du territoire.

Actuellement l'emploi rural de Chiloé est partagé en plus de cinq secteurs de la production et du commerce parmi lesquels on trouve la salmoniculture, la pêche artisanale, les activités de service en zone urbaines et le tourisme. Les paysans des minis domaines, qui représentent la plus grande partie de la population de Chiloé ont abandonné partiellement l'activité agricole et ont adopté un système d'activité assez complexe, caractérisée par la pluriactivité. Ils sont maintenant dans une situation de « nouvelle résidence rurale », rendue possible par l'installation des industries de saumon, la proximité de villes et le développement du tourisme (Salieres *et al.*, 2005). Les résultats de l'enquête indiquent que plus de 40% des agriculteurs ont une activité secondaire. Ces activités secondaires sont principalement réparties dans trois catégories, les services domestiques (35%) dans le monde urbain tels que le jardinage, le tranchement de bois, la charpenterie et la construction. Viennent ensuite le travail dans les entreprises de salmoniculture et aquaculture (33%) et la pêche artisanale (19%).

Le contexte insulaire est favorable aux activités liées à l'exploitation de la mer et c'est traditionnellement vers ce secteur que les paysans s'orientent pour développer une activité secondaire. Le recensement de population et d'habitat (Censo de Población y Vivienda, INE, 2002), signale que la pêche démersale et benthique, la récolte de fruits de mer, d'algues, de produits de la mer en général et l'aquaculture constitue l'activité principale à Chiloé (22,6% de l'activité totale). L'agriculture, l'élevage et la sylviculture n'occupent quant à eux que la cinquième position (9,8% de l'activité totale de Chiloé).

Salieres *et al.* (2005) signalent que 34% de la population active partage sa force de travail entre l'agriculture et d'autres activités et que les revenus des familles paysannes de Chiloé

sont fortement subordonnés à des sources de travail extérieures, étant de ce fait très vulnérables aux évènements externes. Elles ont d'ailleurs été particulièrement touchées au cours de 2008 par la crise économique mondiale qui a causé la perte de 12.000 emplois dans la province de Chiloé. Salieres et al. (2005) ajoutent que seulement 25% des personnes travaillant dans l'agriculture s'y consacrent à temps plein et que ce sont principalement les chefs de famille et que 2/3 des exploitations étudiées à Chiloé présentent une pluriactivité qui implique la participation du chef de famille, d'un fils ou des deux. Dans notre cas nous n'observons que 42% des exploitants qui pratiquent la pluriactivité. La différence est vraisemblablement due à la forte proportion d'exploitants âgés au sein de notre échantillon. Un facteur important de la présence de pluriactivité c'est une surface disponible faible par personne active, l'activité agricole ayant dans ce cas comme fonction principale la réduction des dépenses alimentaires de la famille (Salieres et al. 2005). Nous avons nous aussi observé cette tendance au cours des travaux sur le terrain.

#### 4.1.3. Disponibilité de main d'œuvre dans le territoire.

L'arrivée des entreprises de salmoniculture a eu comme effet général, le fort accroissement du coût de la main d'œuvre. La possibilité de gagner un revenu monétaire décent sans avoir à émigrer loin de l'exploitation, a été une révolution pour les agriculteurs et a eu pour conséquence de fortement diminuer la main d'œuvre disponible pour l'agriculture. La main d'œuvre permanente a diminué fortement lorsqu'un membre de la famille travaillait dans une entreprise de saumon. et la main d'œuvre saisonnière est devenue difficile à trouver ou l'on devait la payer très cher. Ceci a signifié une forte régression des habitudes d'échange de la force de travail. Aujourd'hui, seulement le 23% des exploitants déclarent pratiquer encore le système d'échange de jours de travail pour les lourdes corvées. Les autres doivent payer la main d'œuvre et pour eux, il s'agit d'une dépense qui n'existe pas auparavant. Ce phénomène a impacté la solidarité traditionnelle des paysans qui était une composante essentielle de la vie paysanne traditionnelle de Chiloé. Les travaux les plus lourds (semis et récolte de pomme de terre, battage du blé, etc...) se faisaient ensemble avec les voisins. Dans le cadre des actions solidaires se pratiquait la célèbre « minga » qui est encore un symbole de la culture de Chiloé. La « minga » consiste à aider un voisin à déplacer sa maison de bois d'un site vers un autre (déménagement) en la hissant sur un chariot et tiré ensuite par des. Cela nécessitait la collaboration de tous et devenait une grande fête du voisinage.

Une autre conséquence directe du déplacement de l'activité du secteur agricole vers des secteurs d'activités plus rémunérateurs est la forte migration des jeunes gens vers les

centres urbains en recherche de meilleures perspectives de vie. Ceci se traduit par la forte proportion d'exploitants d'âge avancé que nous avons constatée dans notre échantillon. Sur le sujet spécifique de la conservation *in situ* des variétés natives cette situation est préoccupante car ce sont principalement les agriculteurs âgés qui les maintiennent.

## **4.2. Gestion agronomique de la culture de la pomme de terre dans l'agriculture domestique paysanne (AFC) de Chiloé.**

### 4.2.1. Surfaces affectées à la culture de pommes de terre.

Les données obtenues indiquent que les agriculteurs consacrent une petite surface de leur exploitation (de l'ordre de 6%) à la plantation de pommes de terre, qu'elles soient natives ou améliorées. Il est possible d'observer une corrélation positive (tableau 8) entre la grandeur des exploitations et la surface destinée à la plantation de pommes de terre améliorées ( $r : 0,52$ ). Mais cette corrélation n'existe pas pour les pommes de terre natives, ce qui est cohérent avec le fait que les pommes de terre natives ont un débouché essentiellement domestique alors que les variétés améliorées sont principalement destinées à la vente (cf. figures 14 et 17). .

**Tableau 8.** Coefficient de corrélation entre la grandeur de l'exploitation et la Surface plantée avec des différentes variétés de pomme de terre.

Variables	Surface de L'exploitation	Surface de variétés natives
Surface de variétés natives	0,14 <sup>(NS)</sup>	
Surface de variétés améliorées	0,52 <sup>(***)</sup>	0,18 <sup>(NS)</sup>

<sup>(NS)</sup> non significatif, <sup>(\*)</sup> significatif à 5%, <sup>(\*\*\*)</sup> significatif à 1%

### 4.2.2. Moyens de production, intrants et mécanisation.

Parmi les exploitations étudiées, très peu utilisent des moyens de production modernes pour la culture de pomme de terre. En général, la semence est de faible qualité et il n'existe pas de processus massif de renouvellement de cette semence. Ceci est plus marqué pour les variétés natives que pour les variétés améliorées (cf. tableaux 3 et 6). Les variétés natives pour beaucoup d'entre elles ne bénéficient pas des programmes d'assistance technique dépendants des institutions publiques et elles ne sont maintenues que par les paysans. Les achats et échanges au sein des communautés paysannes sont donc primordiaux pour la

conservation de la plupart des variétés natives. Malgré tout, dans la commune de Curaco de Velez et Quinchao, nous avons trouvé des agriculteurs qui plantaient du matériel natif distribué par l'INIA-Remehue et qui correspondait à la variété Guadacho colorado.

La fertilisation de la culture est assez standard dans toutes les exploitations paysannes, elle consiste en l'application d'un sac de fertilisant (mélange d'engrais) pour chaque sac de semence plantée. On observe en général un usage de faibles doses de fertilisation chimique. La fertilisation est assurée par l'usage d'engrais organiques provenant des basses-cours et des étables présentes dans les exploitations, ainsi que par l'usage d'algues marines (*Sargassum*, *Laminaria*) qui sont récoltées justement à cet effet. Cette pratique devrait se maintenir à cause de la forte hausse des prix des fertilisants chimiques constatée ces dernières années au Chili, en particulier les fertilisants phosphatés.

La mécanisation de la culture est faible, les façons manuelles sont prédominantes pour les semis, les soins aux cultures et les récoltes. Ceci peut s'expliquer par la faible surface destinée à la culture de pomme de terre dans chaque exploitation et par le manque de capital de travail auquel font face les paysans. De plus, l'accès aux crédits pour l'achat de matériel agricole est difficile parce que les institutions financières ne voient pas l'AFC comme une activité rentable.

#### 4.2.3. Position de la pomme de terre dans la rotation

Les résultats indiquent que la plupart des paysans réalisent une certaine rotation entre leurs cultures. La tendance majoritaire (~60%) est de réaliser des rotations de quatre ans. Il y a une conscience de l'importance d'intercaler entre deux cultures de pomme de terre, des cultures d'autres espèces et familles. On remarque dans ces cas la présence d'espèces fourragères qui composent les prairies naturelles et artificielles, avec la présence de différentes espèces de graminées. Néanmoins, les résultats obtenus révèlent aussi la présence de monoculture de pomme de terre chez environ 12% des paysans et 10% supplémentaires pratiquent la culture de pomme de terre tous les deux ans sur la même parcelle. Traditionnellement, la pomme de terre est la première culture de la rotation et elle s'établit après une prairie dégradée. A Chiloé, les exploitations consacrées à l'agriculture traditionnelle suivaient une rotation équilibrée des cultures. La culture de la pomme de terre s'établissait après la « roce y quema » (la brûlée) des buissons ou des bois. La culture était fertilisée avec du fumier de mouton. Dans ce contexte, Salieres *et al.* (2005) signalent que la longueur des rotations dépendait de la surface disponible et du nombre d'habitants sur l'exploitation. Le blé était semé après la récolte des pommes de terre et profitait des résidus

comme fertilisation. Postérieurement, l'année suivante une prairie naturelle était établie. Actuellement ces rotations se maintiennent sur certaines exploitations, sauf que la présence du blé a été substituée par de l'avoine, entre autres espèces fourragères.

#### **4.3. Points spécifiques de la production de variétés natives de pomme de terre.**

##### 4.3.1. Diversité des variétés natives en culture.

On observe qu'actuellement le nombre moyen des variétés natives présentes dans chaque exploitation, est bas. Le nombre observé le plus fréquemment (24% des exploitations) est de trois variétés par exploitation. 30 % des exploitations plantent seulement une ou deux variétés et 18.5% en plantent quatre. Bien que les variétés natives soient présentes dans plus de 80% des exploitations, la diversité maintenue par chaque exploitant est assez faible. Ces résultats coïncident avec ceux reportés par Cárdenas et Villagran (2005), qui signalent que ce ne sont pas plus de trois variétés qui sont actuellement en culture dans les exploitations de Chiloé. Certaines variétés (Lengua, Murta, Pie) et certains groupes de variétés (Michuñes, Guadachos et Clavelas) sont bien représentés, car plus de 20 paysans les cultivent (figure 12). Par contre d'autres variétés ont des situations beaucoup plus problématiques et risquent la disparition. Nous avons répertorié dans notre échantillon de nombreuses variétés cultivées par très peu de paysans. De l'avis des paysans de Chiloé il existe un nombre important de variétés en disparition parmi lesquelles on peut signaler : Azul pullan, Americana, Gueicoña, Natalina, Villarroela, Mantequilla, Condor, Chilca, Pesada, Carrilada, Vilo, Cebara et Gineca. Parmi ces variétés seules Gueicoña et Natalina ont été répertoriées lors de notre enquête. La cause principale de l'abandon de certaines variétés natives se rapporte au fait du non-usage, à la sensibilité aux maladies et à l'absence de demande commerciale.

Vis-à-vis de la résistance à la maladie principale de la culture, le « mildiou », il existe au sein des variétés natives des variétés résistantes et sensibles. Les plus résistantes au mildiou seraient celles de plus de 120 jours de cycle végétatif et sont pour cela appelées « postreras ». Les paysans identifient comme variétés résistantes Michuñe Azul, Michuñe Negra, Quila et Frutilla entre autres. Comme variétés très sensibles, on identifie Murta, Camota et Natalina. Le faible usage de fongicides ne permet pas le contrôle de la maladie sur les variétés sensibles, afin de les maintenir en cultures, il est donc nécessaire d'utiliser des pratiques culturales adéquates. La pratique assez courante (47% des cas) qui consiste à cultiver les variétés en mélange ou en association avec d'autres espèces dans le potager (60% des paysans cultivent les variétés natives dans leur potager) est très certainement à

mettre en rapport avec des stratégies de contrôle des maladies. Nous avons pu aussi noter une autre pratique fréquente chez les paysans qui consiste en l'application de cendres sur le feuillage de la culture.

La perte de matériel natif de pomme de terre a aussi été reportée dans les communautés paysannes du centre primaire d'origine de l'espèce au Pérou. La mise en place du projet d'Innovation et de Compétitivité de la Pomme de Terre (INCOPA) impulsé par le Centre International de la Pomme de Terre dans la communauté d'Andahuilas de l'altiplano du Pérou, pendant une période de cinq ans, a permis d'augmenter la surface plantée avec des variétés natives et d'augmenter le nombre de variétés en culture à l'intérieur de la communauté. Ceci a permis de passer de 7 à 12 variétés natives en culture tout en sauvant quelques variétés qui auraient été perdues autrement.. Le projet cité a cherché à mettre en valeur l'image des pommes de terre natives, en soulignant les avantages comparatifs (biodiversité, originalité ...) donnant un avantage compétitif à ces produits bien différenciés sur un marché d'exigence plus haute (Ordinola, 2009).

Enfin signalons ici le rôle très important des femmes dans la conservation des variétés natives de pomme de terre de Chiloé. Clairement le matériel natif est passé de génération en génération à travers le passage de mères à filles, les pommes de terre natives sont considérées comme un précieux cadeau pour la nouvelle famille. Dans la présente étude, 58% des producteurs sondés correspondaient au genre féminin, ce qui est en rapport direct avec le fait que c'est la femme qui assume le rôle principal du travail au champ pendant que le chef de famille occupe une activité à l'extérieur assurant un revenu financier à la famille. Ce sont les femmes qui en général réalisent l'échange de semences, la plantation et la culture dans le potager, elles connaissent les principaux usages et attributs des différentes variétés, lesquels sont en rapport avec la préparation de différents mets typiques de Chiloé.

#### 4.3.2. Mode et lieu de culture de la pomme de terre native au sein de l'exploitation.

Comme nous l'avons déjà signalé dans 60% des exploitations paysannes les pommes de terre natives sont établies l'intérieur du potager avec des espèces de légumes comme des betteraves, des carottes, des petits pois, des haricots, des laitues, du piment, de l'ail et aussi des cultures de fleurs. Ce qui est à mettre en relation avec la destination principale des variétés natives de pommes de terre qui est la consommation par la famille. Pour les exploitations de surface inférieures à 0,5 hectares, les agriculteurs sondés tendent à surexploiter la surface disponible, tout en gérant une diversité de productions avec lesquels ils peuvent subsister aux nombreuses cultures citées ci-dessus sont associés des animaux,

moutons et volailles dont la production pour la consommation permet la subsistance. Ces caractéristiques, coïncident avec les observations déjà signalées par Thorner (1979) ; Gómez (1982), Schejtman (1980) ; Boeninger *et al.*, (1981), FAO (1992) et le Ministère d'Agriculture du Chili (1990 et 1992).

La pratique des cultures de variétés en mélange (47%) a pour but principal de fortifier la protection sanitaire de la culture, spécialement vis-à-vis du «mildiou». Il s'agit de la plantation simultanée et conjointe de trois à cinq variétés sur la même ligne et / ou parcelle de plantation ou le semis de variétés intercalées. Les paysans de Chiloé appellent ce genre de plantation «semis en Chahuen» ceci voulant dire le semis de plusieurs variétés ensembles dans un même temps et espace. La présence de variétés natives dans les potagers et l'établissement de variétés en mélanges avec différentes formes d'architecture de la plante, phénologies, et degrés de résistance aux maladies, seraient des éléments très importants pour la conservation *in situ* dans les exploitations de l'agriculture domestique campagnarde (AFC) de Chiloé. Maintenir cette forme de polyculture est nécessaire pour la conservation des variétés natives dans le temps.

#### 4.3.3. Niveau de production et destination de la production de pommes de terre natives.

Les doses de semence utilisées sont relativement faibles pour ce type de variété atteignant en moyenne les 1.300 Kg/ha. Ceci peut être expliqué par le petit calibre des tubercules sélectionnés par les paysans pour constituer la semence. Les niveaux de production (Tableau 5) montrent que les rendements d'une culture de variétés natives sont bas, de l'ordre de 6.350 Kg/ha en moyenne. Citons ici comme référence les rendements moyens nationaux en pomme de terre au Chili qui ont varié entre 15 et 22 tonnes à l'hectare sur la période allant de 2005 à 2009 (moyenne sur la période 19t/ha), 2007 ayant été la plus mauvaise année et 2006 la meilleure. Acuña *et al.*, (2007) et Secor (communication personnelle) signalent que le mildiou causé par le champignon *Phytophthora Infestans* a causé de très importantes pertes dans tout le pays durant la saison 2006/2007 (dans l'hémisphère sud le cycle de la pomme de terre est à cheval sur deux années) et en particulier à Chiloé où les pertes ont atteint presque 50% du rendement normal de la culture. En conséquence l'indice de multiplication des pommes de terre natives est faible et atteint seulement une valeur de 4,9. Cette valeur est comparativement plus petite que l'appréciation qu'en font les paysans qui pour la majorité d'entre eux signalent un indice de multiplication de 10.

#### **4.4. Points spécifiques de la production de variétés améliorées de pomme de terre.**

Historiquement, les introductions de variétés de pomme de terre ont commencé à Chiloé dès la fin du XIX<sup>ème</sup> siècle, l'année 1895, avec plusieurs sélections apportées d'Europe par les colons. D'autres variétés extérieures à l'île ont été importées, jusqu'à ce que le « mildiou » des années 50-60 les ait retirées des semis à cause de leur faiblesse face à ce champignon. A la fin des années 60 ces variétés ont été remplacées par la variété « Désirée » toujours très populaire aujourd'hui. A ce sujet, Cubillos (1995) indique que la substitution d'anciennes variétés par des cultures modernes est un phénomène intense et à caractère le plus souvent irréversible.

##### **4.4.1. Origine et diversité du matériel amélioré.**

31% des exploitants déclarent utiliser des semences certifiées provenant des programmes d'assistance technique présents dans le territoire, 16% déclarent même n'utiliser que cette source d'approvisionnement. La situation est donc bien différente de ce qui a été observé pour les variétés natives pour lesquelles seulement 5% des agriculteurs déclaraient utiliser ce type de semences. Ceci s'inscrit en continuité du processus d'introduction des variétés améliorées qui a été initié il y a maintenant plusieurs décennies.

Il existe actuellement, au moins deux organisations paysannes qui se consacrent à la gestion de semenciers communautaires basés sur l'introduction de matériel amélioré et certifié. Ces organisations assurent parallèlement une assistance professionnelle dans ce sujet. Cela confirme l'observation faite par Brush (1992), en ce sens que les variétés améliorées nécessitent un approvisionnement régulier, les agriculteurs qui les utilisent optant très souvent pour un renouvellement de ces semences tous les deux ou trois ans.

En rapport avec la gestion des cultures de variétés améliorées à Chiloé on peut observer que les paysans établissent très souvent une seule variété pour assurer la production de l'année, ce qui a été identifié dans 45% des exploitations. A ces agriculteurs s'ajoutent 35% des exploitants qui ne cultivent que 2 variétés. Parmi les variétés produites certaines sont destinées à la garde et à la consommation en frais, d'autres sont destinées à l'industrie comme « Shepody » et « Yagana ».

En rapport à la diversité du matériel amélioré, les résultats indiquent que le nombre total de variétés améliorées en culture atteint la douzaine, mais avec une très nette prédominance de deux variétés : Romano, la variété la plus fréquemment plantée, est présente dans 75%

des exploitations, et la variété « Désirée » qui est présente dans plus de 60% des exploitations. Les agriculteurs signalent que Romano a été introduite à cause de sa haute résistance au « mildiou », maladie qui a eu une haute incidence ces dernières années, en particulier au cours de la saison 2006/2007..

#### 4.4.2. Rendement et destination de la production de variétés améliorées de pomme de terre.

Les rendements observés pour le matériel amélioré sont comparativement bas pour le potentiel décrit pour elles (Kalazich, 2008) ne dépassant pas en moyenne les 13,5t/ha avec un indice de multiplication bas, lequel atteint seulement une valeur de 6,9. Le rendement observé est légèrement inférieur aux chiffres signalés comme moyenne pour la province de Chiloé, lequel atteint 15 t/ha (INE, 2007).

D'autre part, dans 66% des exploitations, le débouché de la production de variétés améliorées est la vente, qui se réalise principalement sur les marchés locaux des villes de Castro et Quellón.

## **V. CONCLUSIONS**

Les variétés natives s'inscrivaient dans une culture traditionnelle, des relations sociales et une agriculture qui sont en pleine évolution. Selon les résultats obtenus dans cette étude, nous pouvons affirmer que le maintien *in situ* de la diversité des pommes de terre native n'est pas bien conservé à Chiloé. Bien que les variétés natives soient présentes dans plus de 80% des exploitations, la diversité maintenue par chaque exploitant est assez faible. Seules trois variétés sont actuellement cultivées au sein de chaque exploitation à Chiloé. Certaines variétés (Lengua, Murta) et certains groupes de variétés (Michuñes, Guadachos et Clavelas) sont bien représentés, car plus de 20 paysans les cultivent.

Par ailleurs, 47 variétés sont au total rapportées chez les paysans visités. Certains paysans en nombre limité sont intéressés par le maintien de la diversité de leur production de pomme de terre native. On remarque en effet en queue de distribution un groupe de 5 paysans qui cultivent au moins 12 variétés différentes et l'un d'entre eux en maintient 28. A court terme le maintien de la diversité *in-situ* ne semble pas menacé car de nombreux paysans cultivent des variétés natives, coïncidant avec celle indiquée par Javis *et al.*, (2008), soulignent l'importance d'un grand nombre les petites exploitations agricoles en

adoptant des stratégies variétale distinctement divers que force majeure qui maintient la diversité génétique des cultures à la ferme.

D'une part les jeunes se tournent préférentiellement vers des activités en ville et dans les entreprises locales et d'autre part les agriculteurs qui possèdent des exploitations de taille suffisante pour assurer un revenu familial correct se tournent vers les productions dont les débouchés sont assurés au-delà de l'agriculture de subsistance et des marchés locaux. Il existe donc un processus clair de remplacement du matériel natif par du matériel amélioré, notamment en réponse aux conditions du marché qui imposent des types de tubercules bien définis, produits de façon régulière et à des prix compétitifs. Les exigences de volumes plus grands, d'homogénéité, de qualité standardisée ont donc marginalisé les productions traditionnelles de pommes de terre natives qui se retrouvent bien souvent cantonnées à la production vivrière sur de petites surfaces, souvent dans le potager. De plus ces variétés semblent davantage appréciées par les personnes âgées que par les jeunes générations.

A l'absence de débouchés rémunérateurs et capables d'absorbés des volumes suffisants s'ajoutent des facteurs limitants de la production de pomme de terre natives. Les principaux problèmes cités par les agriculteurs sont la sensibilité aux maladies, des rendements faibles, le manque de ressources pour obtenir des fertilisants et le manque de ressources pour engager de la main d'œuvre.

Pour maintenir un intérêt pour la production de pommes de terre natives il faudrait donc trouver un marché suffisamment rémunérateur qui, étant donnée la spécificité du produit, sera un marché de niche. Le produit « Pomme de terre native de Chiloé» pourrait être valorisé pour sa typicité, sa diversité, son histoire, la tradition qu'il représente. Si pour différentes variétés il était possible d'en assurer la disponibilité à un haut niveau de qualité et que des recettes spécifiques leur soient dédiées, tout cela pourrait leur donner un avantage compétitif sur un marché d'exigence plus haute. Le développement du tourisme et de centres urbains et l'attachement aux cultures traditionnelles d'une partie de la population chilienne avec un pouvoir d'achat suffisant constituent sans doute des opportunités pour créer ce type de marché. Ceci étant, comme l'expérience d'innovation et de compétitivité de la pomme de terre (INCOPA) menée au Pérou semble le montrer (Ordinola, 2009), il est peu vraisemblable que ces marchés de niche offrent des débouchés à l'ensemble des variétés natives et il sera sans doute difficile de maintenir beaucoup plus d'une dizaine de variétés. Donc, au-delà des quelques paysans amateurs de diversité qui maintiennent spontanément un grand nombre de variétés il faudrait envisager la création d'un réseau plus large de maintien *in situ* de ces variétés et par sécurité y associer une conservation *ex-situ*. Les

moyens pour pérenniser ce réseau sont à organiser. Des institutions travaillent déjà dans ce sens à Chiloé. La conservation *ex situ* des variétés dans leur forme actuelle devrait par sécurité s'envisager au champ et *in vitro*. Enfin, au-delà de la conservation des variétés en l'état, la conservation de la diversité génétique spécifique de ce matériel pourrait passer par la conservation de semences botaniques.

Enfin, nous pouvons demander quels sont les risques et les opportunités pour maintenir la diversité *in-situ*. Pour les risques sont la pyramide des âges pas très favorable, l' aspiration des jeunes générations à d'autres modes de vie, la manque de débouchés rémunérateurs, la compétition avec d'autres activités (services, pisciculture, tourisme) et l' augmentation de la demande de pommes de terre améliorées. Pour les opportunités sont q' il n'y aura pas à moyen terme un abandon total de l'agriculture paysanne à Chiloé, certains paysans sont intéressés par la diversité des pommes de terre natives, le maintien dans le potager est une opportunité car beaucoup de gens continuent à entretenir un potager même s'il leur profession n'est pas « agriculteur », il existe déjà des associations pour le maintien des variétés natives.

## VI REFERENCES BIBLIOGRAPHIQUE

- Boeninger, E.; M. Franco; H. Ortega; G. Irarrazabal; C. Valdes; O. Valenzuela; S. Perez et J.Morales. 1981. La economía campesina. Ed. Aconcagua, Colección Lautaro, Santiago. 181 p.
- Brush S. B., 1992. Ethnoecology, biodiversity and modernization in Andean potato agriculture. *Journal of Ethnobiology*, 12:161-185.
- Cárdenas, R. 2003. La papa, patrimonio de la humanidad. *Revista de la papa*. Año 5, N° 15:14-15.
- Cárdenas, R. et C. Villagran. 2005. Chiloé botánica de la cotidianidad. Gráfica Lascar. Archivos Bibliográficos y Documental de Chiloé. Consejo Nacional del Libro y la Cultura. Chile.365p.
- Cox, M. 1983. Agricultura chilena 1974-1982. DECAM, Agraria. Santiago, Chile. 193 p.
- Cubillos, A. 1995. Chile: Informe nacional para la conferencia técnica internacional de la FAO sobre los recursos filogenéticos. Santiago. Chile. 107 pp.
- CGIAR. 2007. El cambio climático pone en peligro variedades silvestres de cultivos básicos; se encuentran amenazados recursos genéticos esenciales para resistir a

las sequías y las plagas. Grupo Consultivo sobre Investigaciones Agrícolas Internacionales.

D. Jarvis; A. Brown; P. Cuong; L. Collado-Panduroe; L. Latournerie-Moreno, S. Gyawali; T. Tanto; M. Sawadogo; I. Mar; M. Sadiki; N. Thi-Ngoc Hue; L. Arias-Reyes; Didier Balma; Jwala Bajracharya; F. Castillo; Deepak Rijal; L. Belqadik; R. Rana; S. Saidi; J. Ouedraogo; R. Zangre; K. Rhrib; J. L. Chavez; D. Schoen; B. Sthapit; P. De Santis, Carlo Faddaa and T. Hodgkin. 2008. A global perspective of the richness and evenness of traditional crop-variety diversity maintained by farming communities. PNAS, April 8, 2008. Vol. 105(14): 5326-5331.

Gay, C. 1974. Historia física y política de Chile. Paris. MD CCCLXV. Francia. 443p.

Gómez, S. 1982. Instituciones y procesos agrarios en Chile. FLACSO. Santiago de Chile.

Hair, J., R. Anderson., R. Tatham y W. Black. 1999. Análisis Multivariante. Prentice Hall Iberia. S.R.L. Quinta edición.799p.

Hijmans, R. J. y Spooner, D. M. 2001. Geographic distribution of wild potato species. American Journal of Botany. Vol. 88, no. 11, p. 2101-2112.

INSTITUT NATIONAL DE LA STATISTIQUE (INE). 1977. V Recensement National Agricole.

INSTITUT NATIONAL DE LA STATISTIQUE (INE). 1997. VI Recensement National Agricole.

INSTITUT NATIONAL DE LA STATISTIQUE (INE). 2002. Recensement de Population et du logement.

INSTITUT NATIONAL DE LA STATISTIQUE (INE) 2007. VII Recensement National Agricole.

Kalazich, J. 2008. Variedades aptas para el mercado. Tierra Adentro. Instituto de Investigaciones Agropecuarias. Ministerio de Agricultura. Chile. N° 80. Julio-Agosto 2008. Pp:6-7.

MINISTERIO DE AGRICULTURA. 1990. Ley-18919. Ley Orgánica del Instituto de Desarrollo Agropecuario.

MINISTERIO DE AGRICULTURA. 1992. Políticas y bases programáticas del desarrollo de la pequeña agricultura campesina, la reducción de la pobreza rural y el fortalecimiento de las organizaciones de productores. Proyecto TCP/FAO/CHI/ 0051 (A). 23 p.

MINISTERIO DE AGRICULTURA. 2001. Una política de estado para la agricultura chilena. Período 2000-2010. Gobierno de chile. Ministerio de Agricultura. Santiago. Chile. 140 p.

Montaldo, A. 1984a. Cultivo y mejoramiento de la papa. Instituto Interamericano de Cooperación para la Agricultura. San José. Costa Rica. pp.359-414.

- Montaldo, A. 1984b. Papas de Chile, valor agronómico, utilización y proyecciones futuras. En: Actas de la Cuarta Reunión Nacional de la papa: 49-80. Universidad Austral de Chile, Valdivia, Chile.
- Oordinola, M., Devaux, A., Manrique, K., Fonseca, C., A. Thomann. 2009. Generando Innovaciones para el Desarrollo Competitivo de la Papa en el Perú. Centro Internacional de la Papa. Lima. Perú. 64 pp.
- Ordinola, M. 2009. Biodiversidad e Innovaciones: El caso de las papas nativas en el Perú. EN: VII Simposio de recursos genéticos para América Latina y el caribe. 28-29 y 30 de Octubre de 2009. Pucón. Chile.
- ORGANIZACIÓN DE LAS NACIONES UNIDAS PARA LA ALIMENTACIÓN Y LA AGRICULTURA, (FAO). 1992. Informe de la reunión FAO/CLADES sobre currículos en agroecología y desarrollo rural sostenible. Oficina Regional para América Latina y El Caribe. Consorcio latinoamericano sobre Agroecología y Desarrollo. División Agrícola Conjunta CEPAL/FAO. Santiago, Chile. 91 p.
- ORGANIZACIÓN DE LAS NACIONES UNIDAS PARA LA ALIMENTACION Y LA AGRICULTURA, (FAO). (1996) Conservación y utilización sostenible de los recursos fitogenéticos para la alimentación y la agricultura: Plan de acción mundial e informe sobre el estado de los recursos fitogenéticos en el mundo. Organización de las Naciones Unidas para la Agricultura y la Alimentación, Italia. 10 pp.
- ORGANIZACIÓN DE LAS NACIONES UNIDAS PARA LA ALIMENTACIÓN Y LA AGRICULTURA, (FAO). 2007. La FAO en Chile: 60 años de colaboración 1945-2005. Segunda Edición. Representación de la FAO en Chile. 268 p.
- Salieres, M; M. Le Grix ; W. Vera; R. BillazBILLAZ. 2005. La agricultura familiar chilota en perspectva. Revista LIDER. Vol 13 N° 10:70-104.
- Schejtman, A. 1980. Economía campesina: lógica interna, articulación y persistencia. Revista de la CEPAL: N° 11.
- Spooner, D.; Mclean, K.; Ramsay, G.; Waugh, R. y G. Bryan. 2005. A single domestication for potato based on multilocus amplified fragment length polymorphism genotyping. Proceeding of the National Academy of Sciences, vol. 102, no. 41, p. 14694–14699.
- Thorner D. 1979. La economía campesina. Conceptos para la historia económica. 12-27 pp. Economía campesina. Presentación y Selección de textos. In: plaza, O. DESCO. Lima (Perú). 62 p.
- Vera, W. 2003. La agricultura chilota: de una lenta evolución a una profunda transformación. Puerto Montt: Agraria Los Lagos. 49 p.

## **CHAPITRE III. Evaluation of the morphological diversity of a potato collection (*Solanum tuberosum* L.) native of Chile.**

### **I. INTRODUCTION.**

The Solanaceae contain many domesticated species including several economically important crops. The genus *Solanum* L., which contains an estimated 1500 species includes three major food crops: potato (*S. tuberosum* L.), tomato (*S. lycopersicum* L.) and eggplant (*S. melongena* L.), as well as other minor food, medicinal and ornamental species (Bohs. 2007; Ames and Spooner. 2008).

Brush (1992), indicated four criteria are important in potato classification: ecology (cultivated/wild/weedy; production zone), use (edible; for boiling; for freeze drying), plant and tuber phenotype and degree of polypoidy.

So called "native potatoes" are mainly non commercial varieties of potato (*Solanum tuberosum* L.) that are locally cultivated and express the ability to produce tubers and grow under harsh conditions with limited environmental and agronomic management. For centuries these "native potatoes" have been selected by local farmers in South America, particularly Peru, Bolivia and Chile, to survive in harsh environmental conditions. These farmers are able to select and maintain a high diversity of germplasm with excellent organoleptic qualities, growing native potatoes with different ploidy, disease resistance and biotic stress, in the same cultivation land Brush (1992).

Varieties are primarily distinguished according to tuber characteristic, such as tuber shape (oval, spherical, flat, long), configuration of the tuber's "eyes" (depth, number, location, color), skin color and pattern (white to deep purple, solid color, multicolored), and flesh color and color pattern (solid, ringed, white to deep purple). In rare cases, non tuber characteristic such as stem or flower color distinguish varieties. Tuber characteristics are highly subject to somatic variation (Brush, 1992).

For the Andean potato, evidence of great intraspecific diversity has been well documented. However, very little is known about the actual distribution of diversity either within or between regions or how diversity is affected by changes in agriculture. The measurement of genetic diversity and its distribution in Andean potato agriculture is entangled by the complexity within the group of cultivated *Solanums* and by the great number of phenotypes and

genotypes at the variety level. Somatic variation, introgression between cultivated and wild species and hybridization within cultivated species also pose problems for measuring diversity. Geneticists working with the crops have preferred to work at the ploidy or species level rather than at the variety level. However, advances in biochemical characterization of potatoes may help overcome some of the biological obstacles for assessment of diversity (Hawkes. J. G. 1990, Hawkes and Hjerting, 1989 cited by Brush. 1992; Quiros. et al.. 1990).

Quiros et al.. (1990). found that there is a high degree of correspondence between farmer segregation and identification of tubers and biochemical profiles of tubers that reflects genotype differences. The isozyme analysis is particularly relevant here, since one would not expect any degree of correspondence between a folk taxonomy largely based on one criterion (tuber characteristics) and biochemical identity based on characters that are invisible to Andean farmers. Households are the primary management unit of selection of potato varieties and the primary unit for maintaining diversity. Assessment of the amount of diversity kept by different households is therefore essential to an overall understanding of diversity in the agricultural system and this assessment can rely on farmer identification. Thus, research on diversity can draw directly on folk classification, as long as the unit of analysis is the household.

Chile is considered to be a sub-center of origin for the cultivated potato (Spooner et al.. 2005). There is a remarkable concentration of cultivated and wild forms of potato in the south of Chile with the greatest number of native varieties being located on the island of Chiloe, and they are still preserved in the fields of small farmers. The peculiar characteristics of Chiloe, its natural conditions and its isolation, have allowed the proliferation of a great number of native varieties, of varying qualities and suitable for cultivation at different times in the farming calendar, as well as a number of different forms of preparation and consumption. In this context, the native potatoes of Chiloe are characterized by a wide variety of shapes, sizes, colors and phenological characteristics. This rich genetic patrimony needs to be described and individualized in order to be conserved and used. Unfortunately, more of the native potatoes of Chiloe are being lost every day, because of their replacement by introduced varieties and their phytopathological deterioration, among other causes. As a result, their conservation must be ensured by means of germplasm banks collections. In this context, new advances in molecular biology with the use of more sensitive molecular markers able to detect changes in the genotype of the individuals, would greatly contribute to the knowledge and to the management of our germplasm collection. The conservations and use of this native genetic material will ensure that it does not disappear, but enjoys a

projection for the future with new uses. In addition, potato improvement programs require a basic knowledge of the morphology and genetic nature of the main parts of the plant.

In this paper, we analyze the morphological diversity of a Chilean native potato collection (*Solanum tuberosum* ssp. *tuberosum* L.) of 30 accessions in order to characterize the available phenotypic variability within the collection and to identify varieties with interesting features for direct use or to be incorporated into breeding programs for biotic and abiotic stress. Studies on molecular diversity will be presented in companion papers.

## **II. MATERIAL AND METHODS.**

### **2.1.- Plant material.**

The analysis included 30 accessions of native potato collected on the island of Chiloe. Accessions were chosen to represent the extensive genetic diversity that can be found in *Solanum tuberosum* L. (Table 1). This material was cultivated in the field during the agricultural seasons 2006/07 to 2009/10. The accessions were planted to 70/75 cm between and 30 cm over the crop line. An experimental design of complete randomized blocks was used in the establishment of the varieties in the field, in which the treatments corresponded to the different landraces being evaluated.

### **2.2.- Morphological characters analysis.**

Fifty four characters were analyzed. These were described by Huaman, (1977) using potato varieties, and applied to “Chileanlandraces”; varieties were numerically codified using qualitative multi-status criteria (from 0 to 9. depending on the variables of each character). These were used to design a numbered-data matrix. Some characters of nominal variables included in this analysis were (Table 2): predominant tuber skin color, general tuber shape, leaf characters, sprout and plant characters. Six individuals were morphologically evaluated in each variety. For sprout and leaf characters (Figure 1) the guidelines of the Union Pour la Protection des Abtentions Vegetables for Potato (2004) were also used. The morphological data were subjected to multiple correspondence factorial analyses (AFCM). This analysis is an extension of simple correspondence analysis for two sets of characteristics. It allows the study of interdependence relationship between categorical or qualitative variables, that is, not metric. This model goes beyond the analysis of existing relationships between variables, because it allows learning how this relationship is structured. It was used in correspondence analysis with XLSTAT program version 1.02 (2009). The classification was of the ascendant

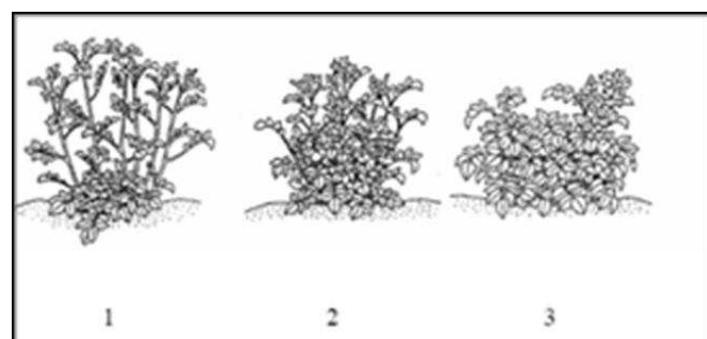
hierarchical type (CAH), by means of Euclidean distances and the aggregation methodology of Ward (Dervin, 1990; 1996).

**Table 1.** Accessions of native of potatoes (*Solanum tuberosum* L.) of Chili.

Dendogram number	Accession	Local name	Origin
1	UCT-11Mgb	Meca gato blanca	Isla grande de Chiloe
2	UCT-14MgRe	Redonda	Isla de grande Chiloe
3	UCT-17Br	Bruja	Isla de Quinchao.
4	UCT- 6Gc	Guadacho colorado	Chonchi. Isla grande de Chiloe
5	UCT-24Tn	Tonta	Castro. Isla grande de Chiloe.
6	UCT-22Cm	Clavela morada	Castro. Isla grande de Chiloe
7	UCT-25Gñ	Guicoña	Quellón. Isla grande de Chiloe
8	UCT- 7Ca	Camota	Isla grande de Chiloe
9	UCT-18Mn	Michuñe negro	Isla grande de Chiloe
10	UCT-26Ach	Azul chañihue	Isla grande de Chiloe
11	UCT-27Mu	Murta	Quellón. Isla grande de Chiloe
12	UCT-28MiR	Michuñe rojo	Isla grande de Chiloe
13	UCT-29Mol	Molejona	Isla grande de Chiloe
14	UCT- 3Cl	Clavela	Los Muermos. Continente
15	UCT- 1Ma	Michuñe azul	Isla grande de Chiloe
16	UCT-16At	Azul table	Isla grande de Chiloe
17	UCT-30Ño	Ñocha	Isla grande de Chiloe
18	UCT-19Aq	Azul de quento	Castro. Isla grande de Chiloe.
19	UCT- 2Lv	Lengua	Castro. Isla grande de Chiloe
20	UTC-31Ob	Ojitos blanco	Ancud. isla grande de Chiloe.
21	UCT-32Ci	Cielito	Castro. Isla grande de Chiloe
22	UCT-20Ro	Rosada	Isla grande de Chiloe
23	UCT-33Cab	Cabrita	Isla grande de Chiloe
24	UCT-15MgRo	Meca gato rojo	Isla grandede Chiloe
25	UCT-21Ac	Azul cristalina	Isla grande de Chiloe
26	UCT-34Cor	Cordillera	Castro.Isla grande de Chiloe
27	UCT-35AzC	Azul cauchequé	Castro. isla grande de Chiloe
28	UCT- 8Gb	Guadacho blanco	Ancud. isla grande de Chiloe
29	UCT- 9MgM	Meca gato morada	Ancud. Isla grande de Chiloe
30	UCT-10MgL	Meca gato morada larga	Los Muermos. Continente

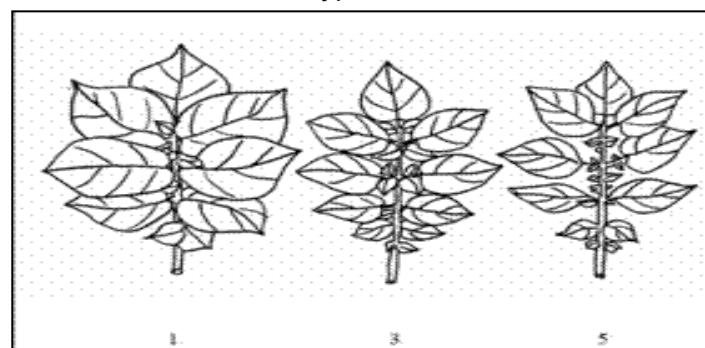
**Table 2.** Descriptive names of the morphological characters examined.

The descriptor list	Morphological characters
Tuber	General tuber shape Unusual tuber shape Predominant tuber skin color Secondary tuber skin color Distribution of secondary tuber color Tuber skin type Predominant tuber flesh color Secondary tuber flesh color Distribution of secondary tuber flesh color Eyes per tuber Depth of tuber eyes Distribution of tuber eyes Presence eyebrows
Sprout characters	Shape Habit of tip Length of lateral shoots Predominant sprout color Pubescence of base Lenticels anthocyanin pigmentation of the sprout
Stem characters	Stem colors Stem cross section Stem wing Stem: Anthocianin coloration
Plant	Foliage structure End bearing
Leaf characters	Leaf: openness Presence of secondary leaflets Abaxial leaf pubescent Adaxial leaf pubescent Green color Anthocyanin coloration on midrib of upper side Terminal and lateral leaflets: frequency of coalescence Leaflet: waviness of margin Leaflet: depth of veins Glossiness of the upperside Leaf dissection Terminal leaflets: size Second pair lateral leaflets: size Second pair of lateral leaflets: width in relation to length
Flower	Calyx color Calyx symmetry Corolla shape Predominant flower color Secondary flower color Distribution of secondary flower color Anther pigments Stamen formation Pistil pigments Pistil morphology Stigma shape Degree of flowering Premature flower abscission Number of flower per inflorescence
Phenology	Maturation cycle



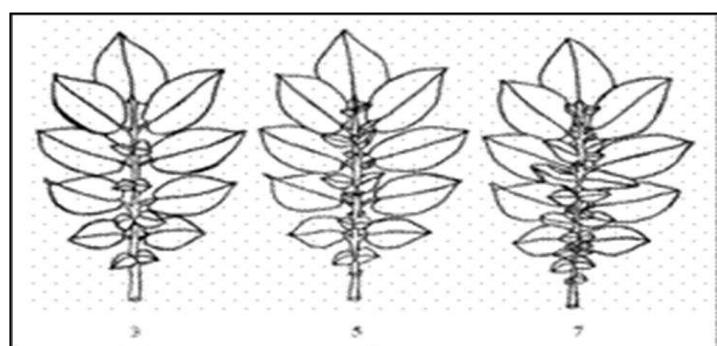
Foliage plant structure

- 1: branched type
- 2: intermediate type
- 3: leaf type



Leaf: openness

- 1: closed
- 3: intermediate
- 5: open



Presence of secondary leaflets

- 3: weak
- 5: medium
- 7: strong

**Figure 1.** Plant foliage characters evaluated.

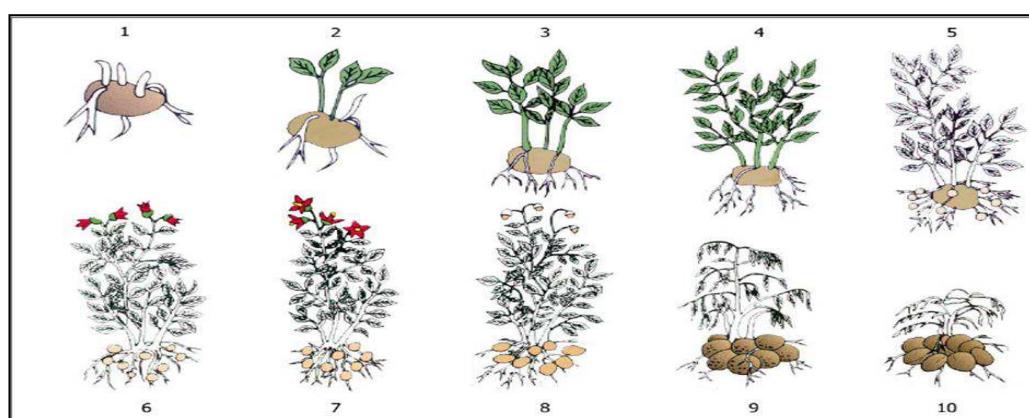
### **2.3.- Cycle of maturation.**

The ripening of different accessions of potato was evaluated periodically throughout the growing stage level (Table 3), which described 10 stages of development (Figure 2). The stage describes the phenological time in cultivation since the time that has been established (Dacom Plant Plus, 2003).

**Table 3.** Crop stages scale.

<b>Observation value</b>	<b>Crop stage</b>
1	Description and/or condition
2	Sprouting
3	Crop emergence
4	Developing leafs and stems
5	Growth in crop length
6	Closing crop canopy
7	Developing flower buds
8	Flowering stage
9	Developing of seeds
10	Die off crop
	Ripening tubers

**Source:** Dacom Plan Plus, (2003)



**Figure 2.** Crop stages of potato.

**Source:** Dacom Plan Plus, (2003).

### **III. RESULTS.**

#### **3.1.- Morphological data.**

Table 4, presents some morphological characters of the tuber, sprout and bloom observed for the native potato accessions studied. There is wide variability in tuber shape, type of habit and length of lateral shoots and flower color. Within the tuber shape, shape ranges from regular and odd-shaped oval to rare. The latter shape are typical fusiform accessions UCT-18Mn, UCT-31Ob, UCT-1Ma, UCT-9MgM and UCT-28MiR to local origin popularly called "guadacho potatoes", "michuñes potatoes" and "chunk potatoes". Round and oval tubers are representative of the accessions with regular shapes and are typical of UCT-3Cl, UCT-22cm, belonging to the locally called "clavelas potatoes". In relation to the shape of the shoots, there is a wide variability of forms, these being ovoid, spherical, cylindrical and conical. The habit of shoots opening are widely heterogeneous, ranging from open to closed leaves. The length of the lateral branches of the shoots varied from short to long. In relation to flowers color, these showed a gradient of colors from deep purple to white

Moreover, Table 5 shows a wide variability in plant height and average number of main stems. Plant ranged from short to tall from 48.65 to 101.7 cm. The average number of main stems per plant ranged from 2.80 to 5.40. Lower values were presented in accessions UCT-6Gc and UCT-15MgRo; the highest number corresponded to accession UCT-17Br. Furthermore, accessions studied predominantly showed a branched plant-type structure (23 accessions), semi-upright habit and leaf intermediates leaf opening. The presence of secondary leaflets showed a wide variability from weak to strong leaflets.

**Table 4.** Morphological characters of tuber, and flower bud observed in native potato accessions.

Accessions	Tuber shape	Sprout shape	Habit of tip	Length of lateral shoots	Flower color
UCT-11Mgb	elongated	wide cylindrical	intermediate	short	light purple
UCT-14MgRe	round	Ovoid	open	long	white
UCT-17Br	oblong	wide cylindrical	closed	medium	white
UCT- 6Gc	elongated	wide cylindrical	closed	short	intense purple
UCT-24Tn	round	Ovoid	open	short	white
UCT-22Cm	oval	Cónica	open	short	intense purple
UCT-25Gñ	round	wide cylindrical	intermediate	short	light blue
UCT- 7Ca	oval	Spherical	intermediate	short	light purple
UCT-18Mn	fusiform	Cónica	intermediate	long	light purple
UCT-26Ach	oval	narrow cylindrical	intermediate	short	intense purple
UCT-27Mu	round	Ovoid	closed	medium	light purple
UCT-28MiR	fusiform	wide cylindrical	intermediate	medium	light purple
UCT-29Mol	round	Cónica	intermediate	medium	light purple
UCT- 3Cl	Oval	wide cylindrical	open	short	light blue
UCT- 1Ma	fusiform	Cónica	closed	medium	light purple
UCT-16At	long-oblong	Ovoide	intermediate	medium	white
UCT-30Ño	elongate	Cónica	intermediate	short	white
UCT-19Aq	round	Cónica	intermediate	short	intense purple
UCT- 2Lv	elliptical. long	oval -	Ovoid	open	medium
UTC-31Ob	fusiform	wide cylindrical	intermediate	short	light purple
UCT-32Ci	round	Cónica	intermediate	long	intense purple
UCT-20Ro	round	Ovoid	open	short	white
UCT-33Cab	oval	wide cylindrical	open	medium	-
UCT-15MgRo	elongated	wide cylindrical	intermediate	medium	light purple
UCT-21Ac	rounded	Cónica	intermediate	long	intense purple
UCT-34Cor	round	wide cylindrical	intermediate	short	intense purple
UCT-35AzC	ovate	Ovoid	open	long	white
UCT- 8Gb	elongated	wide cylindrical	closed	medium	intense purple
UCT- 9MgM	fusiform	Narrow cylindrical	Intermedio	Corto	intense purple
UCT-10MgL	elongated	wide cylindrical	Intermedio	Media	light purple

**Table 5.** Plant morphological traits observed in native potato accessions.

Accessions	Plant height (cm)	Main stems per plant (average)	Foliage structure	Growth habit	Leaf openness	Presence of secondary leaflets
UCT-11Mgb	medium (61.30)	3.90	intermediate	semi erect	intermediate	strong
UCT-14MgRe	high (101.70)	3.15	branched	erect	intermediate	medium
UCT-17Br	medium (73.85)	5.40	intermediate	semi erect	intermediate	weak
UCT- 6Gc	low (55.40)	2.80	branched	erect	intermediate	weak
UCT-24Tn	low (54.55)	3.45	intermediate	semi erect	intermediate	medium
UCT-22Cm	low (50.70)	3.85	intermediate	semi erect	intermediate	weak
UCT-25Gñ	low (56.25)	3.70	intermediate	semi erect	intermediate	medium
UCT- 7Ca	medium (63.80)	4.10	branched	erect	intermediate	weak
UCT-18Mn	medium (76.45)	3.30	intermediate	semi erect	intermediate	medium
UCT-26Ach	medium (67.35)	3.35	intermediate	semi erect	closed	weak
UCT-27Mu	medium (69.65)	4.00	intermediate	Semi erect	intermediate	medium
UCT-28MiR	medium (57.18)	2.00	branched	erect	closed	weak
UCT-29Mol	low (49.20)	4.00	intermediate	semi erect	closed	weak
UCT- 3Cl	low (48.65)	4.00	intermediate	semi erect	intermediate	weak
UCT- 1Ma	high (92.30)	3.90	intermediate	semi erect	intermediate	weak
UCT-16At	medium (61.30)	4.00	intermediate	semi erect	closed	weak
UCT-30Ño	medium (64.30)	3.55	intermediate	semi erect	intermediate	weak
UCT-19Aq	low (53.85)	3.75	intermediate	semi erect	closed	weak
UCT- 2Lv	medium (60.50)	3.80	intermediate	semi erect	intermediate	medium
UTC-31Ob	medium (76.90)	3.10	intermediate	semi erect	closed	medium
UCT-32Ci	medium (61.80)	4.30	foliar	creeping	intermediate	weak
UCT-20Ro	medium (61.95)	3.50	intermediate	semi erect	intermediate	weak
UCT-33Cab	medium (61.35)	4.30	intermediate	semi erect	intermediate	medium
UCT-15MgRo	medium (69.50)	2.80	branched	semi erect	intermediate	weak
UCT-21Ac	medium (66.25)	4.90	intermediate	semi erect	intermediate	medium
UCT-34Cor	medium (59.85)	3.45	intermediate	semi erect	intermediate	medium
UCT-35AzC	high (89.45)	3.25	branched	erect	closed	medium
UCT- 8Gb	medium (60.80)	3.60	intermediate	semi erect	open	strong
UCT- 9MgM	medium (74.20)	3.75	intermediate	semi erect	intermediate	weak
UCT-10MgL	medium (67.35)	3.95	intermediate	semi erect	intermediate	weak

In relation to the stages of growth and ripening of the material under study (Table 6), we observed a wide variability of the cycle, which varied between 100-day growing cycle for the earliest and 150/160 days for the latest accessions. Early accessions were UCT-24Tn, UCT-33Cab and UCT-32Ci and late accessions were UCT-27Mu, UCT-34Cor, UCT-28MiR, UCT-15MgRo, UCT-26Ach, UCT-6Gc, UCT-22cm, UCT-30Ño, UCT-21Ac and UCT-29Mol.

The early accessions, matured at the end of January and early February, after 100 to 107 days. The late accession matured at the end of February, after 150 days. The later accessions corresponded to the popularly called "potato postreras".

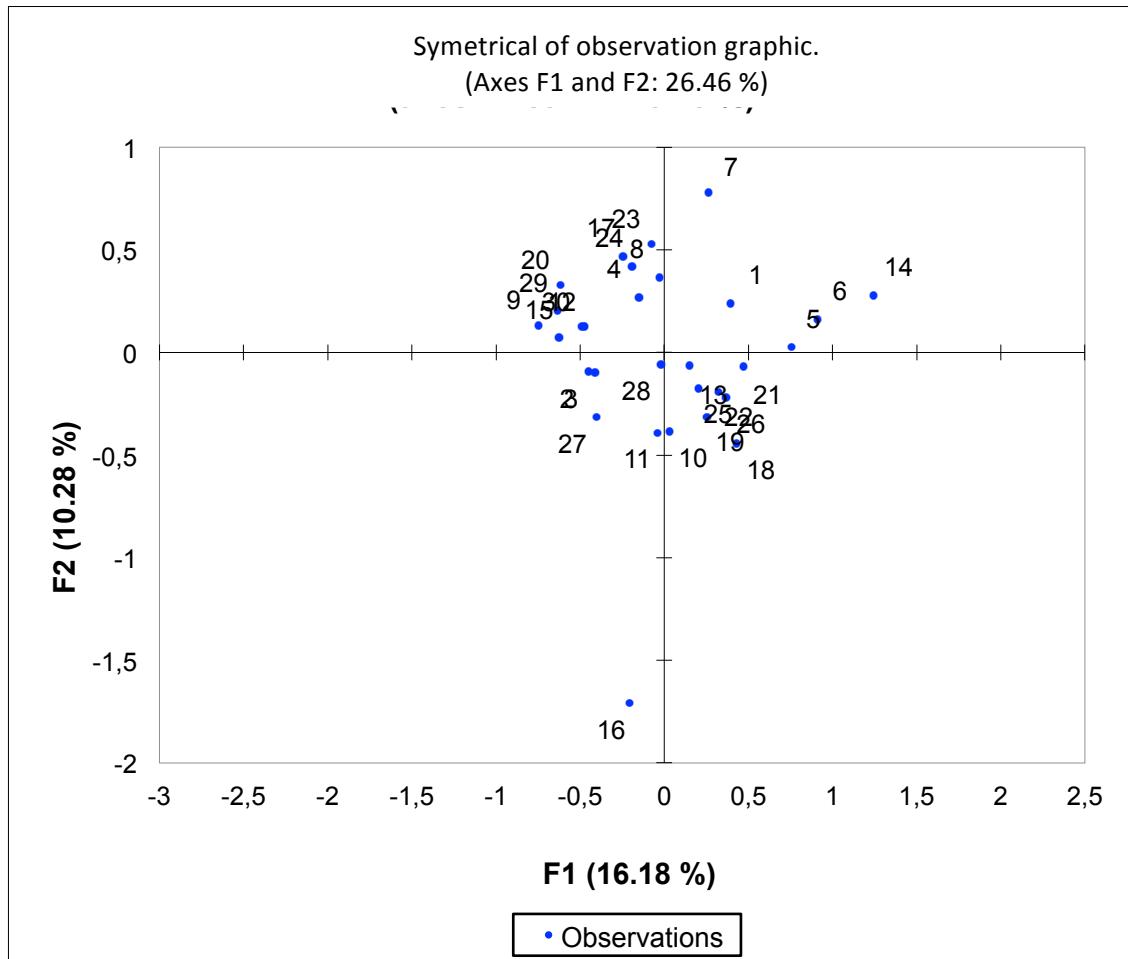
**Table 6.** State of cultivation and maturation cycle of different accessions of native potato.

Accessions	Average values of growth stages							
	13-12-08	27-12-08	5-1-2009	15-1-09	23-1-09	29-1-09	6-2-09	20-2-09
UCT-11Mgb	5.6	7.2	6.9	7.7	8.3	8.6	9.1	9.6
UCT-14MgRe	7.0	7.0	7.3	7.1	8.1	8.7	8.8	9.5
UCT-17Br	5.4	7.0	7.1	8.0	8.3	8.8	9.3	9.7
UCT- 6Gc	4.2	6.6	7.2	7.0	7.5	8.2	7.6	8.3
UCT-24Tn	5.1	6.8	8.1	8.6	8.8	9.2	10.0	10.0
UCT-22Cm	4.6	6.0	7.1	8.2	8.1	8.7	9.1	9.6
UCT-25Gñ	4.8	6.6	7.7	8.6	9.6	9.3	9.8	9.8
UCT- 7Ca	5.4	7.0	7.5	7.6	7.8	8.6	9.0	9.3
UCT-18Mn	5.0	7.0	7.1	8.1	8.1	8.7	9.0	9.5
UCT-26Ach	4.0	7.5	7.3	7.8	8.0	8.5	8.5	8.8
UCT-27Mu	5.2	7.2	7.1	7.6	7.8	8.5	8.6	9.0
UCT-28MiR	4.1	6.1	5.6	8.0	8.3	9.2	9.6	9.5
UCT-29Mol	4.7	6.0	7.6	8.7	9.0	9.0	9.5	9.7
UCT- 3Cl	4.5	6.5	7.2	8.1	8.0	8.7	9.1	9.6
UCT- 1Ma	5.1	7.1	7.3	8.0	8.1	8.5	8.5	9.5
UCT-16At	5.2	7.0	7.0	8.0	8.5	9.1	9.5	9.5
UCT-30Ño	4.7	7.0	7.0	7.5	8.0	8.5	8.6	9.0
UCT-19Aq	5.7	7.1	7.3	8.0	8.3	8.5	9.1	9.6
UCT- 2Lv	5.1	7.1	7.7	8.3	8.7	9.0	9.6	9.8
UTC-31Ob	4.9	7.0	7.8	8.0	8.0	8.5	8.6	9.2
UCT-32Ci	5.7	7.2	7.5	8.3	9.5	9.5	10.0	10.0
UCT-20Ro	5.1	7.2	7.3	7.8	8.3	8.8	8.8	9.6
UCT-33Cab	5.2	5.7	7.6	8.7	9.3	9.5	9.6	10.0
UCT-15MgRo	4.7	6.5	7.2	7.2	7.5	8.3	7.6	8.7
UCT-21Ac	5.5	6.7	7.1	8.1	8.5	9.1	9.5	9.8
UCT-34Cor	4.5	6.2	7.5	7.1	7.6	8.3	8.3	8.5
UCT-35AzC	6.5	7.0	7.7	7.8	8.7	8.7	9.0	9.6
UCT- 8Gb	5.0	6.8	7.2	8.0	8.1	8.6	9.1	9.2
UCT- 9MgM	5.0	7.2	7.3	7.8	7.8	8.5	8.5	9.3
UCT-10MgL	4.8	6.3	7.1	8.1	8.1	8.5	8.6	9.5

### 3.2.- Multiple correspondence factorial analyses (AFCM).

Figure 3, presents the plane engendered by the first two axes (F1 and F2) of the multiple correspondence factorial analysis. This first plane takes into account for 26.46% of the inertia of the observations. It is noted that accession UCT-16At occupied a position distant

from the rest of the materials due to the long-oblong tuber shape, white color of the flower and other characteristics.



**Figure 3.** Graph of the observations represented in the first plane of the multiple correspondence factorial analysis.

The multiple correspondence factorial analysis allowed the separation of three defined group (Table 7). The first group included twenty one accessions, the second group included eight accessions and a third group included only one accession (Figure 4). Results by class showed that intra-class variance for the first, second and third group was 2.786. 2.091 and 0, respectively.

**Table 7.** Results by class formed from multiple correspondence factorial analyses

Classes	Group 1	Group 2	Group 3
Objects	21	8	1
Sum of weights	21	8	1
Intra-class variance	2.786	2.091	0.0
Minimum distance to centroid	0.99	1.15	0.0
Mean distance from centroid	1.60	1.34	0.0
Maximum distance to centroid	2.01	1.57	0.0

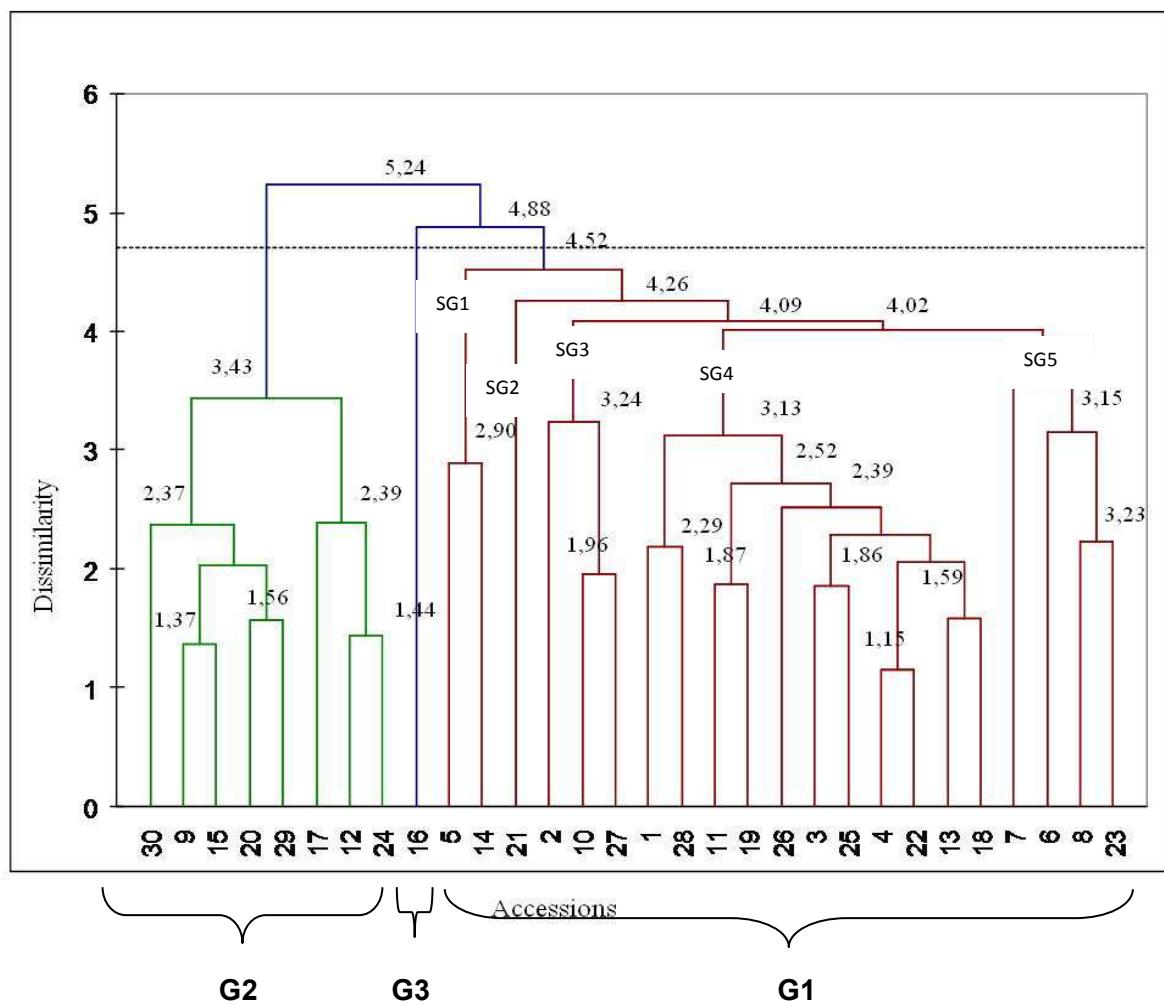
Figure 4 presents the dendrogram formed for the 30 accessions of native potatoes studied. The correspondence between dendrogram numbers and accession numbers are presented in table1. The first Group comprises twenty one accessions. This cluster comprises mostly tuber accessions having primary round, oval and elongated shapes, with moderate to low amount of variable eyes depth. These accessions are UCT-24Tn, UCT-3Cl, UCT-32Ci, UCT-14MgRe, UCT-26Ach, UCT-35AzC, UCT-11Mgb, UCT-8Gb, UCT-27Mu, UCT-2Lv, UCT-34Cor, UCT-17Br, UCT-21Ac, UCT-6Gc, UCT-20Ro, UCT-29Mol, UCT-19Aq, UCT-25Gñ, UCT-22cm, UCT-7Ca and UCT-33Cab. According to the observed cluster nodes within the group, it clearly divided into 5 homogeneous subgroups that correspond to: Sub group 1 with two accessions: UCT-24Tn. UCT-3Cl. These are two widely grown varieties among farmers of Chiloe. Their tubers are characterized by a predominance round-oval shape, predominant skin color is yellow and creamy colored pulp. The plants display low or semi-erect habit and intermediate foliage semi-open. One of the main features of both is their rapid growth and early cycle and semi-early cycle, respectively. Sub group 2 with one accession, UCT-32Ci. This accession, presents round tubers, brownish skin and white flesh. Plants are of medium height, creeping habit and closed-canopy leaf type with deep purple flowers, corresponding to one of the earliest native varieties. Sub group 3, with three accessions: UCT-14MgRe, UCT-26Ach and UCT-35AzC. These accessions have round-ovate tubers, with an average number of eyes of less than 8.0, evenly distributed throughout the tuber, soft skin, and white pulp or cream distributed in the vascular ring and pith. The plants have open-end foliage branching structure bearing erect or semi-erect flowers of varying colors. All present late ripening cycles. Sub group 4 with eleven accessions: UCT-11Mgb, UCT-8Gb, UCT-27Mu, UCT-2Lv, UCT-34Cor, UCT-17Br, UCT-21Ac, UCT-6Gc, UCT-20Ro, UCT-29Mol and UCT - 19Aq is quite heterogeneous. The accessions of this sub group present tubers which are characterized by rounded and elongated shapes. The rounded shapes have mostly clear skin colors (white, cream and yellow), whereas elongated tubers are dark in colors (red and purple) with the exception of accession UCT-8Gb which is white. There is no presence in these accessions of rare tuber shapes. The range of eyes per tuber fluctuates between 4.6

and 10.2 distributed throughout the tuber. The pulp is white, creamy-white and yellow. All plants in this group are semi-erect or medium height. Predominantly present purple flowers in varying degrees of intensity, however, accessions UCT-20Ro and UCT-17Br have white flowers. All are late maturing with the exception of UCT-33Cab that is early. Sub group 5 with four accessions UCT-25Gñ, UCT-22Cm, UCT-7Ca and UCT-33Cab. These accessions are characterized by bringing together strongly variegated tubers, primary purple colored and yellow skin. The flesh is heavily pigmented, purple ring at the level of vascular and bone. They have generally oval and round eyes evenly distributed with number below 10. The flowers of these accessions are colored ranging from light to intense purple.

The second group included eight accessions, all characterized by elongated and/or fusiform constrained tuber shapes. In addition, the skin of most of them is colored, from white with red to deep purple through red. The accessions included in this group are UCT-10MgL, UCT-18Mn, UCT-1Ma, UCT-31Ob, UCT-9MgM, UCT-30Ño, UCT-28MiR and UCT-15MgRo. The pulp is heavily pigmented, presenting white as a predominant color and violet, purple and red as secondary colors. This is distributed mainly around the vascular ring and pith, except UCT-10MgL, which shows it only in the medulla. In most of these accessions the number of eyes per tuber was 10, evenly distributed. Buds are variably moving from cylindrical to conical. Plants are of medium height with the exception of UCT-1Ma which was high. The structure of plant is intermediate with semi opened foliage, except UCT-28MiR and UCT-15MgRo which are branched. In general bearing at the end of the plant is semi-erect except UCT-28MiR which is erect. In all accessions, the plants show weakly dissected leaves with large lateral leaflets and terminal medium to large. The presence of secondary leaflets is weak. Flowers are predominantly light purple in color; however, accessions UCT-15MgRo and UCT-9MgM are deep purple. In this group are the local native varieties that are popularly called "chunk potatoes".

The third group, only contained the accession UCT-16At. This accession has the local name of "Azul tabla". This accession was characterized by presenting a long-oblong tuber shape and a flat shape that gives the vernacular name. Its skin is smooth and dark purple – black; predominantly the pulp is creamy, with strong presence of violet in the vascular ring. It has an intermediate eye number per tuber, with an average of 11 eyes, these are of medium depth and evenly distributed throughout the tuber. Buds are ovoid. The plant is of medium height, has an intermediate semi open foliage and is of semi-erect structure. Stems are green with purple knots, with wavy wings and anthocyanin pigmentation with some green. Average number of main stems observed is four. The leaves are dissected, light green, but

more heavily pigmented in the central nerve of the upper side. This accession has shown a late growth cycle.



**Figure 4.** Dendrogram of potato accessions using morphological data.

## **IV. DISCUSSION**

Results obtained using morphological data and multiple factorial correspondences analysis indicate a good clustering of native potato accessions into groups that correspond to the popular names and the attributes assigned by local farmers themselves, especially those related to tuber characteristics and morphology of the plant. The grouping is consistent with local names that are recognized for different varieties at the local level in Chiloe. For example, the second group formed for eight accessions, all characterized by elongated and/or fusiform constrained tuber shapes with skin of most of them is colored, from white with red to deep purple through red. The pulp is heavily pigmented, presenting white as a predominant color and violet, purple and red as secondary colors. This is distributed mainly around the vascular ring and pith. This group is the local native varieties that are popularly called "chunk potatoes", specifically in response to fusiform constrained tubers shape. In the same manner, we can say that the vernacular name of the accession UCT-16 At is in response to flat shape of tuber.

This agrees with the point made by Brush (1992) in relation to the taxonomy of the Andean potato popular in the central and upper areas of Peru. Varieties are primarily distinguished according to tuber characteristics, such as tuber shape (oval, spherical, flat, long), configuration of tubers "eyes" (depth, Number, location, color), skin color, and pattern (white to deep purple, solid color, multicolored) and flesh color and color pattern (solid, ringed, white to deep purple). In rare cases, non tuber characteristic such as stem or flower color distinguish varieties. The days to maturity were between 140 and 170 days with an average of 155 days. The number of tubers per plant was between 2 and 99 depending on location and variety. Yield fluctuated between 0.05 and 2.3 kg per plant. Days to post-harvest tuber sprouting ranged between 2 and 139 days.

In summary, characters evaluated in the collection presented considerable variability. This material could be used directly or as parents in breeding new varieties.

## **V. CONCLUSION**

There is a great morphological diversity in the potato collection evaluated. Highlights the facts that no are accessions duplicate within this collection.

Wide variability was observed for several traits, such as tuber shape and color, shape and colour of the sprout, type of habit sprout, length of lateral branches of the sprout, the color of the flower and several characteristics of the leaf. The forms of the tubercle were from regular to oval shapes as rare as fusiform. The shapes of the sprout were ovoid, spherical, cylindrical and conical. The habits of opening the sprout were very heterogeneous, showing a change from open to closed leaves. The length of the lateral branches of the sprout varied from short to long. The colour of the flowers showed a wide variation of colors, forming a garden of colors from deep purple to white. Overwhelmingly, the accessions studied, showed a structure of branched-type plant, semi-upright habit and leaf opening intermediates. The maturation cycle in the material under study showed large variability. This ranged from 100 days of growth cycle for earliest accessions and 150/160 to later accessions.

The three-group structure is not very easy to interpret, due to descriptor that takes into account the characteristic of different organs and plant phenology. The first group comprehend mostly tubers accessions have primary form round, oval and elongated, with moderate to low amount of variable depth eyes. The second group, submit all characterized by elongated forms and/or fusiform-fusiform constrained tuber. In addition, the skin of most of them is colored, from white with red to deep purple through red. In this group are local native varieties that are popularly called “cacho potatoes”. The third group, alone container the accession UCT-16At. This accession was characterized by to present a long-oblong form of tuber and a pleated look that gives the vernacular name.

## **VI.- REFERENCES**

- Brush S. B. 1992. Ethnoecology, biodiversity and modernization in Andean potato agriculture. *Journal of Ethnobiology*. 12:161-185.
- Dacom Plant Plus. 2003. Plant plus manual. Using plant-plus on the internet. Available at <http://www.dacom.nl/ppo/ppo.php?>. Accessed 18 july 2006.
- Dervin, C. 1990. Comment interpréter les résultats d'une analyse factorielle des correspondances ?. Publication ITCF. Institut Technique des Céréales et des Fourrages. 75p.
- Dervin, C. 1996. Comment interpréter les résultats d'une classification automatique. Publication ITCF. Colecction STAT-ITCF. ITCF Céréaliers De France. 70p.
- Hawkes. J. G. 1990. The potato. Evolution, biodiversity and genetic resources. Belhaven Press. London, UK. 178p.
- Huaman. Z., J. Williams., W. Salhuana., and N. Vincent. 1977. Descriptors for the cultivated potato and for the maintenance and distribution of germoplasm collections. Consultative Group on International Agricultural Research. Rome. International Board for Plant Genetic Resources (I.B.P.G.R.). p 47.
- Quiros, C.. B. Brush., D. Douches., K. Zimmerer and G. Huestis. 1990. Biochemical and folk assessment of variability of Andean cultivated potatoes. *Economic Botany* 44:254-266.
- Spooner. D., K. Mclean., G. Ramsay., R. Waug and G. Bryan. 2005. A single domestication for potato based on multilocus amplified fragment length polymorphism genotyping. *Proceeding of the National Academy of Sciences*. vol. 102., no. 41, p. 14694–14699.

## **CHAPITRE IV. Genetic diversity of Solanum landraces in Chile, using simple sequence repeats markers (SSR).**

### **I.- INTRODUCTION**

Chile is considered to be a sub-center of origin for the cultivated potato (Spooner *et al.*, 2005b). In this context, the native potatoes of Chiloe are characterized by a rich variety of forms, sizes, colors and phenological characteristics. This rich genetic patrimony needs to be described and individualized in order to be conserved and used. There is a remarkable concentration of cultivated and wild forms of potato in the southern Chile with the greatest number of native varieties being located in the island of Chiloe, and they are still preserved in the fields of small farmers. The peculiar characteristics of Chiloe, its natural conditions and its isolation, have allowed the proliferation of a great number of native varieties, of varying qualities and suitable for cultivation at different times in the farming calendar, as well as a number of different forms of preparation and consumption. Unfortunately, the native potatoes of Chiloe are being lost, partially because of the phytopathological deterioration of certain landraces but mainly because of their replacement by introduced new improved commercial varieties. As a result, their conservation must be ensured by means of germplasm banks collections. The conservations and use of this native genetic material will ensure that it does not disappear, but enjoys a projection for the future with new uses. In addition, potato improvement programs require a basic knowledge about morphology and genetic diversity. In this context, new advances in molecular biology with the use of more sensitive molecular markers able to detect changes in the genotype of the individuals, would greatly contribute to the generation of this important information.

Until the mid 1960s, the markers used in genetic studies and improvement were those controlled by genes associated with morphological characteristics in easily identified phenotypes (Hijmans and Spooner, 2001). Potato germplasm has been described using morphological traits (Huamán *et al.* 1977; Ortiz and Huamán, 1994). Since 1975, it has been known in Europe and North America that potato cultivars could be identified by their protein and enzyme patterns (Stegemann and Loeschecke, 1976).

With the modern technologies provided by molecular biology, various methods arise for the direct detection of genetic polymorphism at the DNA level using molecular markers. Detection and analysis of genetic variation can help us to understand the molecular bases of various biological phenomena in plant. Genetic or DNA based marker techniques such as

RFLP, RAPD, SSR and AFLP are routinely being used in ecological, evolutionary, taxonomical, phylogenetic and genetic studies of plant sciences (Agarwal *et al.*, 2008).

Molecular markers have contributed to a greater genetic knowledge of many plant species, including potato. In addition, these markers have been used in the Solanum genus for the analysis of biodiversity and for phylogenetic studies (Ritter, 2000; Ritter *et al.*, 2004; Spooner *et al.*, 2005a). Recently, efforts have been devoted to achieve a less subjective description of cultivars, based on Random Amplified Polymorphic DNA (RAPD) (Miller and Spooner, 1999; Isenegger *et al.*, 2001; Sun *et al.*, 2003), and microsatellites (Ashkenazi *et al.* 2001; Raker and Spooner, 2002). Interrupted Simple Sequence Repeats (ISSR) has also been used (Milbourne *et al.*, 1998; Bornet *et al.*, 2002).

In a previous research, Solano *et al.* (2007) determined the genetic relationships existing among twenty varieties of potatoes from the Chiloe Island using AFLP markers. A similarity tree-diagram was made, based on all the AFLP bands..The results indicated that all the potatoes analyzed differ in their degrees of similarity, but no relationship has been found with the morphological diversity of the varieties. New molecular tools such as SSRs which are highly polymorphic at numerous loci with a co-dominant behavior may be more discriminant and provide the geneticists with more efficient tools to look for potential relation between morphological and molecular diversity.

The present study aimed at the development of SSRs markers to describe the genetic diversity of thirty native potato varieties collected in the isle of Chiloe.The specific objective was to compare the native material with commercial cultivars available in the country.

### **1.1.- Microsatellite-based marker technique.**

After the invention of polymerase chain reaction (PCR) technology (Mullis and Falloona 1987), a large number of approaches for generation of molecular markers based on PCR were detailed, primarily due to its apparent simplicity and high probability of success. Usage of random primers overcame the limitation of prior sequence knowledge for PCR analysis and facilitated the development of genetic markers for a variety of purposes. PCR-based techniques can further be subdivided into two subcategories: (1) arbitrarily primer PCR-based techniques (or nonspecific sequence) and (2) sequence targeted PCR-based techniques(or specific sequence).

Microsatellite or short tandem repeats or simple sequences repeats (SSRs) are monotonous repetitions of very short (one to five) nucleotide motifs, which occur as interspersed repetitive elements in all eukaryotic genomes (Tautz and Renz 1984). Variation in the number of tandem repeated units is mainly due to strand slippage during DNA replication where the repeats allow matching via excision or addition of repeats. As slippage in replication is more likely than point mutations, microsatellite loci tend to be hypervariable (Levinson and Gutman, 1987). Microsatellite assays show extensive inter-individual length polymorphisms during PCR analysis of unique loci using discriminatory primers sets (Schlötterer and Tautz, 1992).

SSRs have greater technological simplicity relative to RFLP and AFLP, and do not require high concentration and quality of DNA. SSRs have codominant genetic behavior and usually can detect all possible alleles of a locus. Generally the degree of polymorphism revealed at a locus increases with the mean total length of the SSR, and although the SSR repeat motif might not be specific of a locus, the flanking regions are. So a pair of primers defined in the flanking regions will amplify a unique target microsatellite (Ortiz *et al.*, 2000).

The Table1 shows different applications in genetic analysis of microsatellite markers cited by Ferreira and Grattapaglia (1998).

**Table 1.** Applications in genetic analysis of microsatellite markers.

Application	Adequacy and / or response
Identification of genotypes	Very high
Germplasm evaluation	High
Genetic mapping	Very high
Mapping directed to specific regions	Moderate
Comparative mapping	High
Phylogenetic analysis	High

**Source:** Ferreira and Grattapaglia (1998).

The reproducibility of microsatellites is such that, they can be used efficiently by different research laboratories to produce consistent data. Locus-specific microsatellite-based markers have been reported from many plant species such as tomato (*Solanum lycopersicum* L.) (Benor *et al.*, 2008), pea (Gong, *et al.*, 2010), lettuce (*Lactuca sativa* L.) (van de Wiel *et al.* 1999), barley (*Hordeum vulgare* L.) (Saghai Maroof *et al.*, 1994), rice

(*Oryza sativa* L.) (Wu and Tanksley 1993), and potato (Feingold *et al.*, 2005; Ghislain *et al.*, 2004; Ghislain *et al.*, 2006; Ghislain *et al.*, 2009a; Ghislain *et al.*, 2009b; Ispizúa *et al.*, 2007; Mathias *et al.*, 2007 ; Milbourne, *et al.*, 1998 ; Spooner *et al.*, 2007).

## II.- MATERIALS AND METHODS

### 2.1.- Plant material

A collection of 40 potato accession was studied. This included 30 accessions of native potatoes, collected on the island of Chiloe in previous works. This material is maintained *ex-situ* as a field collection by clonal propagation at the Catholic University of Temuco experimental station since 2005, and represents the extensive genetic diversity that can be found in *Solanum tuberosum* ssp. *tuberosum* in Chiloe Island(Table 2). A set of nine commercial cultivars broadly grown in Chile was included for comparative purposes: Desirée, Karú, Shepody, Baraka, Híbrido LT-8 x TS-9, Rosara, Yagana, Pukará and Rodeo (Table 3). An accession of *Solanum fernandezianum* collected on the Juan Fernandez Archipelago was included as outgroup. This herbaceous and diploid endemic species has been conserved *ex-situ* as botanic seed at the laboratory. The native and commercial groups, belong to the Tuberose serie ( $2n=4x=48$ ), whereas *S. fernandezianum* belong to the Etuberosa serie ( $2n=2x=24$ ).

**Table 2.** Description of Chiloe native potato collection under study.

Laboratory number	Accession	name	Geografic origin
<b>Native group</b>			
1	UCT-11Mgb	Meca gato blanca	Isla grande, Chiloe
2	UCT-14MgRe	Redonda	Isla grande, Chiloe
3	UCT-17Br	Bruja	Isla Quinchao.
4	UCT- 6Gc	Guadacho colorado	Chonchi. Isla grande, Chiloe
5	UCT-24Tn	Tonta	Castro. Isla grande, Chiloe.
6	UCT-22Cm	Clavela morada	Castro. Isla grande, Chiloe
7	UCT-25Gñ	Guicoña	Quellón. Isla grande, Chiloe
8	UCT- 7Ca	Camota	Isla grande, Chiloe
9	UCT-18Mn	Michuñe negro	Isla grande, Chiloe
10	UCT-26Ach	Azul chañihue	Isla grande, Chiloe
11	UCT-27Mu	Murta	Quellón. Isla grande, Chiloe
12	UCT-28MiR	Michuñe rojo	Isla grande, Chiloe
13	UCT-29Mol	Molejona	Isla grande, Chiloe
14	UCT- 3Cl	Clavela	Los Muermos. Continent
15	UCT- 1Ma	Michuñe azul	Isla grande, Chiloe
16	UCT-16At	Azul table	Isla grande, Chiloe
17	UCT-30Ño	Ñocha	Isla grande, Chiloe
18	UCT-19Aq	Azul de quento	Castro. Isla grande, Chiloe
19	UCT- 2Lv	Lengua	Castro. Isla grande, Chiloe
20	UTC-31Ob	Ojitos blanco	Ancud. isla grande, Chiloe.
21	UCT-32Ci	Cielito	Castro. Isla grande, Chiloe
22	UCT-20Ro	Rosada	Isla grande, Chiloe
23	UCT-33Cab	Cabrita	Isla grande, Chiloe
24	UCT-15MgRo	Meca gato rojo	Isla grande, Chiloe
25	UCT-21Ac	Azul cristalina	Isla grande, Chiloe
26	UCT-34Cor	Cordillera	Castro.Isla grande, Chiloe
27	UCT-35AzC	Azul caucheque	Castro. isla grande, Chiloe
28	UCT- 8Gb	Guadacho blanco	Ancud. isla grande, Chiloe
29	UCT- 9MgM	Meca gato morada	Ancud. Isla grande, Chiloe
30	UCT-10MgL	Meca gato morada larga	Los muermos, continent

**Table 3.** Description of commercial cultivar and *Solanum fernandezianum* species evaluated.

Laboratory number	Name	Geographic origin	Year of release	Parents
<b>Commercial group</b>				
31	Desirée	Cultivar introduced ZPC – Holanda	1962	Urgenta x Despeche
32	Karú	Cultivar of Chile	2002	Yagana x Fanfare
33	Shepody	Cultivar introduced Canada	1980	Bake-King x F58050
34	Baraka	Cultivar introduced		SVP 50-358 x Avenir
35	Híbrida	Hybrid LT8 xTS-9	----	LT-8 x TS-9
36	Rosara	Cultivar introduced Solana – Alemania	1990	Secura x 2605 77
37	Yagana	Cultivar of Chile	1983	Hydra x 904/61
38	Pukará	Cultivar of Chile	1993	Cleopatra x Yagana
39	Rodeo	Cultivar introduced		
<b>S. fernandezinum group</b>				
40	<i>Solanum fernandezianum</i>	Endemic specie	-----	Juan Fernandez Archipelago

## 2.2.- DNA extraction

DNA was extracted from young fresh leaves. Approximately 100-300 mg of leaf tissue were freeze-dried and ground in liquid nitrogen with a mortar and pestle. Genomic DNA was isolated with Plant DNAzol® (Invitrogen) following the manufacturer's instructions. The pellet was recovered in 70 µl of T.E Buffer.

The quality of DNA was evaluated by agarose gel electrophoresis (1%), using standard DNA marker DL 15.000 bp. The concentration of DNA was quantified using a digital Spectrophotometers (marc Thermo Spectronic GENESYS™ 10) based on 260 nm absorbance. DNA quality was inferred by 260/280 nm ratio. The concentration of the DNA was adjusted to approximately 300 ng/ml, with the addition of sterile deionised water.

## 2.3.- SSRs loci and primers

In this study eleven microsatellites markers (Table 4) were selected by their high level of polymorphism. Five of them were reported by Moisan-Thiery *et al.*, (2005) for the identification of the 286 potato cultivars belonging to national seed collection maintained at

Hanvec by FNPPPT (Fédération Nationale des Producteurs de Plant de Pommes de Terre). Whereas the remaining six loci were selected from the kit proposed by Ghislain *et al.*, (2009b). All of these primers are recommended for genetic studies in potato due to: good stability and quality of amplification, high polymorphic content and localization on different chromosomes of the species (Ghislain *et al.*, 2009b).

**Table 4.** General description of SSRs used in genetic characterization of the potato collection.

Locus	Repeat Motif	Location	Approximate fragment size (bp)	N° of alleles	Polymorphism Information Content	Power discrimination	Bibliographic Source
SSR1	(TCAC) <sub>n</sub>	Chr. VIII	230-194	11	--	0,91	Moisan-Thiery <i>et al.</i> , (2005).
STM2005	(CTGTTG) <sub>n</sub>	Chr. XI	193-160	5	--	0,79	Moisan-Thiery <i>et al.</i> , (2005).
LEMALX	(ATT) <sub>n</sub>	Chr. V	140-120	4	--	0,84	Moisan-Thiery <i>et al.</i> , (2005).
STM1097	(CGTTT) <sub>n</sub>	Chr. XII	281-234	6	--	0,82	Moisan-Thiery <i>et al.</i> , (2005).
STM2020	(TAA) <sub>n</sub>	Chr. I	193-160	10	--	-	Moisan-Thiery <i>et al.</i> , (2005).
STM0019a	(AT) <sub>n</sub> (GT) <sub>n</sub> (AT) <sub>n</sub> (GT) <sub>n</sub>	Chr VI	155-212	17	0,854	--	Ghislain <i>et al.</i> , (2009b).
STG0010	(TG) <sub>n</sub>	Chr III	175-192	11	0,685	--	Ghislain <i>et al.</i> , (2009b).
STG0016	(AGA) <sub>n</sub>	Chr I	137-174	14	0,773	--	Ghislain <i>et al.</i> , (2009b).
STI0003	(ACC) <sub>n</sub>	Chr VIII	137-188	16	0,747	--	Ghislain <i>et al.</i> , (2009b).
STI0030	(ATT) <sub>n</sub>	Chr XII	94-137	17	0,811	--	Ghislain <i>et al.</i> , (2009b).
STM0031	(AC) <sub>n</sub> (GCAC) <sub>n</sub>	Chr VII	185-211	10	0,721	--	Ghislain <i>et al.</i> , (2009b).

#### 2.4.- PCR amplification for microsatellite markers.

The reaction was performed in a volume of 20 µl, with 1U of *Taq* DNA polymerase (Fermentas), 30 ng of template DNA, 2.4 µM of each primer, 0.1 µM of each dNTP, 1X of 10XPCR-buffer (20 Mm Tris, 50 mM KCl, pH 8,4), 1.2 to 2.0 mM MgCl<sub>2</sub>. The mixtures were then subjected to PCR in a Tempra Thermocycler model MG96G version 3.23 using the following program: 1 cycle of 4 min at 95°C, followed by 30 cycles of 1 min at 95°C, 1 min at specific annealing temperature (Annexe 1) and 2 min at 72°C, with a final extension step of 10 min at 72°C.

## **2.5.- Electrophoresis of PCR products.**

After thermal cycling, 7 µl loading dye was added to 14 µl the reaction mixture; the product were then denatured for 5 min at 95°C and, after 30 min pre-run, were loaded on a 6 % denaturing polyacrylamide gel (8 M urea) buffered with 1 X TBE. 60 W constant power was applied for 4 h using a DNA sequencing gel electrophoresis apparatus Biorad, Model Sequi-Gen Cell.38XX 50 of 100 watt (vertical electrophoresis apparatus). The time of electrophoresis run was adjusted depending of the SSRs product size.

Polyacrylamide gels were obtained by polymerizing 120ml of a stock solution of 6% polyacrylamide (19:1 acrylamide: bisacrylamide, TrispH8) prepared in advance and stored in darkness at 4°C. The polymerization was carried out by adding 330µL of 10% ammonium persulfate and 33µL of Temed (C6H16N2) catalyst and initiator of the reaction. One of the glass side (the gel is polymerized between two glass sides) was treated with a binding solution (1mL of ethanol 95 °, 5 µL glacial acetic acid and 3 µL of Silane Bind). The other electrophoretic glass side was treated with repellent solution Sigmicode.

## **2.6.- Silver staining.**

Gels were stained with the method of Bassam *et al.*, (1991) modified as follows: gel were fixed for 30 min in 3 l of 10% acetic acid; washed twice with distilled water; stained for 30 min in 3 l of freshly prepared 0,1% AgNO<sub>3</sub>, with 4,5 ml of formaldehyde added immediately prior to use; rinsed for 7-8 s with 3 l distilled water; developed for about 10 min in 2 l of freshly prepared 3% Na<sub>2</sub>CO<sub>3</sub> with 500 ul of Na<sub>2</sub>SO<sub>4</sub> and 3 ml of formaldehyde added immediately prior to use; and fixed for 5 min in 1=% acetic acid. Gels were left to dry on the bench overnight before scanning.

## **2.7.- Data analysis and quantification of genetic diversity.**

SSR marker alleles were scored for presence (1) or absence (0) of the band for each accession. The approximate size of the alleles was determined by comparison of mobility in the gel with the molecular size marker GeneRuler™ 50bp DNA Ladder (Biolabs).

For the quantification of genetic diversity of *S. tuberosum* and *S. fernandezianum* studied, the following parameters were calculated: total alleles number, average number of alleles per locus, polymorphic information content (PIC), and genetic distance between pairs of genotypes. Cluster analysis was then carried out.

### 2.7.1.- Allelic characterization.

The total number of alleles is the sum of all alleles detected in all loci. The average number of alleles per locus is the sum of all detected alleles in all loci, divided by the total number of loci (formula 1), where,  $K$  = number of loci and  $n_i$  = number of alleles detected per locus. This measure provides information about the level of polymorphism.

$$n_{\cdot} = \frac{1}{K} \sum_{i=1}^K n_i \quad (\text{Formula 1})$$

### 2.7.2.- Polymorphic information content (PIC).

For the measurement of each marker locus polymorphism, the polymorphic information content (PIC) for each SSR was calculated according to the formula 2. Were  $p_i$  is the frequency of the  $i^{th}$  allele detected over all accessions and  $N$  represents the total number of alleles observed at the locus (Nei, 1973). Polymorphic information content (PIC) is a parameter frequently used to measure the discriminatory power of loci and their values vary between 0 and 1.

$$\text{PIC} = 1 - \sum_{i=1}^N (p_i)^2 \quad (\text{Formula 2})$$

In the cases of monogenic and tetragenic profiles the relation between the observed genotype and the allelic composition was clear and non ambiguous. As for digenic and trigenic profiles the different dosages of the alleles were not easy to evaluate on the gels. So we were unable to discriminate digenic simplex from digenic duplex or to determine which allele should be recorded as double dose in trigenics. We decided to approximate the allele frequencies by giving the same weight to the different recorded alleles, one half (1/2) in digenic profiles and one third (1/3) in trigenic. This approximation contributed to slightly homogenize the allelic frequencies estimations towards a more even distribution. As a consequence the PIC values we calculated were somewhat overestimated.

### 2.7.3.- Genetic similarity and cluster analysis.

The bands of selected primer obtained for each clone were identified and ordered in accordance with their molecular weight, the first being the smallest size band. The band pattern obtained was translated into binary matrix of 0 and 1 (Mohammadi and Prasanna, 2003). Genetic distances between individuals were calculated using the similarity coefficient based on presence or absence of alleles. Genetic analysis was performed by using the DARwin 5.0 program (Pierrer and Jacquemoud, 2006). A dissimilarity matrix was calculated by using Sokal and Sneath 2 coefficient (formula 3). This index gives greater weight to mismatches and do not take into account the joint absences.

$$D_{ij} = \frac{2(b + c)}{a + 2(b + c)}$$

(Formula 3)

With “i” and “j” the index of the two compared genotypes, “a” the number over all loci of cases when an allele is present in both genotypes, “b” and “c” the number of cases when an allele is present in one genotype and absent in the other.

A bootstrap analysis was conducted using 1000 replicates with DARwin software, with 60% of minimal proportion of valid data required for each unit pair. The dendrogram was built by using the Neighbor – joining method (Saitou and Nei, 1987). The Neighbor-joining method estimates phylogenetic trees attempting to find a tree that is usually close to the true phylogenetic tree. In the present work the tree was rooted using *S. fernandezianum* as outgroup. This method is proposed for reconstructing phylogenetic trees from evolutionary distance data. The principle of this method is to find pairs of operational taxonomic units (OTU) that minimize the total branch length at each stage of clustering of OTUs starting with a starlike tree. The branch lengths as well as the topology of a parsimonious tree can quickly be obtained by using this method.

## **2.8.- Comparison of allelic richness of samples of different sizes.**

Allelic richness was computed using the rarefaction method as described in Hurlbert, (1971) when samples sizes are unequal (formula 4). We adapted this method to population genetics according to the proposed for El Mousadik and Petit, (1996). The allelic richness  $r(n)$  expected at a locus in a sample of size  $n$  from a population of size  $N$  is:

$$r(n) = \sum_i [1 - \left(\frac{n}{N-N_i}\right)/\binom{n}{N}] \quad (\text{Formula 4})$$

Where  $N_i$  represent the number of occurrences of the allele  $i$  among the  $N$  sampled genes. We assume that each individual represents a sample of four genes (=absence of inbreeding) and the total population is represented by (30+9) varieties tested. Where :

$$\begin{aligned} N &= (39 \times 4) = 156 \\ n_{\text{nat}} &= (30 \times 4) = 120 \\ n_{\text{com}} &= (9 \times 4) = 36 \end{aligned}$$

## **III.- RESULTS**

### **3.1.- DNA extraction and PCR amplification products.**

In general, for most genotypes the DNA was intact and of good quality (Annexe 2). From the total SSR markers used, only seven markers showed up scoreable PCR products (Annexe 3). The other SSRs were discarded due to failure in several assay or because the band were weak and presented unclear pattern (Annexe 4).

### **3.2.- Characteristics of SSR markers.**

From the initial set of SSRs primers, a set of seven was finally used to amplify the DNA of the complete potato collection (Table 2 and 3). The DNA profiles on the “0” and “1” matrixes obtained for each SSRs locus.

The SSR1 marker revealed the presence of 8 alleles found (Annexe 5), many of them were quite common to all materials tested. Allele 8, was present in 93.3% of native accessions and 100% of commercial cultivars. Allele 5 was present in 73.3% of the native accessions and in 77.7% of commercial cultivars. The allele identified as 1, of approximately 172bp, was

found exclusively in the UCT-30Ño accession. For the STM marker 2005, the alleles identified as 7, 8, 10 and 12, were present in respectively 93%, 68%, 55% and 82% of native accessions. Whereas alleles 8 and 10 were present respectively in 100% and 90% of the commercial cultivars. For this marker, allele 5 of approximately 150bp, was unique to the diploid species of *Solanum fernandezianum* (Annexe 6). The marker STM1097, revealed the presence of 5 alleles which were quite common in all materials tested. Allele1, of approximately 224bp, was found exclusively in two native accessions, these accessions were UCT-25Gñ and UCT-23cab (Annexe 7). The marker LEMALX, showed a total of 12 alleles between 120 and 140bp for the tetraploid material. Alleles 4, 5, 6, 7 were present in 63%, 30%, 50% and 26% of the native accessions. The alleles 6, 8 and 10 were present in 100%, 100% and 56% of commercial cultivars. Moreover, a 12<sup>th</sup> allele identified as 1 of approximately 118bp was unique to the diploid species *Solanum fernandezianum* (Annexo 8). The marker STI 0030, was a very polymorphic marker, which revealed the presence of 9 alleles. The most common alleles in the native material were 8, 3 and 9 with an occurrence of 67%, 40% and 30% respectively.. In the commercial cultivars the most common alleles were 8, 1 and 2 present in 90%, 56% and 56% of the materials. Allele 5, approximately, 98bp, was unique to the species *Solanum fernandezianum* (Annexe 9).

For the STM 2020 marker, allele 6 of approximately 156 bp was unique to native potato accessions and was present in 33% of them. Another fairly common allele, although not exclusive of the native material, was the allele 4. Alleles 3, 4 and 5, were present in 56%, 33% and 56% of commercial cultivars included in the study. Allele1, of approximately 140bp, was present uniquely in the diploid species *Solanum fernandezianum*. For STG 0016 marker, the allele 2, 3, 6. 7 and 11 were unique to native potato materials. The remaining alleles were common throughout the material evaluated.

### **3.3.- Genetic diversity analysis.**

To assess the genetic diversity of the accessions of the species *S. tuberosum* spp *tuberosum* and *S. fernandezianum*, we calculated the parameters of total number of alleles, number of alleles per locus, polymorphic content and genetic distances.

#### 3.3.1.-Total alleles number.

*S. tuberosum* spp *tuberosum* and *S. fernandezianum* material evaluated using seven microsatellite markers (SSRs) was comprised of native potato accessions collected in southern Chile, represented by a group of native varieties of the Chiloé Archipelago, a group

of commercial cultivars and the diploid species *Solanum fernandezianum*. A total of 64 allelic variants were observed in the complete potato collection (Table 6).

**Table 6.** Total number and approximate size of alleles.

Locus SSR	Alleles	
	Number	Aproximate fragment size (bp)
STM 2020	6	160-140
SSR 1	8	220-172
STM 2005	12	193-110
LEMALX	12	140-118
STM 1097	5	280-224
STG 0016	12	166-125
STI 0030	9	112-85
Total	64	
Average	9,16	

The group exhibiting the highest number of alleles was the native varieties, with 57 alleles, corresponding to 89% of total alleles detected. The group of commercial cultivars generated a total of 39 alleles, corresponding to 60.9% of the total found. 21 alleles were found to be unique to the group of native potatoes that corresponded at 33% of the total number of alleles, while 34 of them (53% of the total) were shared with commercial cultivars included in the study. Only 5 alleles (8% of the total) were specific to the group of commercial varieties compared with the native potatoes. This indicates that the native material of Chiloe Archipelago may constitute an interesting source of alleles of the species *Solanum tuberosum* spp. *Tuberosum*.

*Solanum fernandezianum* species generated a total of 8 alleles, corresponding to 12.5% of total alleles found. Of these, 2 alleles were shared with the native material of Chiloe and 2 different alleles were shared with the commercial cultivars and not with the native material, while the other 4 alleles were unique and exclusive alleles of the diploid species, which is endemic to archipelago Juan Fernandez (Table 7).

**Table 7.** Total and average number of alleles, percentage of total number and exclusive alleles for native accessions, cultivars and *Solanum fernandezianum*.

Groups	Total alleles number	Average alleles number	Percentage of total	Exclusive alleles for group
Native accessions	57	8,14	89,0	21
Commercial cultivars	39	5,57	60,9	3
<i>Solanum fernandezianum</i>	8	1,60	12,5	4

### 3.3.2.- Average of alleles number per locus.

The SSRs loci generated an average of 9.16 alleles / locus. The number of alleles obtained per marker ranged between 5 and 12 for STM1097 and LEMALX, respectively. The native group of accessions averaged 8.14 alleles per locus, commercial cultivars 5.57 alleles per locus, and the diploid species *Solanum fernandezianum*, averaging 1.6 alleles per locus.

The markers with the highest number of alleles on native potato accessions corresponded to the locus STG0016 and LEMALX with 12 and 10 alleles respectively (table 8). On the commercial cultivars, the markers STG0016, LEMALX and STM2005 were those who generated the greatest number of alleles, reaching 7 alleles each. Allelic richness observed at the different loci was correlated between the two tetraploid origins ( $r=0.91$ ,  $Pvalue_{5\ dof} = 0.0024$ ), which is consistent with the already known results that allelic diversity is highly dependent on the SSRs marker loci and motives. *S. fernandezianum* was found heterozygous at three loci (STM 2005, LEMALX and STI 0030), homozygous at two loci (STM 2020 and STG 0016) and no band was amplified at the two remaining loci (SSR1 and STM 1097) (Table 8).

The lower number of alleles found in commercially available cultivars in southern Chile relative to the native material can be interpreted as a reduction of variability associated with the selection process which has been subjected in conventional breeding programs in Europe and Chile. But it can also be due, at least partly, to the different sizes of the samples studied (30 native varieties vs 9 commercial).

**Table 8.** Number of alleles per locus of accessions native commercial cultivars and *Solanum fernandezianum*.

Locus SSR	Accessions Native	Commercial	<i>Solanum</i>
		cultivars	<i>fernandezianum</i>
STM 2020	5	4	1
SSR 1	8	5	0
STM 2005	9	7	2
LEMALX	10	7	2
STM 1097	5	3	0
STG 0016	12	7	1
STI 0030	8	6	2

### 3.3.3.-Polymorphic information content (PIC).

In table 9 is shown the polymorphic information content (PIC) for data obtained with the selected 7 SSR markers on accessions and commercials potatoes cultivars. The markers were scored across all cultivar groups. Approximate PIC values of SSR markers ranged from 0,63 to 0, 89.

**Table 9.** SSR loci used to distinguish the 40 genotypes of potatoes: number of alleles, and PIC.

Locus	Repeat motif	Number of	PIC
		alleles	Aproximate
STM2020	(TAA)	6	0.76
SSR1	(TCAC)	8	0.77
STM2005	(CTGTTG)	12	0.78
LEMALX	(ATT)	12	0.87
STM1097	(CGTTT)	5	0.63
STG0016	(AGA)	12	0.89
STG0030	(ATT)	9	0.83

Diversity is a value that depends on the number of alleles found and the frequencies of them. Based on the PIC formula, a group with a high degree of diversity is characterized by a high number of alleles at similar frequencies. The marker STM 1097 was the one with the lowest diversity for all groups, with a PIC value of 0.63 in the native material and 0.44 in the

commercial. Conversely, the more diverse was the STG0016 marker with a PIC of 0.87 and 0.80 in the native material and trade respectively (Table 10). The group with the highest PIC was the native potato accessions collected in the archipelago of Chiloe, followed by those belonging to commercial cultivars currently in cultivation at the continental level in southern Chile. High PIC values found indicate that the native material Chiloe corresponds to low related material and represents a wide genetic diversity.

The high values of PIC, the total number of alleles and alleles per locus found, and unique restricted alleles, show remarkable value of the native material of Chiloe, which reinforces the need to evaluate and use these valuable genetic resources. On the other hand, the sole and exclusive alleles found for the species *Solanum fernandezianum*, evidenced the original genotype status of this endemic species of Juan Fernández Archipelago, assessment should be strengthened, because at present this species is considered endangered in the country.

**Table 10.** Polymorphic information content at seven microsatellites markers for native accessions and commercial cultivars.

Material groups	STM 2020	SSR1 2005	STM LEMALX	STM 1097	STG 0016	STI 0030	Average
Native accesiones	0.70	0.79	0.77	0.86	0.66	0.87	0.81
nativas							0.78
Commercial cultivars	0.68	0.68	0.68	0.79	0.44	0.80	0.77
							0.69

### 3.3.4.-Genetic distance levels.

The longest distance was between the species *S. fernandezianum* with native accessions and commercial cultivars (Figure 1). The average genetic dissimilarity between *S. fernandezianum* and the accessions of *S. tuberosum* was 0.96, ranging from 0.90 to 1.00. Accessions UCT-34Cor and UCT-26Ach diverged markedly from the rest of native accessions and commercial material included in this study. The average genetic dissimilarity among the accessions of native potato evaluated was 0.78, with the values ranging from 0.12 between UCT-10MgL and UCT-1Ma to 0.96 between UCT-34Cor and UCT30Ño (Figure 1) . The average genetic dissimilarity among commercial varieties was estimated a little lower to 0.69 but it remained quite high, which proved a broad genetic base of the commercial varieties cultivated in the south of Chile. The minimum and maximum dissimilarities among commercial varieties was evaluated to 0.35 and 0.84 respectively. The

average dissimilarity between native and commercial material was evaluated to 0.84 with a minimum of 0.55 and a maximum of 0.96.

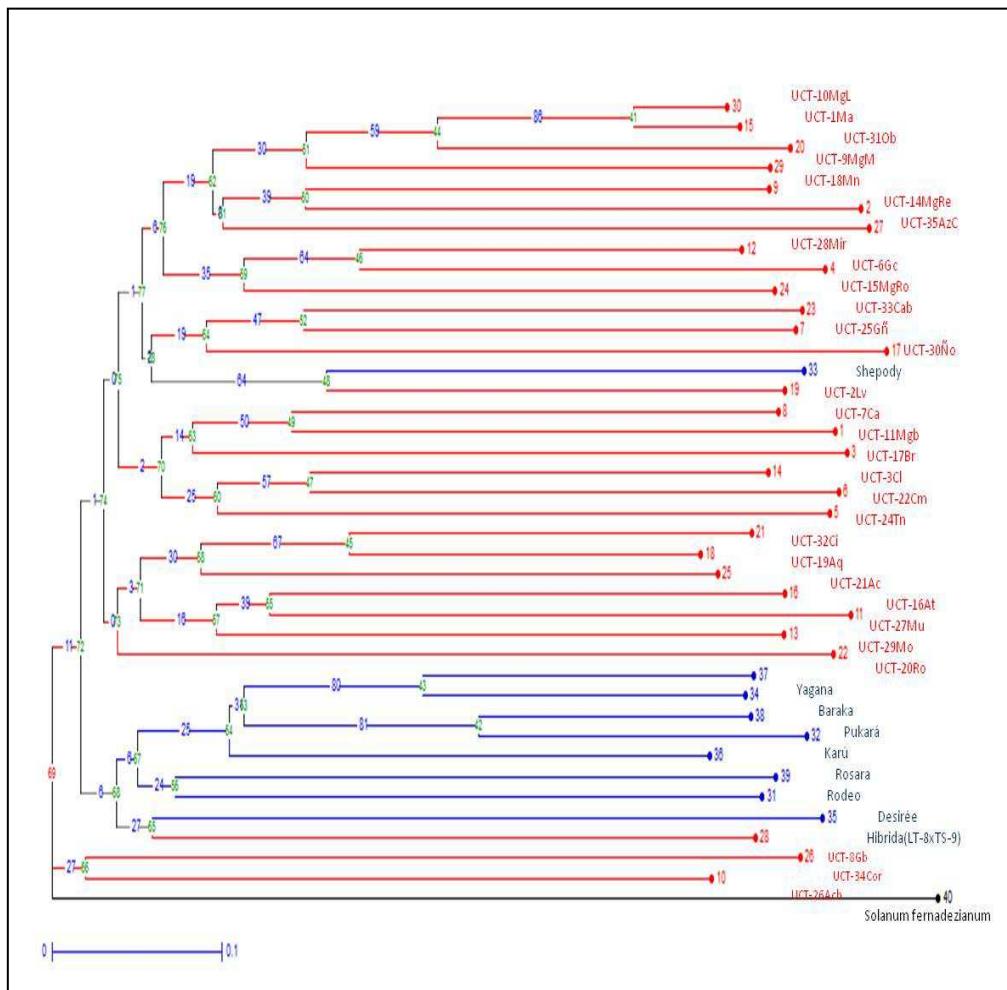
	UCT-11Mgb	UCT-14MgRe	UCT-17Br	UCT-6Gc	UCT-24Tn	UCT-22Cm	UCT-25Gr	UCT-22cm	UCT-25Gr	UCT-27Mu	UCT-28MnR	UCT-18Mn	UCT-29Mol	UCT-3CI	UCT-1Ma	UCT-16At	UCT-30No	UCT-19Aq	UCT-21Lx	UCT-33Cab	UCT-15MgRo	UCT-21Ac	UCT-34Cor	UCT-35Azc	UCT-8Gb	UCT-9MgBm	UCT-10Mgb	Désirée	Karú	Shepody	Baraka	Hibrida	Rosara	Yagana	Pukará	Rodeo	S. fernand.	
UCT-11Mgb	0.77																																					
UCT-14MgRe	0.76	0.80																																				
UCT-17Br	0.72	0.79	0.78																																			
UCT-6Gc	0.73	0.81	0.75	0.76																																		
UCT-24Tn	0.76	0.83	0.83	0.78	0.69																																	
UCT-22Cm	0.78	0.80	0.82	0.78	0.77	0.71																																
UCT-25Gr	0.80	0.80	0.82	0.78	0.77	0.71																																
UCT-7Ca	0.61	0.75	0.74	0.74	0.84	0.78	0.73																															
UCT-18Mn	0.82	0.60	0.81	0.72	0.73	0.80	0.67	0.76																														
UCT-26Ach	0.76	0.83	0.86	0.85	0.90	0.91	0.88	0.78	0.83																													
UCT-27Mu	0.84	0.84	0.84	0.86	0.91	0.86	0.84	0.83	0.84	0.86																												
UCT-28MnR	0.74	0.77	0.86	0.50	0.78	0.72	0.71	0.63	0.65	0.80	0.77																											
UCT-29Mol	0.89	0.78	0.81	0.89	0.90	0.88	0.88	0.83	0.82	0.71	0.67	0.81																										
UCT-3CI	0.73	0.76	0.80	0.71	0.72	0.58	0.78	0.79	0.77	0.85	0.72	0.70	0.72																									
UCT-1Ma	0.84	0.76	0.76	0.70	0.81	0.86	0.64	0.79	0.52	0.79	0.87	0.63	0.81	0.80																								
UCT-16At	0.88	0.87	0.71	0.86	0.81	0.83	0.82	0.79	0.72	0.86	0.65	0.77	0.71	0.72	0.71																							
UCT-30No	0.86	0.85	0.87	0.87	0.89	0.88	0.71	0.81	0.83	0.87	0.88	0.79	0.86	0.87	0.78	0.88																						
UCT-19Aq	0.79	0.78	0.81	0.77	0.74	0.81	0.81	0.68	0.70	0.77	0.78	0.61	0.62	0.74	0.69	0.63	0.83																					
UCT-2Lx	0.83	0.82	0.87	0.77	0.79	0.81	0.75	0.84	0.79	0.77	0.85	0.67	0.79	0.74	0.69	0.82	0.80	0.76																				
UCT-31Ob	0.86	0.82	0.77	0.77	0.79	0.88	0.69	0.77	0.88	0.81	0.86	0.82	0.85	0.69	0.76	0.72	0.76																					
UCT-32Ci	0.83	0.82	0.81	0.79	0.81	0.82	0.63	0.75	0.77	0.78	0.67	0.73	0.78	0.78	0.69	0.86	0.44	0.83	0.72																			
UCT-20Ro	0.88	0.90	0.88	0.86	0.82	0.84	0.86	0.76	0.82	0.89	0.88	0.74	0.83	0.81	0.84	0.81	0.93	0.75	0.83	0.86	0.75																	
UCT-33Cab	0.79	0.83	0.88	0.77	0.87	0.82	0.58	0.67	0.84	0.82	0.89	0.69	0.84	0.83	0.74	0.86	0.79	0.71	0.67	0.80	0.80	0.80																
UCT-15MgRo	0.81	0.80	0.86	0.67	0.88	0.88	0.79	0.76	0.77	0.71	0.80	0.59	0.76	0.77	0.67	0.83	0.85	0.70	0.74	0.74	0.74	0.84	0.74															
UCT-21Ac	0.88	0.77	0.81	0.80	0.78	0.80	0.71	0.76	0.69	0.80	0.84	0.70	0.73	0.81	0.67	0.77	0.88	0.58	0.75	0.75	0.75	0.65	0.78	0.76	0.73													
UCT-34Cor	0.88	0.94	0.92	0.86	0.92	0.90	0.92	0.94	0.88	0.79	0.88	0.86	0.86	0.87	0.91	0.91	0.96	0.88	0.89	0.94	0.88	0.88	0.91	0.83	0.81													
UCT-35Azc	0.91	0.72	0.88	0.83	0.89	0.87	0.80	0.86	0.74	0.86	0.90	0.82	0.84	0.87	0.67	0.84	0.86	0.78	0.79	0.75	0.86	0.91	0.80	0.81	0.78	0.96												
UCT-8Gb	0.77	0.90	0.86	0.88	0.81	0.83	0.77	0.83	0.84	0.79	0.90	0.84	0.85	0.76	0.80	0.84	0.82	0.85	0.82	0.78	0.85	0.88	0.82	0.76	0.81	0.83	0.90											
UCT-9MgM	0.84	0.80	0.84	0.75	0.81	0.85	0.79	0.83	0.60	0.83	0.87	0.68	0.81	0.80	0.48	0.71	0.88	0.63	0.74	0.63	0.78	0.81	0.69	0.67	0.72	0.88	0.72	0.80										
UCT-10Mgb	0.84	0.74	0.79	0.69	0.83	0.86	0.69	0.78	0.48	0.78	0.86	0.61	0.80	0.79	0.12	0.69	0.81	0.67	0.67	0.42	0.77	0.84	0.73	0.65	0.60	0.65	0.79	0.43										
Désirée	0.83	0.93	0.85	0.84	0.83	0.81	0.84	0.81	0.88	0.77	0.93	0.82	0.89	0.85	0.82	0.85	0.86	0.83	0.80	0.83	0.83	0.75	0.81	0.78	0.83	0.86	0.74	0.85	0.81									
Karú	0.92	0.96	0.86	0.93	0.87	0.88	0.89	0.90	0.89	0.88	0.89	0.91	0.84	0.88	0.86	0.82	0.90	0.87	0.84	0.87	0.87	0.83	0.80	0.89	0.78	0.86	0.85	0.65										
Shepody	0.90	0.80	0.83	0.83	0.81	0.85	0.79	0.86	0.81	0.83	0.87	0.81	0.81	0.76	0.76	0.80	0.85	0.82	0.55	0.78	0.82	0.88	0.78	0.76	0.77	0.80	0.76	0.74	0.78	0.78								
Baraka	0.91	0.93	0.81	0.82	0.87	0.88	0.84	0.87	0.83	0.85	0.79	0.80	0.77	0.76	0.75	0.75	0.87	0.84	0.73	0.77	0.81	0.86	0.84	0.89	0.87	0.75	0.79	0.74	0.81	0.67	0.65							
Hibrida	0.93	0.91	0.90	0.92	0.90	0.88	0.86	0.90	0.89	0.87	0.91	0.88	0.87	0.85	0.86	0.88	0.84	0.87	0.87	0.87	0.89	0.86	0.88	0.86	0.75	0.83	0.85	0.84	0.77	0.83	0.78							
Rosara	0.91	0.90	0.87	0.86	0.89	0.90	0.84	0.86	0.85	0.80	0.81	0.82	0.62	0.81	0.81	0.81	0.86	0.75	0.75	0.86	0.79	0.82	0.79	0.73	0.69	0.80	0.85	0.72	0.77	0.80	0.75	0.62	0.67	0.61	0.72			
Yagana	0.88	0.93	0.81	0.81	0.89	0.90	0.84	0.87	0.86	0.81	0.74	0.82	0.71	0.82	0.78	0.74	0.86	0.83	0.76	0.83	0.86	0.88	0.80	0.78	0.78	0.77	0.80	0.65	0.69	0.38	0.84	0.58						
Pukará	0.87	0.93	0.85	0.88	0.84	0.86	0.83	0.88	0.84	0.82	0.83	0.86	0.81	0.86	0.79	0.79	0.87	0.80	0.76	0.84	0.84	0.82	0.75	0.86	0.88	0.77	0.71	0.70	0.69	0.62	0.80	0.58	0.52					
Rodeo	0.92	0.93	0.89	0.85	0.84	0.83	0.82	0.88	0.77	0.88	0.89	0.84	0.81	0.80	0.86	0.83	0.80	0.85	0.85	0.82	0.81	0.83	0.80	0.86	0.70	0.69	0.71	0.82	0.68	0.74	0.74							
S. fernand.	0.97	1.00	1.00	0.97	1.00	0.97	0.97	0.97	0.94	0.90	0.95	0.97	0.95	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.94	0.94	0.97	0.95	0.93	0.97	0.94	0.97	0.94	0.97	0.94	0.95	0.92	0.95	0.95	0.92	0.95	0.95

**Figure 1.** Dissimilarity matrix using Sokal and Sneath 2 coefficient calculated with DARwin 5.0 program.

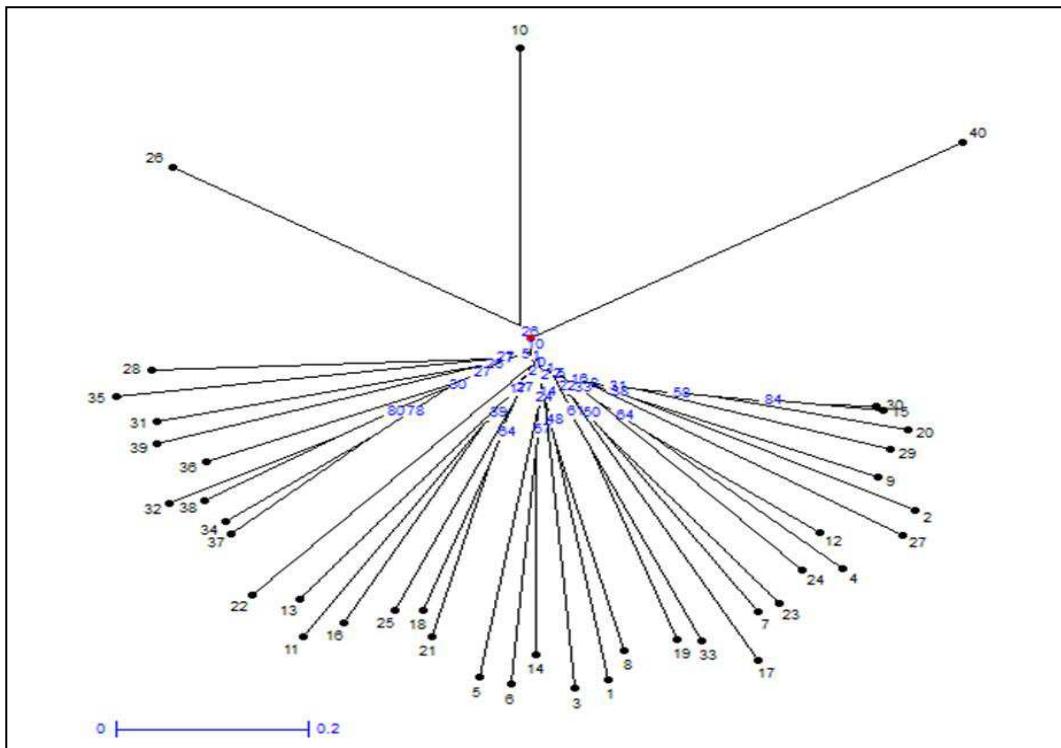
### 3.3.5.-Cluster analysis.

Based on the dissimilarity matrix the cluster analysis by Neighbor – joining method of the 30 accessions of native potato, 9 commercial cultivars and one diploid species, using the seven polymorphic loci resulted in the dendograms shown in Figures 2 and 3. Even if the different nodes of figures 2 and 3 were poorly supported by bootstrap values, the neighbor-joining clustering analyses almost completely separated native potatoes from the commercial potatoes and from *Solanum fernandezianum*. Most of the native potatoes were clustered together in accord with their geographical location, and thus, might have a genetic similarity background. Commercial cultivars, were clustered together in accord with their breeding program in Chile and Europe at the exception of Shepody cultivar (n° 33) which origin is from North America (Canada).

In addition, the high values of polymorphic information content (PIC) found within groups indicated that the native material of Chiloe as well as the sample of improved varieties corresponded to a barely related material and represented a wide genetic diversity. Few genotypes within each group were related. Based on the dissimilarity matrix (figure 9), the genotypes UCT-1Ma (15) and UCT-10MgL (30) were the closest (dissimilarity coefficient of 0.12). They grouped with two other close varieties UCT-310b (20) and UCT-9MgM (29). Interestingly based on morphological distances these four varieties were also grouped together (chapter III). Within the commercial varieties, Karú (32) and Pukará (38) on the one hand, and Baraka (34) and Yagana (37) on the other hand formed two different groups of close varieties. Other varieties appeared quite distant from one another.



**Figure 2.** Dendrogram rooted of accessions natives, commercial cultivars and *Solanum fernandezianum* product of the allelic variation of seven microsatellite markers. The colors are: black for *S. fernandezianum*, red for native potatoes and blue for commercial. Blue figures are bootstrap probabilities of the different nodes.



**Figure 3.** Radial representation of groups of native accessions, commercial cultivars and *Solanum fernandezian*, based on the allelic variation of seven microsatellite markers. Figures in blue represent the bootstrap probabilities of the different nodes.

#### **IV.-DISCUSSION**

In this study, 64 allelic variants were identified their approximate sizes ranging between 110 and 289 bp. The size of alleles found in this study for *Solanum tuberosum*, were within the range reported by other authors (Moisan-Thiery 2005; Mathias et al., 2007; Ghislain 2009b). 21 alleles were found specific of native accessions (*Solanum tuberosum* spp *tuberosum*) and only 3 were specific of the commercial varieties (Table 6). In this study, *Solanum fernandezianum* presented the lower number of alleles per locus compared with *Solanum tuberosum*. The reasons were the diploid status of the species, the only genotype in the sample and two loci (SSR 1 and STM 1092) out of the 7 SSR markers didn't amplified any detectable band. This lack of amplification may indicate divergences between *S. fernandezianum* and *Solanum tuberosum* leading to modification of the flanking sequences themselves and/or insertions of long enough DNA fragments that prevented any PCR amplification. Nevertheless 4 alleles out of the 8 (50%) found in this species were exclusive of *S. fernandezianum*.

Data obtained in the present work, showed that the number of alleles per locus ranged from 5 and 12 (Table 6). Ghislain et al., (2009b), reported a range from 2 to 21 for 742 potato landraces with 51 SSR markers. Mathias et al. (2007), found that 21 SSR markers showed up scoreable products and the allele number ranged between 2 and 17. Moreover, Marchezi et al., (2010), detected that number of alleles per locus varied from 1 to 27 with 127 alleles in total for 14 potato cultivars in Brazil. Feingold et al., (2005) obtained a range from 1 to 16 alleles using 61 microsatellites in 30 genotypes. McGregor et al. (2000) using five microsatellite loci detected 39 alleles in 39 genotypes. On the other hand, Martinez et al., (2010) found a total of 90 alleles with 16 markers, with markers ranging between 3 and 9 alleles.

The lowest number of alleles per locus observed in commercial cultivars could be explained by the smaller size of the sample of commercial varieties. Indeed the number of distinct alleles and the number of private alleles depend on sample size and they can be difficult to interpret when sample sizes differ across populations. Software based on the rarefaction method like ADZE (University of Michigan) or HP-Rare (University of Montana) should help to conclude, but we didn't use any yet.

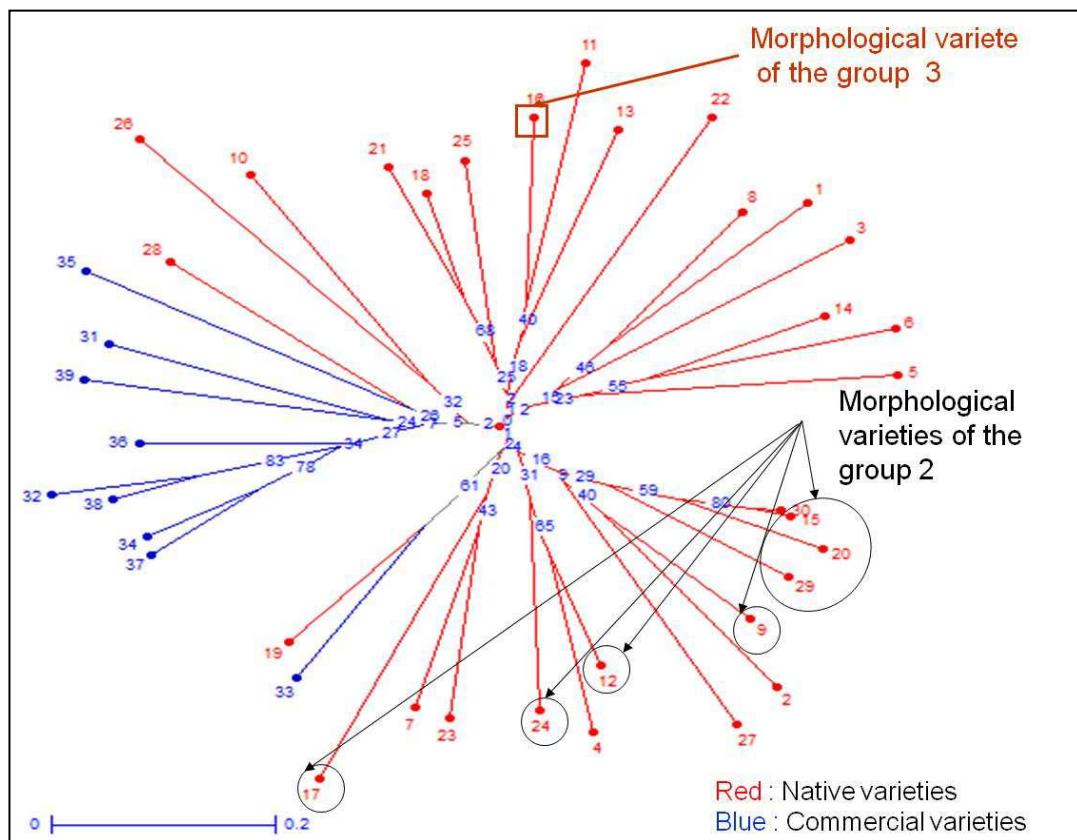
The polymorphic information content (PIC) of the SSR markers ranged from 0.63 to 0.89. The average PIC was 0.79 which confirms that SSRs markers are highly informative. These values are consistent with those reported by Martinez et al., (2010) and Ispizúa et al. (2005)

and higher than those published by Atencio *et al.*, (2010) and Ghislain *et al.*, (2009). Martinez *et al.* (2010) studied the genetic diversity of potato landraces of northwestern Argentina with four SSRs, they found PIC values which ranged from 0.80 to 0.92. Ispizúa *et al.*, (2005), with four SSRs found PIC values ranging from 0.80 to 0.92 for potato landraces of north-western Argentina. When the PIC values were analyzed for each SSR and for each local variety, some markers were more informative than others. Atencio *et al.*, (2010) evaluated the polymorphism of a local variety "Collajera" (*Solanum tuberosum* ssp. *andigena*) at the provincial level (Jujuy) versus Andean farmer level. They obtained PIC values from 0.72 to 0.31. On the other hand, Ghislain *et al.*, (2009), reported in 742 potato landraces PIC values of 0.250 to 0.88 evaluated with 51 SSR markers

Our results based on 7 SSRs markers showed a high discriminatory power, none of the 40 studied genotypes had the same profile. This is consistent with previous results of Marchezi *et al.*, (2010), who observed that a set of only two microsatellites was able to identify and differentiate 14 potato cultivars in Brazil. Reid and Kerr (2007) used six microsatellites to identify approximately 400 genotypes of potato. Our results also coincide with those reported by Moisan-Thiery *et al.*, (2005), who report that sequential amplifications with SSR1, STM2005, LEMALX, STM1097 and STM2020, all markers used in the present study, are able to complete discrimination between all the commercials cultivars (286) in France. Molecular markers based on Simple Sequence Repeats (SSR) are very efficient tool for potato identification and can be very useful for germplasm conservation and management.

#### **4.1.- Comparison between morphological and genetic diversity.**

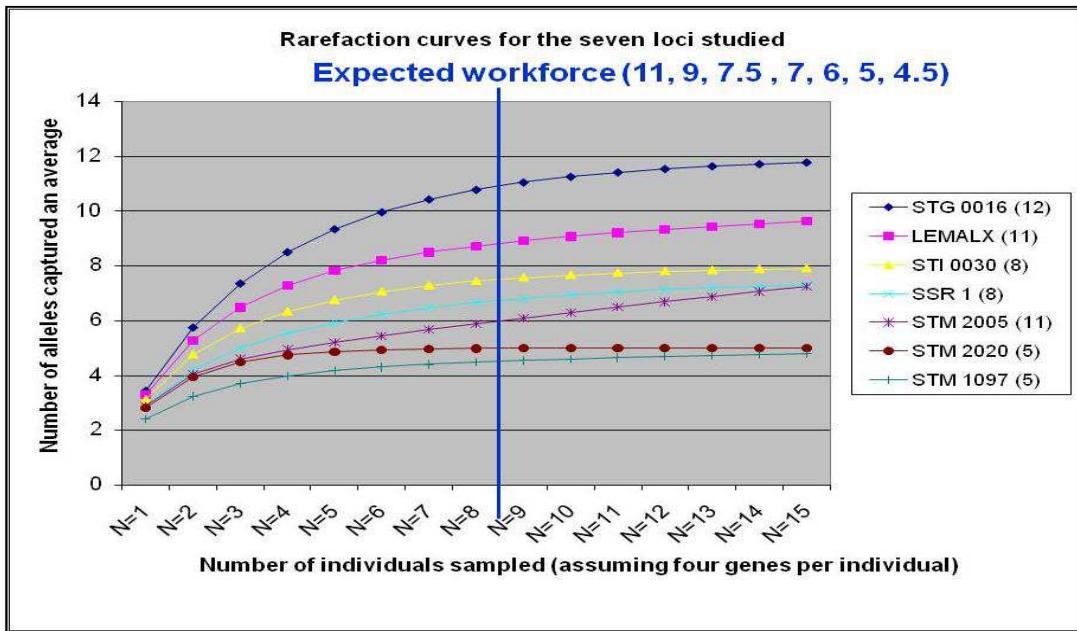
The present work is the first published result about SSRs fingerprint of the Chilean native potato germplasm we collected. In general, in agreement with other authors in potato, there is low coherence between morphological and genetic diversity (Spooner *et al.*, 2005b). Apart from the cluster [UCT-1Ma (15), UCT-10MgL (30), UCT-310b (20) and UCT-9MgM (29)] which coincide with a cluster based on morphological distances (chapter III), the dissimilarity matrix based on SSR markers gave additional and quite independent information compared with the morphological description of the native potatoes (Figure 4). This absence of concordance also has been observed by Federici *et al.* (2001), Roldán-Ruiz *et al.* (2001), Martínez *et al.* (2003), in varieties of weedy rice, ryegrass and azuki beans respectively. So, the genetic distance information reported in this study might be used by breeders when planning future crosses to combine interesting traits among these potato accessions native or commercial while minimizing relatedness between parents.



**Figure 4.** Comparison between morphological and genetic diversity

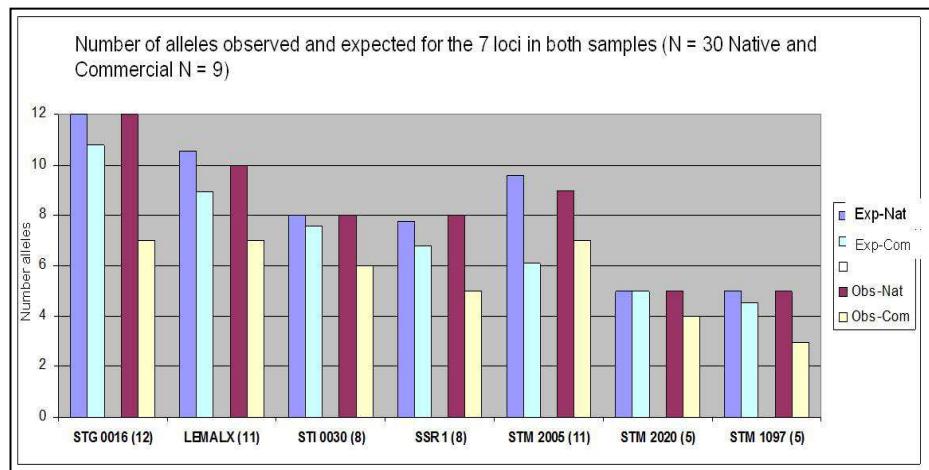
#### 4.2.- Comparison of allelic richness of samples of different sizes.

The figure 5 shows the rarefactions curves for the seven loci studied. It is noted that in the case of a sample of 9 individuals, the total number of alleles observed is higher than expected number of alleles at all loci tested.



**Figure 5.** Rarefacción curves for the seven loci evaluated.

On the other hand, the comparison between the number of alleles observed and expected for the commercial potato material (Figure 6) allows to point out that for six of the seven loci, the expected number of alleles exceeds the number of alleles observed, corresponded to the locus STM2005 exception. In the case of native accessions, only the locus SSR1 the number of alleles observed exceeded expected. For example, we noted that the locus of STG0016 12 alleles found, 5 (individual alleles as 2, 3, 6, 7 and 11) are exclusively present in native material of Chiloe. On the contrary, it has been observed in the STM2005 locus, that of the 12 alleles, 4 alleles (alleles individualized as 2, 3, 4 and 11) are exclusively present in the native potato material, whereas 2 allele (allele 1 and 6) are unique to the commercial potato material.



**Figure 6.** Number of alleles observed and expected for native and commercial potatoes.

In Table 11, shows the test of homogeneity of distributions allelic among native and commercial potatoes. The differences are highly significant, therefore reject the hypothesis of homogeneity between the two samples for five of the seven loci studied. The allelic richness of commercial varieties is lower than expected under the sole effect of the smaller size of sample, is therefore possible to determine that the commercial varieties do not have the same genetic variability that native varieties. On average, they have fewer alleles. For the alleles common to both sources the frequencies are significantly different between the two origins.

**Table 11.** Test of homogeneity of distributions allelic among natives and commercial potatoes.

LOCUS	Chi <sup>2</sup>	Pc
STG0016 (12>10)	33,6	1.03E-04
LEMALX (11>7)	26,2	2.02E-04
STI0030(8>7)	33,6	7.95E-06
SSR1 (8>5)	3,4	0.50
STM 2005 (11 > 4)	18,8	4.79E-04
STM 2020 (5 > 5)	38,8	7.80E-08
STM 1097 (5 > 4)	9,1	0.03

Tests performed on the alleles such as Ni> = 8 that is to say, p> 0.05.

## V.-REFERENCES.

- Agarwal, M., N. Shrivastava and H. Padh. 2008. Advances in molecular marker techniques and their applications in plant sciences. *Plant Cell Rep.* 27:617-631.
- Atencio, M., A. Clausen, V. Ispizua and S. Feingold. 2009. Collajera potato genetic diversity (*Solanum tuberosum* ssp. *andigena*) assessed with microsatellites in Argentina. In: VII Symposium on Genetic Resources for Latin America and the Caribbean. Pucon, Chile. Pp: 297-298.
- Ashkenazi, V., E. Chansi, U. Lavi., D. Levy., J. Hillel and R. Veilleux. 2001. Development of microsatellite markers in potato and their use in phylogenetic and fingerprinting analyses. *Genome*, vol. 44, no. 1, p. 50-62.
- Bassam, B. J., Caetano-Anolles and P.M. Gresshoff. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. *Annals of Biochemistry* 196:80-83.
- Benor, S., M. Zhang., Z. Wang and H. Zhang. 2008. Assessment of genetic variation in tomato (*Solanum lycopersicum* L.) inbred lines using SSR molecular markers. *Journal of Genetic and Genomics*. 35: 373-379.
- Bornet, B., F. Goraguier., G. Joly and M. Branchard. 2002. Genetic diversity in European and Argentinian cultivated potatoes (*Solanum tuberosum* subsp. *tuberosum*) detected by inter-simple sequence repeats (ISSRs). *Genome*, vol. 45, no. 3, p. 481-484.
- El Mousadik, A. and R. Petit. 1996. High level of genetic differentiation for allelic richness among populations of the argan tree (*Argania spinosa* (L.) Skeels) endemic to Morocco. *Theor. Appl. Genet.* 92: 832-839.
- Federici, M.T.; D. Vaughan; N. Tomooka; A. Kaga; X. Wang Wang; K. Doi; M. Francis; G. Zorrilla and N. Saldain. 2001. Analysis of Uruguayan weedy rice genetic diversity using AFLP molecular markers. *Electronical Journal Biotechnology*, vol. 4, no. 3, p. 130-145.
- Feingold, S., J. Lloyd., N. Norero., M . Bonierbale and J. Lorenzen. 2005. Mapping and characterization of new EST-derived microsatellites for potato (*Solanum tuberosum* L.) *Theor. Appl. Genet.* 111: 456-466.
- Ferreira M. E. and D. Grattapaglia. 1998. Introducción al uso de marcadores moleculares en el análisis genético. 1 ed. Brasilia. Ministerio da Agricultura e o Abastecimiento (M.A.), Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA), Centro Nacional de Pesquisa de Recursos Genéticos e Biotecnología (CENARGEN).
- Ghislain. M., D. Spooner., F. Rodriguez., F. Villamón., J. Nuñez., C. Vásquez., R. Waugh and M. Bonierbale. 2004. Selection of highly informative and user-friendly

- microsatellites (SSRs) for genotyping of cultivated potato. *Theor Appl. Gent.* 108:881-890. Doi:10.1007/s00122-003-1494-7.
- Ghislain. M., D. Andrade., F. Rodríguez., R. Hijmans and D. Spooner. 2006. Genetic analysis of the cultivated potato *Solanum tuberosum* L. Phureja Group using RAPDs and nuclear SSRs. *Theor Appl Genet.* 113:1515-1527.
- Ghislain. M., J. Nuñez. M del R. Herrera and D. Spooner. 2009a. The single Andigenum origin of Neo-Tuberosum potato materials is not supported by microsatellite and plastid marker analyses. *Theor. Appl. Genet.* 118:693-696.
- Ghislain. M., J. Nuñez. M del R. Herrera., J. Pignataro., F. Guzman., M. Bonierbale and D. Spooner. 2009b. Robust and highly informative microsatellite-based genetic identity kit for potato. *Mol. Breeding.* 23:377-388.
- Gong, Y., S. Xu., W. Mao., Q. Hu., G. Zhang., J. Ding and Y. Li. 2010. Developing new SSR markers from ESTs of pea (*Pisum sativum* L.). *Biomed & Biotechnol.* 11(9):702-707.
- Hijmans, R. J. and D. M. Spooner. 2001. Geographic distribution of wild potato species. *American Journal of Botany.*; vol. 88, no. 11, p. 2101-2112.
- Hurlbert, S. 1971. The nonconcept of species diversity: a critique and alternative parameters. *Ecology* 52:577-586.
- Ispizúa, V., I. Guma., S. Feingold and A. Clausen. 2007. Genetic diversity of potato landraces from northwestern Argentina assessed with simple sequence repeats (SSRs). *Genetic Resources Crop Evolution.* 54:1833-1848.
- Levinson, G. and G.A. Gutman. 1987. Slipped-strand mispairing: A major mechanism for DNA sequence evolution. *Mol. Biol. Evol.* \$:203-221.
- Marchezi, P., T. de Campos., A. Barbosa de Sousa., D. Sforca, G. Montan., and A. Pereira de Souza. 2010. Potato cultivar identification using molecular markers. *Pesq. Agropec. Bras., Brasília.* v.45, n.1, p.110-113
- Mathias, M., B. Sagredo and K. Kalazich. 2007. SSR molecular markers for identification of potato germplasm in the breeding program at INIA in Chile. *Agricultura Técnica (Chile)* 67(1):3-15.
- Martinez, L.; P. Cavagnaro; R. Masuelli and J. Rodriguez. 2003. Evaluation of diversity among Argentine grapevine (*Vitis vinifera* L.) varietes using morphological data and AFLP markers. *Electronical Journal Biotechnology*, vol. 6, no. 3, p. 241-250.
- McGregor, C., M. Greyling., C. Lambert., J. Louw and L. Warnich. 2000. A comparative assessment of DNA fingerprinting techniques (RAPS, ISSR, AFLP and SSR) in tetraploid potato (*Solanum tuberosum* L.) germplasm. *Euphytica*, vol. 113, p. 135-144.

- Milbourne, D., R. Meyer., A. Collins., L. Ramsay., C. Gebhardt and R. Waugh. 1988. Isolation, characterisation and mapping of simple sequence repeat loci in potato. Molecular and General Genetics, vol. 259, n°. 3, p. 233-245.
- Miller, J. T. and D. M Spooner. 1999. Collapse of species boundaries in the wild potato *Solanum brevicaule* complex (Solanaceae, sp. Sect. Petota): molecular data. Plant Systematic and Evolution, vol. 214, p. 103-130.
- Mohammadi, S.A. and B. M. Prasanna. 2003. Analysis of genetic diversity in crop plants – salient statistical tools and considerations. Crop Science 43:1235-1248.
- Moisan-Thiersy, M., S. Marhadour., M. Kerlan., N. Dessenne., M. Perramant., T. Gokelaere and Y. Le Hingrat. 2005. Potato cultivar identification using simple sequence repeats markers (SSR). Potato Research 48: 191-200.
- Mullis, K. B, and F. Falloona. 1987. Specific synthesis of DNA in vitro via polymerase chain reaction. Methods Enzymol 155:350–355.
- Nei. M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA. Vo.70 (12):3321-3323.
- Ortiz, R. and Z. Huaman. 1994. Inheritance of morphological and tuber characteristics. In: CAB International. Potato Genetics. Bradshaw, J. E. y Mackay, G. R. (Eds). Pp: 263-279.
- Ortiz, J., I. Aguimagalde and J. Martín. 2000. Varietal identification. P. 515-560. In: F. Nuez y J.M. Carrillo (eds). Los marcadores genéticos en la mejora vegetal. Editprial de la UVP, Valencia, España.
- Pierrer, X, .and J.P. Jacquemoud-Collet, 2006. DARwin software, (<http://darwin.cirad.fr/darwin/Home.php>) version 5.0.
- Raker, C. M. and D. M. Spooner. 2002. Chilean tetraploid cultivated potato, *Solanum tuberosum*, is distinc from Andean the populations: microsatellite data. Crop Science. vol. 42, p. 1451-1458.
- Reid, A. and E. M. Kerr. 2007. A rapid simple sequence repeat (SSR)-based identification method for potato cultivars. Plant Genetic Resources: Characterization and Utilization, vol.5, p7-13.
- Ritter, A. Aplicación de la biotecnología a la mejora genética de la patata. In: PATATA 2000. Vitoria-Gasteiz, España. 2000, p. 8.
- Ritter, E., F. Lucca., I. Sanchez., J. Ruiz de Galarreta., A. Aragones., S. Castañon., G. Bryan., R. Waugh., V. Lefebve., F. Rousselle-Bourgoise., C. Gebhardt, H. Van Eck, H., Van Os, J. Taco and J. Bakker. 2004. Recursos genómicos en la papa y posibilidades de su explotación. Suplemento Revista Latinoamericana de la Papa. p. 17.

- Roldán-Ruiz, I.; F. A. Van Eeuwijk; T.J. Gilliland; P. Dubreil; C. Dillman; J. Lallemand; M. De Loose and C.P. Baril. 2001 A comparative study of molecular and morphological methods of describing relationships between perennial ryegrass (*Lolium Perenne* L.) varietes. *Theoretical and Applied Genetics*, vol. 103, no. 8, p. 1138-1150.
- Saghai Maroof M. A., R. M. Biyashev., G. P. Yang., Q. Zhang and R. W. Allard. 1994. Extraordinarily polymorphic microsatellite DNA in barley: species diversity, chromosomal locations, and population dynamics. *Proc. Natl. Acad. Sci. USA*. 91:5466–5470.
- Saitou, N and M. Nei. 1987. The Neighbor-Joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*. 4(4):406-425.
- Schlotterer, C. and D. Tautz. 1992. Slippage synthesis of simple sequence DNA. *Nucleic Acids Res* 20:2211–2215.
- Spooner, D.M., I. Peralta. and S. Knapp. 2005a. Comparison of AFLPs with other markers for phylogenetic Inference in wild tomatoes (*Solanum* L. Section *Lycopersicon* [MILL.] Wettst). *Taxon*, vol. 54, p. 43-61.
- Spooner, D., K. Mclean., G. Ramsay., R. Waugh and G. Bryan. 2005b. A single domestication for potato based on multilocus amplified fragment length polymorphism genotyping. *Proceeding of the National Academy of Sciences*, vol. 102, no. 41, p. 14694–14699.
- Spooner, D., J. Nuñez,, G. Trujillo., M del R. Herrera., F. Guzman and M. Ghislain. 2007. Extensive simple sequence repeat genotyping of potato landraces support a major reevaluation of their gene pool structure and classification. *PNAS* 104 (49): 19398-19403.
- Stegemann, H. and V. Loeschecke. 1976. Index. *Europäischer Kartoffelsorten; Bestimmung durch elecktrophoretische Spektren*. Paul Parey, Berlín. p. 214.
- Sun, G., G. Wang-Pruski., M. Mayich and H. Jong. 2003. RAPD and pedigree-based genetic diversity estimates in cultivated diploid potato hybrids. *Theoretical and Applied Genetics*, vol. 107, no. 1, p. 110-115.
- Tautz, D. and M. Renz. 1984. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. *Nucleic Acids Res* 12(10):4127–4138.
- Van de Wiel, C., P. Arens and B. Vosman. 1999. Microsatellite retrieval in lettuce (*Lactuca sativa* L.). *Genome* 42:139–149.
- Wu, S. D. and K. S. Tanksley. 1993. Abundance, polymorphism and genetic mapping of microsatellites in rice. *Molecular and General Genetics*, vol 241:225–235.

## ANNEXES CHAPITRE IV.

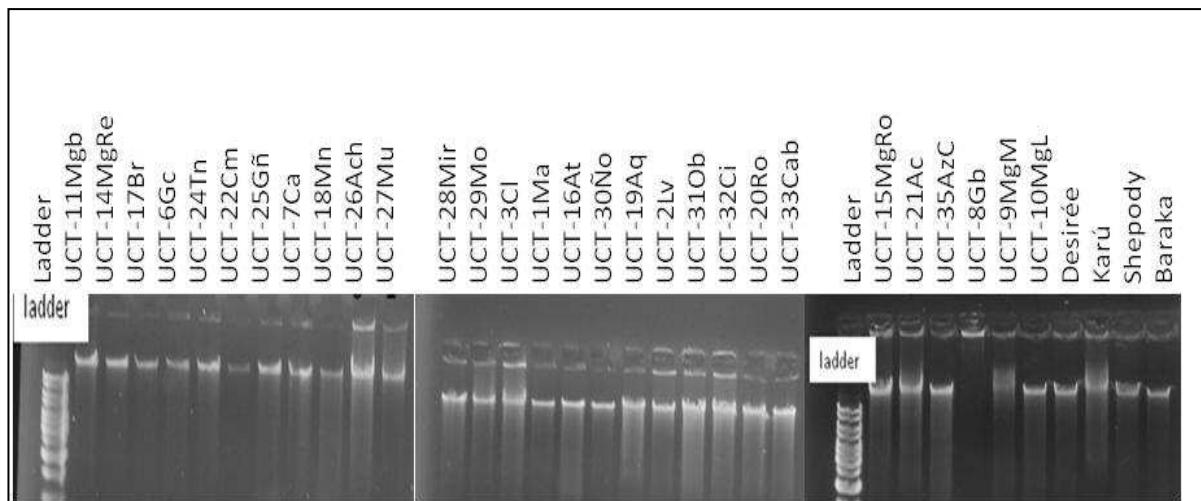
### ANNEXE 1.

Annealing temperatures and primer sequences for microsatellite.

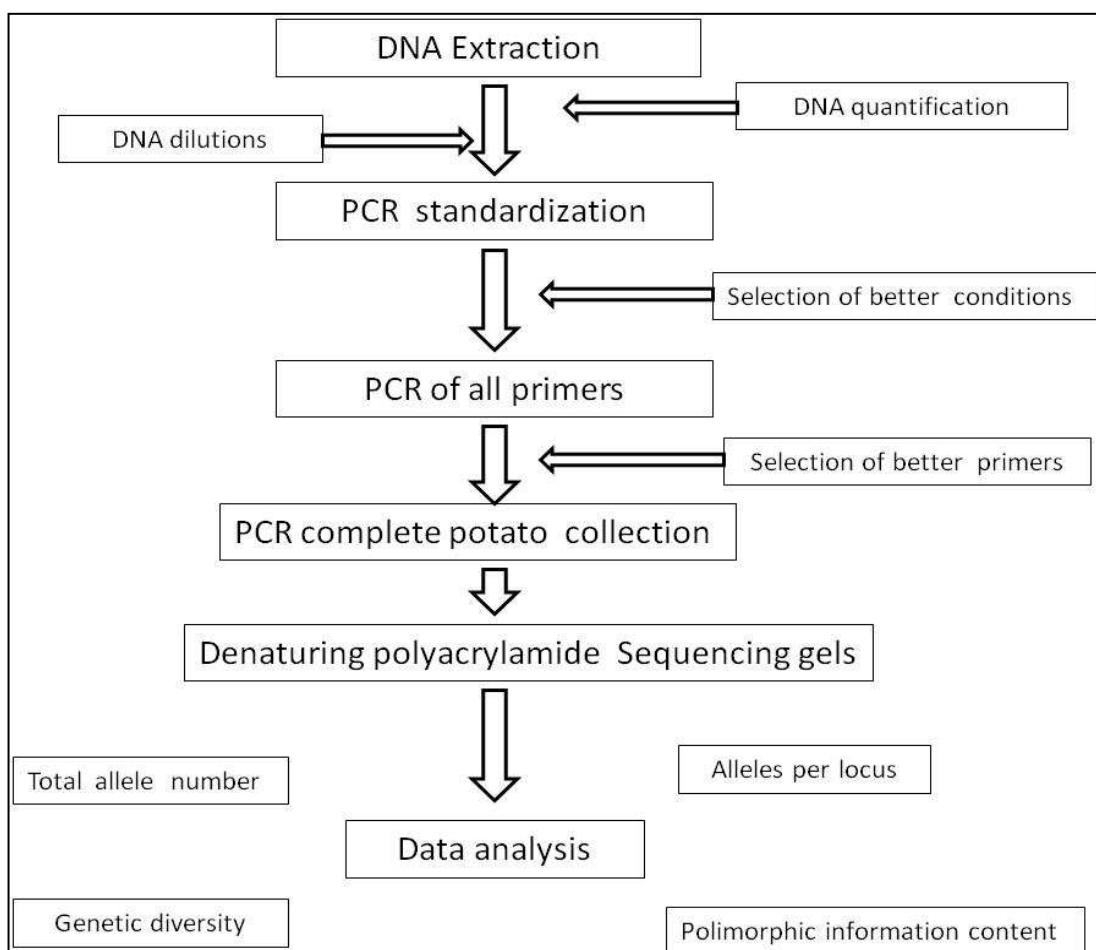
Locus	Annealing temperature (Ta °C)	Primer sequences “forward (F)” and “reverse (R)”
SSR1	54	F. 5'- GATGAGATGAGATATGAAACAAACG 3' R. 5'- CGCAATTCTCTTGCACACGTGTCACTGAAAC3'
STM2005	54	F. 5' - TTTAAGTTCTCAGTTCTGCAGGG 3' R. 5' - GTCATAAACCTTACCATGCTGGG 3'
LEMALX	54	F. 5'- CTCACCCCACAAAGAAAATTC 3' R. 5' - CTAACAAACATTGTACAACAATAATC 3'
STM1097	54	F. 5' - GTTCACAGCCTTCGTGAACG 3' R. 5' - ATTCAAACACTCAGCCAGCAGC 3'
STM2020	54	F. 5' - CCTTCCCCTTAAATACAATAACCC 3' R. 5' - CATGGAGAAGTGAACACGTCTG 3'
STM0019a	47	F. 5'- AATAGGTGTACTGACTCTCAATG 3' R. 5'- TTGAAGTAAAAGTCCTAGTATGTG 3'
STG0010	55	F. 5'- CGATCTCTGCTTGCAAGGTA 3' R. 5'- GTTCATCACTACCGCCGACT 3'
STG0016	53	F. 5'- AGCTGCTCAGCATCAAGAGA 3' R. 5'- ACCACCTCAGGCACTTCATC 3'
STI0003	54	F. 5'- ACCATCCACCATGTCAATGC 3' R. 5'- CTCATGGATGGTGTATTGG 3'
STI0030	60	F. 5'- TTGACCCTCCAACATATAGATTCTTC 3' R. 5'- TGACAACTTTAAAGCATATGTCAGC 3'
STM0031	57	F. 5'- CATA CGCACGCACGTACAC 3' R. 5'- TTCAACCTATCATTGTGAGTCG 3 '

## ANNEXE 2.

DNA of native potato accessions, immediately after its extraction.

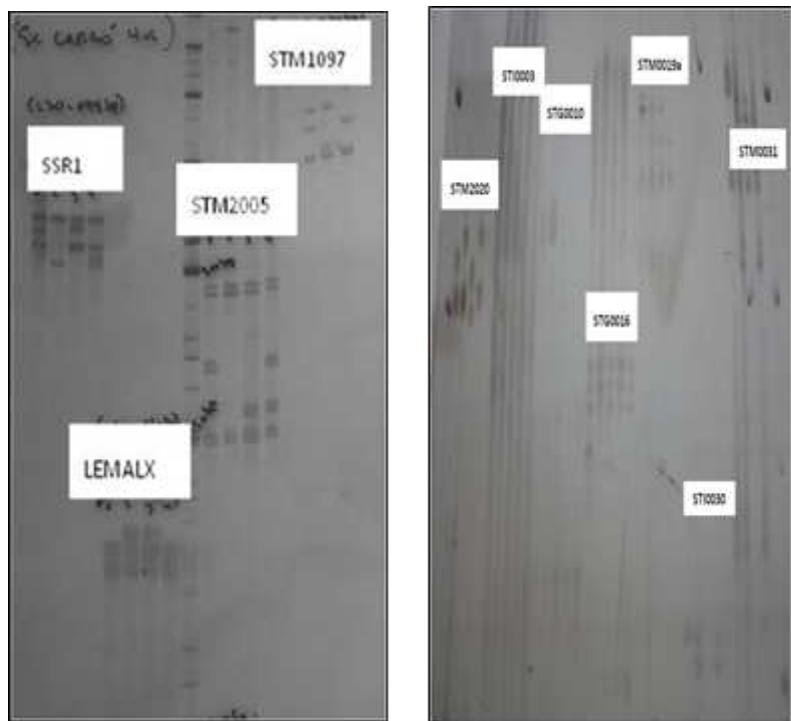


**ANNEXE 3.**  
Laboratory: Micosatellite markers protocol.



**ANNEXE 4.**  
**Standardization SSRs.**

- a) SSR1, STM1097, STM2005, LEMALX;  
b) STM2020, STI0003, STG0010, STG0016, STM0019a, STI0030, and STM0031

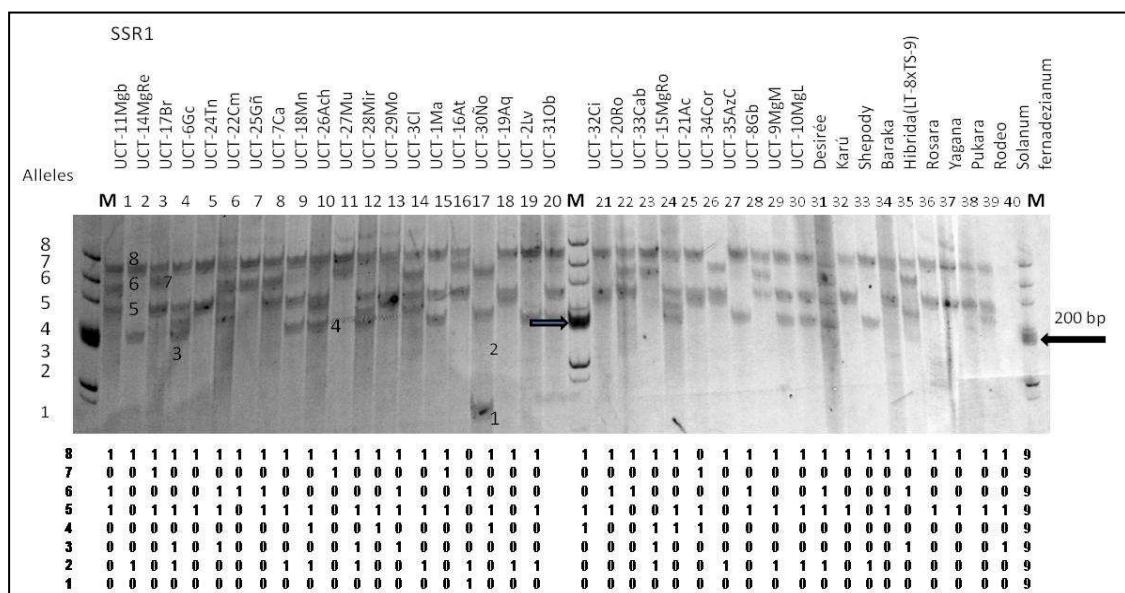


a)

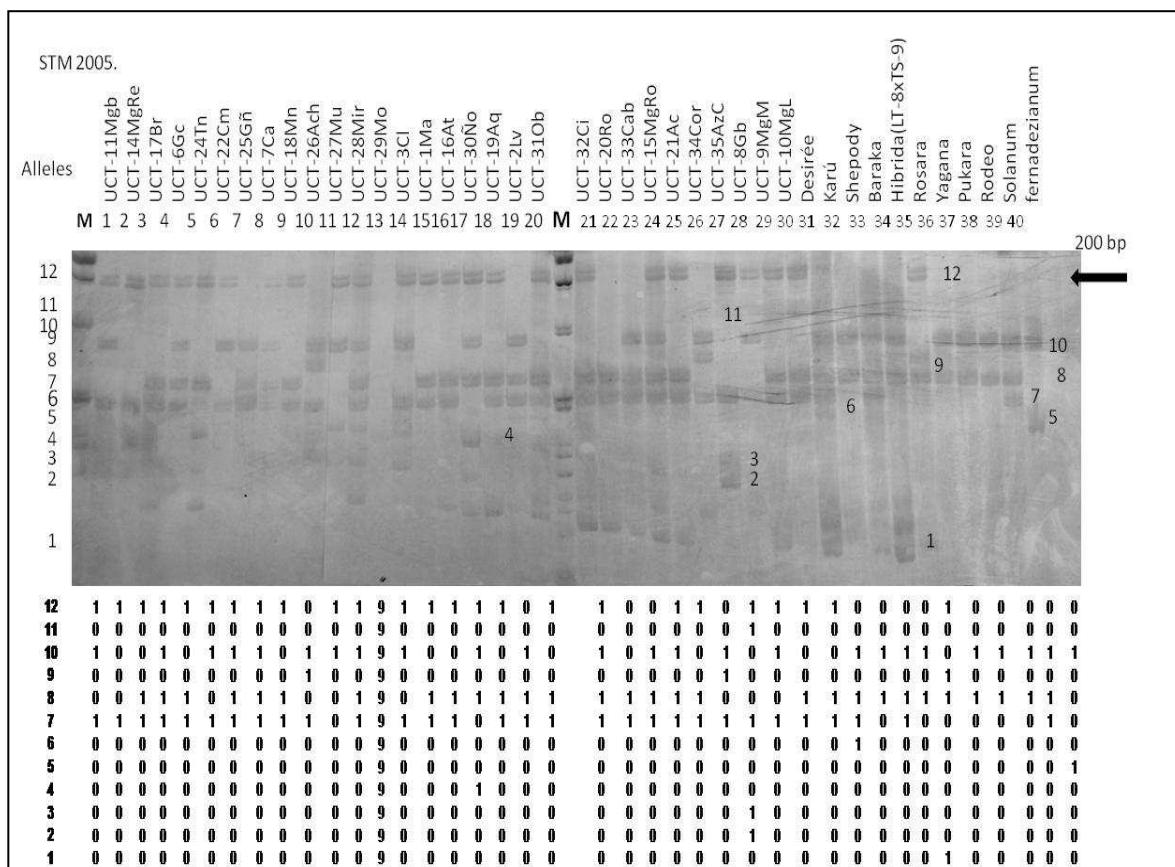
b)

### Annexe 5.

Amplified alleles, with microsatellite SSR1.

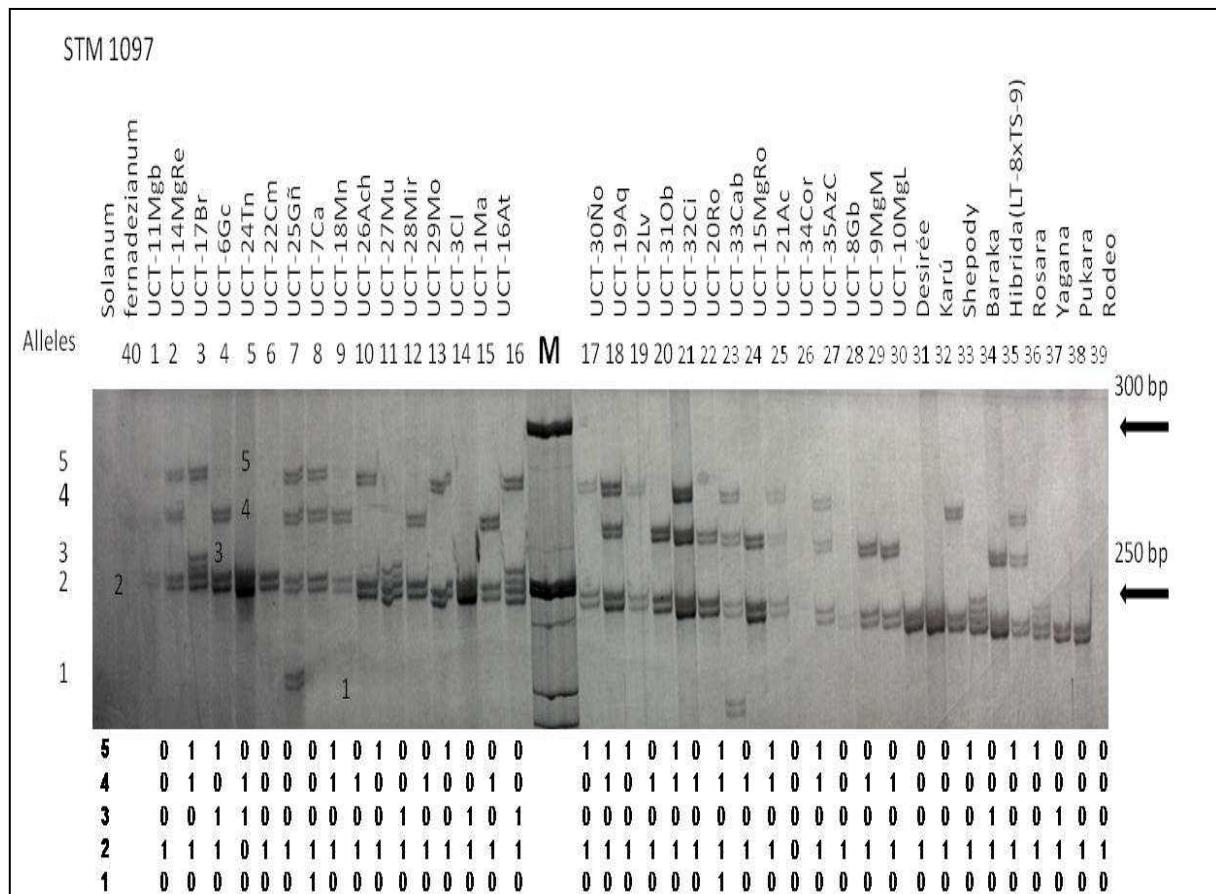


**ANNEXE 6.**  
Amplified alleles, with the microsatellite STM2005.



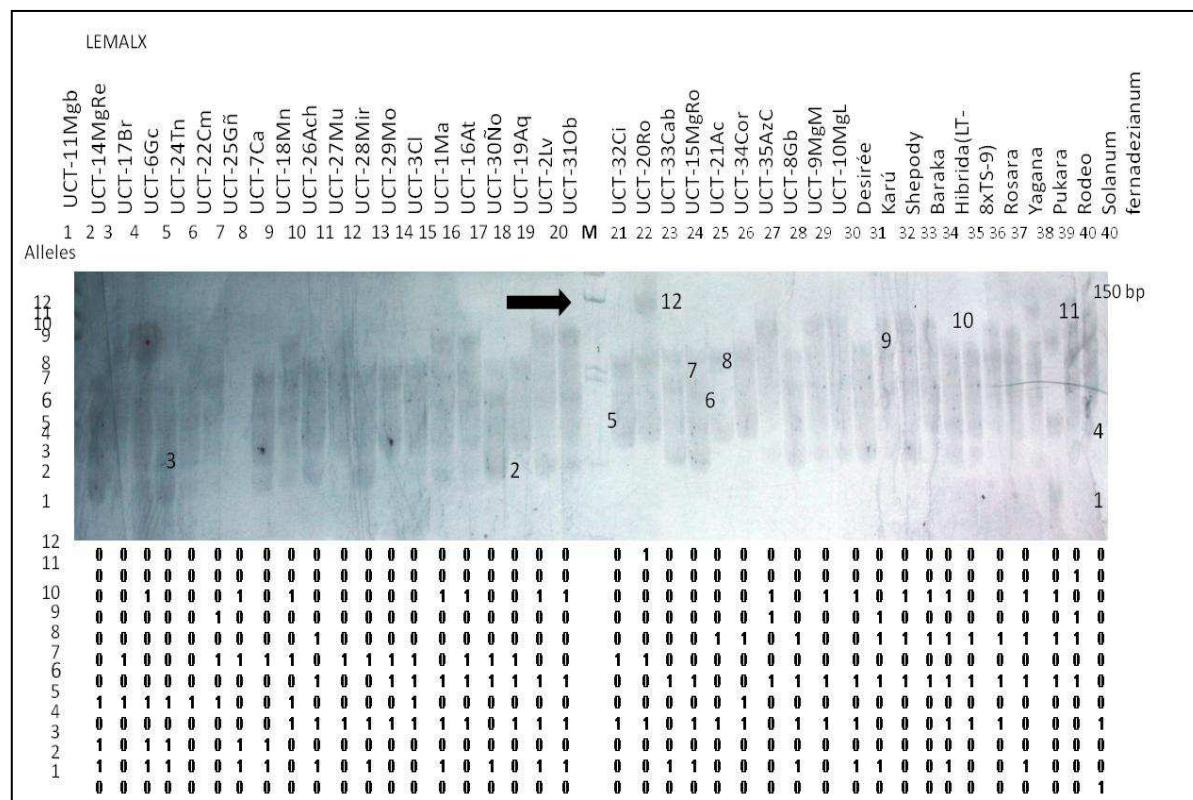
## ANNEXE 7.

Amplified alleles, with the microsatellite STM1097.

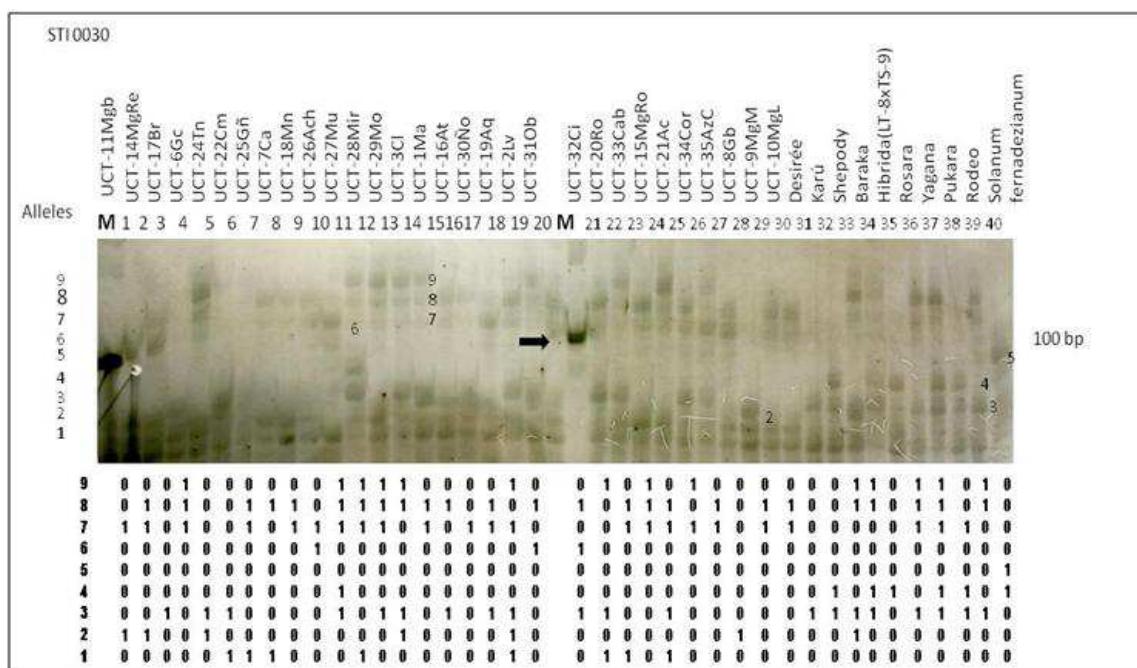


## ANNEXE 8.

Amplified alleles, with the microsatellite Lemalx.



**ANNEXE 9.**  
Amplified alleles, with the microsatellite STI0030.



**CHAPITRE V. ce chapitre correspond à l'article : “molecular description and similarity relationships among native germplasm potatoes (*solanum tuberosum* ssp. *tuberosum* L.) using morphological data and aflp (amplified fragment length polymorphism) markers.”**

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***RESEARCH ARTICLE***

**A la suite de cet article nous avons ajouté une discussion sur la comparaison des résultats obtenus grâce aux deux types de marqueurs, SSRs et AFLP**

## Résumé

Le Chili est considéré comme un centre secondaire d'origine de la pomme de terre cultivée dans lequel des variétés indigènes et des variétés introduites coexistent. Les différentes variétés présentes dans l'île de Chiloé sont caractérisées par une grande diversité de formes, de tailles, de couleurs et de caractéristiques phénologiques. Dans l'étude que nous présentons, le niveau de polymorphisme et les distances génétiques entre variétés ont été étudiées au moyen de marqueurs moléculaires en utilisant la technique AFLP et de vingt-sept caractères morphologiques. Vingt variétés de pommes de terre natives de l'île de Chiloé et la variété commerciale Desirée ont été analysées. Une espèce de la série Etuberosa, *Solanum fernandezianum*, recueillie dans l'île Juan Fernandez a été incluse comme groupe externe. Deux arbres de classification ont été construits, l'un à partir des données morphologiques et l'autre à partir de la matrice de similitude fondée sur toutes les bandes AFLP générées dans la gamme comprise entre 65 et 290 paires de bases. Avec ces outils, il a été possible d'identifier les différences et les similitudes moléculaires qui pourraient être associés à d'importants caractères morphologiques comme la forme prédominante du tubercule, couleur de la fleur et la résistance aux maladies.

**Molecular description and similarity relationships among native germplasm potatoes  
(*Solanum tuberosum* ssp. *tuberosum* L.) using morphological data and AFLP  
(Amplified Fragment Length Polymorphism) markers.**

**ABSTRACT**

Chile is considered to be a sub-center of origin for the cultivated potato, with native and introduced genetic material coexisting in the country. Thus, the different varieties present in Chiloé Island are characterized by a rich diversity of forms, sizes, colors and phenological characteristics. In the present work, the level of polymorphism and the genetic relationship were studied by means of molecular markers using the AFLP technique and twenty-seven morphological characters. Twenty varieties of potatoes from the Chiloé Island were analyzed. The commercial variety Desirée and one specie from the Etuberosa series, *Solanum fernandezianum*, collected in the Juan Fernandez Island were included as controls. A similarity tree-diagram was made, based on all the AFLP bands generated in the range between 65 and 290 base pairs. With these tools, it was possible to identify molecular differences and similarities that might be associated with important morphological traits such as the predominant forms of the tuber, flower color and resistance to disease.

## I.-INTRODUCTION

The potato is one of the four most important crops in the country and constitutes a basic food in the diet of the population. Chile is considered to be a sub-center of origin for the cultivated potato (Spooner *et al.* 2005b). In this context, the native potatoes of Chiloé are characterized by a rich variety of forms, sizes, colors and phenological characteristics. This rich genetic patrimony needs to be described and individualized in order to be conserved and used. Unfortunately, more of the native potatoes of Chiloé are being lost every day, because of their replacement by introduced varieties and their phytopathological deterioration among other causes. As a result, their conservation must be ensured by means of germplasm banks collections. The conservations and use of this native genetic material will ensure that it does not disappear, but enjoys a projection for the future with new uses. In addition, potato improvement programs require a basic knowledge of the morphology and genetic nature of the main parts of the plant. In this context, new advances in molecular biology with the use of more sensitive molecular markers able to detect changes in the genotype of the individuals, greatly contributed to the generation of this important information.

There is a remarkable concentration of cultivated and wild forms of potato in the south of Chile with the greatest number of native varieties being located in the island of Chiloé, and they are still preserved in the fields of small farmers. The peculiar characteristics of Chiloé, its natural conditions and its isolation, have allowed the proliferation of a great number of native varieties, of varying qualities and suitable for cultivation at different times in the farming calendar, as well as a number of different forms of preparation and consumption (Contreras *et al.* 1981). Until the mid 1960s, the markers used in genetic studies and improvement were those controlled by genes associated with morphological characteristics in easily identified phenotypes (Hijmans and Spooner, 2001). Potato germplasm has been described using morphological elements (Huamán *et al.* 1977; Ortiz and Huamán, 1994). Since 1975, it has been known in Europe and North America that potato cultivars could be identified by their protein and enzyme patterns (Stegemann and Loeschecke, 1976). With the modern technologies provided by molecular biology, various methods arise for the direct detection of genetic polymorphism at the DNA level using molecular markers. Molecular markers have contributed to a greater genetic knowledge of many vegetable species, including potato (Ritter *et al.* 2004). In addition, these markers have been used in the Solanum genus for the analysis of biodiversity and for phylogenetic studies (Ritter, 2000; Spooner *et al.* 2005a). Recently, efforts have been devoted to achieve a less subjective

description of cultivars, based on Random Amplified Polymorphic DNA (RAPD) (Miller and Spooner, 1999; Isenegger *et al.* 2001; Sun *et al.* 2003), and microsatellites (Ashkenazi *et al.* 2001; Raker and Spooner, 2002). Interrupted Simple Sequence Repeats (ISSR) has also been used (Milbourne *et al.* 1998; Bornet *et al.* 2002). AFLP analysis represents the most recent technology for obtaining a great number of molecular markers in the genomes of prokaryotes and eukaryotes (Savelkoul *et al.* 1999). The AFLP technique (Vos *et al.* 1995) generally produces between 50 and 100 scorable fragments per PCR reaction (Maughan *et al.* 1996). This technique has been widely used in various crops, including cultivated potatoes (Powell *et al.* 1996; Milbourne *et al.* 1997; McGregor *et al.*, 2000; Avrova *et al.* 2002; Straadt and Rasmussen, 2003; Furini and Wunder 2004) and their wild relatives (Kardolus *et al.* 1998). In the last years, the AFLP technology was used to differentiate genotypes used as ancestors in potato genetic improvement programs. This allowed establish exactly the differences between the most important varieties used in these programs. In this context, Spooner *et al.* (2005b) used AFLP molecular data, suggest a single origin rather than multiple origins of cultivated potato.

In this paper, we have analyzed the molecular diversity of Chilean native potato germplasm (*Solanum tuberosum* ssp. *tuberosum* L.) using AFLP markers and determined the genetic relationships existing among the genotypes included in the study.

## **II.- MATERIALS AND METHODS.**

### **2.1.- Plant material.**

The analysis included twenty accessions of native potato collected on the island of Chiloé, one improved cultivar Desiree, and one accession from the Etuberosa series, *Solanum fernandezianum* collected in the Juan Fernandez Island, Chile. Accessions were chosen to represent the extensive genetic diversity that can be found in *Solanum tuberosum* (Table 1). This material was cultivated in the field during agricultural season 2003/2004. The culture in the field began October 2003 and ended with the harvest in May 2004. The varieties were planted to 80 cm. between and 40 cm. over the crop line. An experimental design of complete blocks in random order was used in the establishment of the varieties in the field, in which the treatments corresponded to the different varieties being evaluated.

### **2.2.- Morphological characters analysis.**

Twenty-seven characters, analyzed and described by Huaman (1977) using potatoes varieties, and applied to "Chilean or native" varieties were numerically codified using a qualitative multi-status criteria (from 0 to 9, depending on the variables of each character). These were used to design a numbered-data matrix. Some characters included in this analysis were (Table 2): predominant tuber skin colour, general tuber shape, leaf characters, flower and fruit characters, phenologic data and reaction to fungi (*Phytophtora infestans*). Ten individuals were morphologically evaluated in each variety.

The morphologic data were subjected to cluster analysis. A standardization of the data by means of the Z-scores function was made. Average was applied to the technique of hierarchical conglomerate linkage using a matrix of quadratic similarity by means of Euclidean distances. The clustering method was made using the statistical software SPSS version 12.0 for windows. *S. fernandezianum* was not included since it is a non-bearing species. Bruja, Michuñe negra and Clavela morada were not included because none of them bloomed and therefore several characters related to flowering and fructification could not be sufficiently registered.

**Table 1.** Accessions included in the analysis.

Sample Nº	Accession	Common name	Specie	Location	Material
1	UCT1Ma	Michuñe azul	<i>S. tuberosum</i>	Island of Chiloé.	Tuber
2	UCT2Lv	Lengua vaca	<i>S. tuberosum</i>	Island of Chiloé	Tuber
3	UCT3Cl	Clavela	<i>S. tuberosum</i>	Los muermos.	Tuber
5	UCT5De	Desireé	<i>S. tuberosum</i>	Temuco, Huichahue.	Tuber
6	UCT6Gc	Guadacho colorado	<i>S. tuberosum</i>	Island of Chiloé	Tuber
7	UCT7Ca	Camota	<i>S. tuberosum</i>	Island of Chiloé	Tuber
8	UCT8Gb	Guadacho blanco	<i>S. tuberosum</i>	Island of Chiloé	Tuber
9	UCT9MgM	Meca gato Morada	<i>S. tuberosum</i>	Island of Chiloé	Tuber
10	UCT10MgL	Meca gato morada larga	<i>S. tuberosum</i>	Los Muermos	Tuber
11	UCT11Mgb	Meca gato blanca	<i>S. tuberosum</i>	Island of Chiloé	Tuber
12	UCT12Co	Coktail	<i>S. tuberosum</i>	Island of Chiloé	Tuber
13	UCT13MpB	Meca gato morada puntos blancos	<i>S. tuberosum</i>	Island of Chiloé	Tuber
14	UCT14MgRe	Meca gato redonda	<i>S. tuberosum</i>	Los Muermos	Tuber
15	UCT15MgRo	Meca gato roja	<i>S. tuberosum</i>	Island of Chiloé	Tuber
16	UCT16At	Azul tabla	<i>S. tuberosum</i>	Island of Chiloé	Tuber
17	UCT17Br	Bruja	<i>S. tuberosum</i>	Island of Chiloé	Tuber
18	UCT18Mn	Michuñe negro	<i>S. tuberosum</i>	Island of Chiloé	Tuber
19	UCT19Aq	Azul de Quento	<i>S. tuberosum</i>	Island of Chiloé	Tuber
20	UCT20Ro	Rosada	<i>S. tuberosum</i>	Island of Chiloé	Tuber
21	UCT21Ac	Azul cristalina	<i>S. tuberosum</i>	Island of Chiloé	Tuber
22	UCT22Cm	Clavela morada	<i>S. tuberosum</i>	Island of Chiloé	Tuber
23	UCT23Sf		<i>S. fernandezianum</i>	Island Juan Fernández. Plazoleta Yunque.	Botanical seed

### 2.3.- DNA isolation.

Approximately 100-200 mg of leaf tissue were freeze-dried and ground in liquid nitrogen with a mortar and pestle. Genomic DNA was isolated with Plant DNAzol® (Invitrogen) following the manufacturer's instructions. RNA was further eliminated by treatment with RNase. The quality and concentration of DNA was evaluated by agarose gel electrophoresis and spectrophotometry. Two independent extractions were performed on each accession.

### 2.4.- AFLP analysis.

AFLP reactions were carried out using the AFLP Analysis System I kit (Invitrogen Life Technologies) according to the manufacturer's instructions. Each reaction was repeated at least once to verify the AFLP patterns generated.

Approximately 500 ng of genomic DNA was digested for 2 h at 37° C using 2  $\mu$ l Eco RI/MseI restriction enzyme solution. The AFLP procedure (Vos *et al.* 1995) was carried out as described by Arens *et al.* (1998) with slight modifications. Briefly, the entire genomic DNA (400–500 ng) was digested with *Eco*RI and *Mse*I, followed by ligation of the adapters. Pre-amplification was performed using a single adenine (A) selective nucleotide for each primer. For selective amplification, an *Eco*RI primer, with three selective nucleotides, was used in combination with a *Mse*I primer with three selective nucleotides. For both the pre-amplification and selective amplification the following amplification profile was used: an initial cycle of 94°C for 30 s, 65°C for 30 s, 72°C for 1 min, followed by 12 touchdown cycles in which the annealing temperature was reduced by 0.7°C per cycle. The annealing temperature was then kept constant at 56°C for the remaining 23 cycles. Amplification products were separated on a 6% polyacrylamide gel, and made visible by silver staining. Twenty-three primer combinations were tested for their ability to generate reproducible AFLP profiles that could be scored unambiguously. Reproducibility of the primer combinations was tested by comparing the AFLP profiles of four DNA samples collected from the same individual. Five combinations were specifically chosen for their ability to generate a large number of bands in order to increase resolution for the identification of possible identical plants (Table 2). For each primer combination, the presence or absence of a band in each sample was visually scored. Data were set in a binary matrix (Paul *et al.* 1997; Yee *et al.* 1999). Genetic similarities were calculated using the similarity coefficient and tree-diagrams obtained by clustering according to the UPGMA method using the NTSYSpc 2.0.1 program (Applied Biostatistics Inc., NY, USA). The correspondence between the morphological and AFLP similarity coefficient matrices was tested on the basis of correlation analysis for Mantel test using the Mxcomp procedure.

**Table 2.** Descriptive names of the 27 morphological characters examined.

The descriptor list	Morphological Characters
Plant	Growth habit type
	Plant Height at flowering stage
Tuber	General tube shape Unusual tuber shape Predominant tuber skin colour Secondary tuber skin colour Distribution of secondary tuber colour Tuber skin type Predominant tuber flesh colour Secondary tuber flesh colour Distribution of secondary tuber flesh colour Depth of tuber eyes Note of eyes per tuber
Leaf	Abaxial leaf pubescent Adaxial leaf pubescent
Stems	Number of the primary stem Stem colour Stem cross section
Phenology	Day to formation of the first floral button Day at beginning of flowering Day at beginning of berry formation Day to harvest maturity
Flower	Calix colour Predominant flower colour Secondary flower colour
Fruit	Fruit colour Number of fruits Fruition presence
Disease reaction data	Evaluation to potato late blight ( <i>Phytophthora infestans</i> )

### **III.-RESULTS AND DISCUSSION**

#### **3.1.- Morphological data.**

The morphologic data cluster analysis allowed the separation of two defined groups (Figure 1). The first group included potatoes with semi late vegetative cycle and elongate tubers, and skins of coloration dark purple. The stems of this group present angular sections and green colors and node of reddish and purples colors. The flowers show an intense red color. Some varieties included in this are UCT8Gb, UCT11Mgb, UCT15MgRo, UCT6Gc, UCT9MgM and UCT1Ma. The second group presented semi early cycle and tubers of round forms, with skins principally purple color and secondary white color, distributed through all tuber or located around the eyes. This material presented dark green stems of angular sections and flowers intensely purple. This group was formed by the accessions UCT7Ca, UCT13MpB, UCT20Ro, UCT21Ac and UCT16At. Four accessions were not integrated in any of the previous groups. These are UCT19Aq, UCT5De, UCT14 MgRe and UCT 12Co, which morphologically are different. Comparatively these were less erect, more branching and smaller plant. Their tubers were predominantly oval-elongate to round, with a greater number of principal stems by plant and a clear dominion of flowers of white coloration.

#### **3.2.- AFLP data.**

The DNA samples were amplified with five primer combinations generating 281 AFLP markers positions, of which 253 (90.04%) were polymorphic (Table 3). The number of polymorphic markers per primer varied from 26 to 71. Primer pair *Eco*RI-ACC /*Mse*I-CAT contributed the highest number of profiles obtaining 100 per cent of polymorphic bands. These results concurred with those obtained by Savelkoul *et al.* (1999), who indicated that analysis using AFLP markers ensured a high level of polymorphic bands.

**Table 3.** Primer combinations used and polymorphic bands generated.

Primer combination	N° of bands	N° of monomorphic bands	N° polymorphic bands	% of polymorphic bands
EcoRI-ACC /Msel-CAT	66	0	66	100
EcoRI-AGG /Msel-CTT	34	8	26	76.47
EcoRI-AAG/Msel-CAC	56	9	47	83.93
EcoRI-ACC/Msel-CAA	48	5	43	89.58
EcoRI-ACA/Msel-CTG	77	6	71	92.21
TOTOL	281	28	253	90.04
		9.96%	90.04%	

Furthermore, these results agree with those reported by Kim *et al.* (1998), who obtained a total of 84 polymorphic bands using a single primer combination among 12 potato cultivars. Mc Gregor *et al.* (2002) indicate that AFLP is a very informative technique for difference potato germplasm, since they obtained 130 polymorphic DNA bands from two pair of combinations. Different combinations of primers achieved different degree of efficiency in the detection of polymorphism (Table 3). It was observed that the combination EcoRI-AGG /Msel-CTT displayed the lowest efficiency in the detection of polymorphisms with just 26 polymorphic bands (76.47%). The most efficient combination was EcoRI-ACC/Msel-CAT, which detected a total of 66 polymorphic bands (100%). Rouppe van der Voort *et al.* (1998), report that only one combination revealed sufficient information in an analysis of five genotypes of potato. These authors add that the number of monomorphic AFLP markers increases with the number of combinations of primers evaluated and the number of genotypes analyzed. Nevertheless, using different restriction enzymes, different pre-selective and selective nucleotide combinations increase the probability of finding useful polymorphisms; however, a greater number of selective bases lower the detection of polymorphisms. Our results indicated that all the matches analyzed differ in their degrees of similarity. The accessions "UCT5De" and "UCT10MgL" presented the highest genetic similarity (0.89), likewise the accessions "UCT9MgM" and "UCT13MpB" (Table 4). With respect to the accessions from Chiloé, the one, which displayed the least similarity to the rest, was "UCT1Ma". On the other hand, when comparing all accessions included in the study, it appears that they presented on average high genetic diversity (0.73) with similarities ranging between 0.32 and 0.89 (Table 4).

The similarity tree-diagram (Figure 2) derived from the register of all AFLP bands was generated by the use of combinations ACC/CAT, AGG/CTT, ACC/CAT, AAG/CAC and

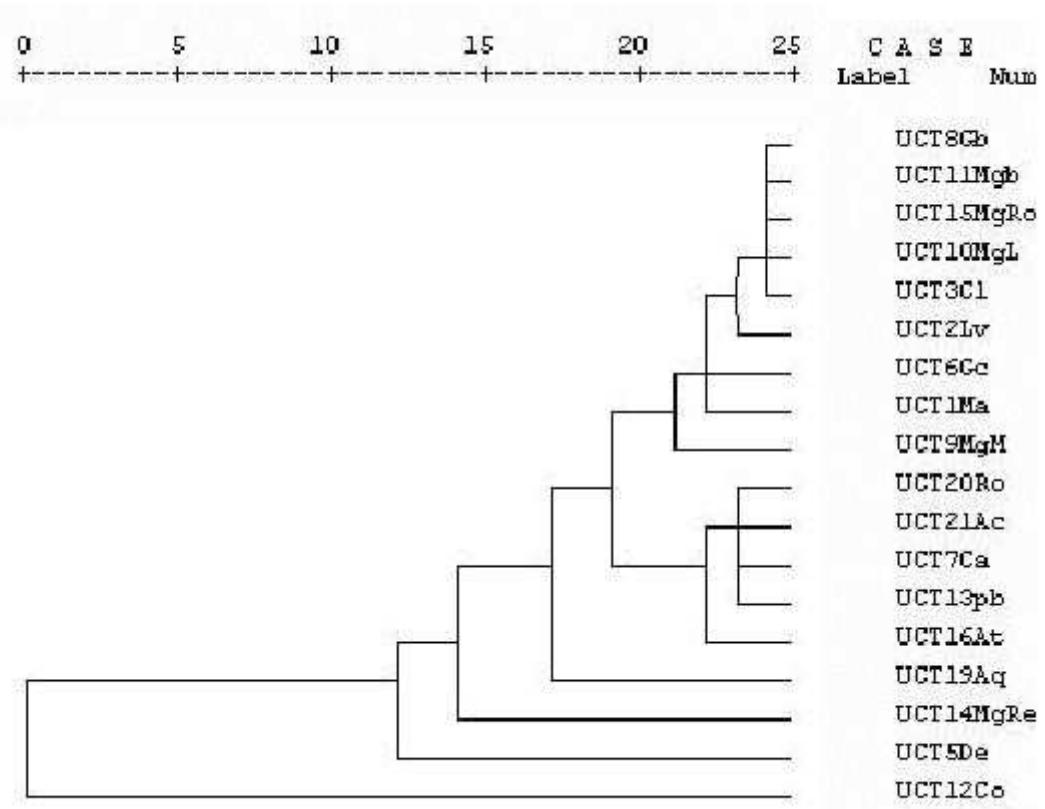
ACA/CTG. Nevertheless a single combination of primers (ACC/CAT) was sufficient to establish a level of optimal polymorphism. Four clusters were defined from the cluster analysis. The first cluster included only the accession UCT1Ma (A) (common name "michuñe azul"), which has a strongly restricted fusiform tuber of an intense blue color. The second group (B) corresponded to tuber potatoes which have a generally oval form and a light-colored skin, some with the presence of secondary colors. The flowers of this group are blue or pale-blue in color. The third group (C) was made up of varieties which are long and smooth in shape, with strong pigmentation. This group presented flowers with strong colors, predominantly purple and intense red. Finally, the last group included the control diploid species, *Solanum fernandezianum* (D), which confirms the fact that this species belongs to the Etuberosa series.

### **3.3.- Comparison between morphological and AFLP data.**

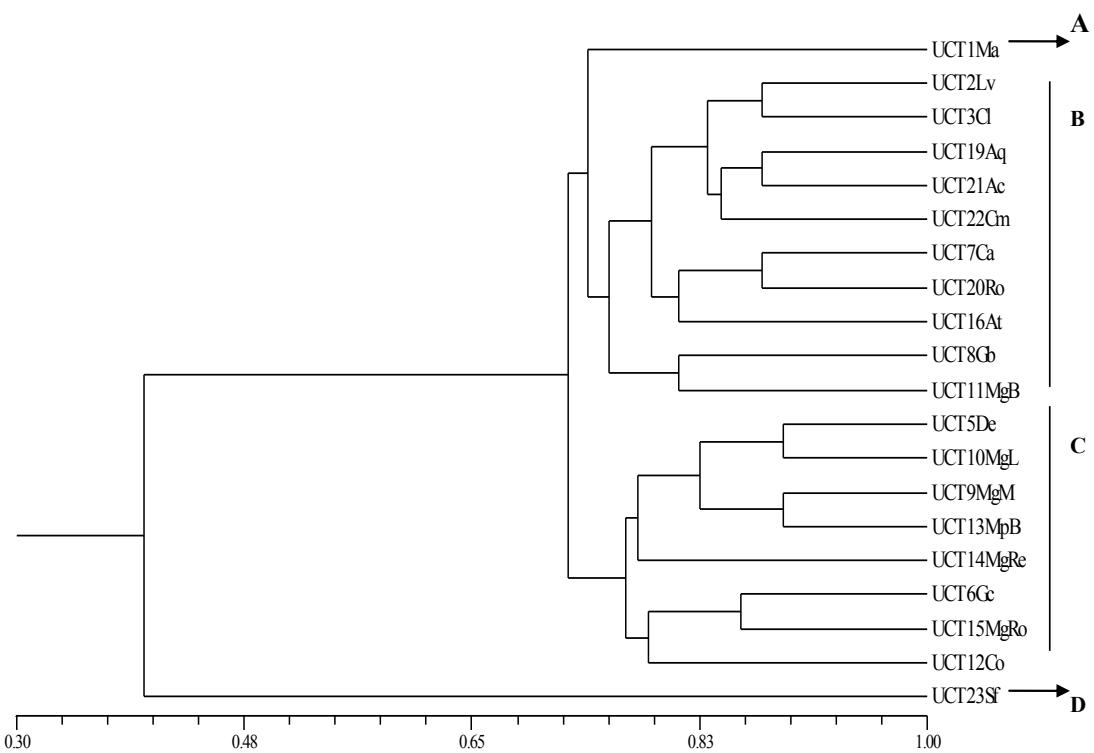
The Mantel test showed quite low correlations between the morphological and the molecular (AFLP) dendograms obtained ( $r = -0,09$ ). Both the morphological and genetic analysis allowed to separate defines groups of native potato germplasm. No significant concordance between AFLP and morphology cluster analyses was observed. In the morphologic analysis, the commercial variety Desiree appeared like an independent variety whereas in the AFLP analysis it integrated group C. The morphological traits of this group are not present in variety Desiree; however, AFLP data indicating that exists a high similarity with the native material of potato of the Chilote group. This result agrees with the hypothesis of the existence of ancestral genes in improvement cultivars of potato. This absence of concordance also has been observed by Xu *et al.* (2000), Federici *et al.* (2001), Roldán-Ruiz *et al.* (2001), Martínez *et al.* (2003), in varieties of grapevines, weedy rice, ryegrass and azuki beans respectively. Finally, Spooner *et al.* (2005b), report that techniques of DNA fingerprinting are better than morphological data for discrimination of related genotypes and in the analysis of the genetic similarity. Our results confirm that DNA analysis by AFLP is an efficient method for the exploration of genetic diversity in potato populations. According to our background this is the first study using AFLP markers in Chile to assess the great variability that existing among Chilean germplasm of potato.

**Table 4.** Genetic similarity values of potato native varieties using SM coefficient with AFLP markers.

	UCT 1Ma	UCT 2Lv	UCT 3Cl	UCT 5De	UCT 6Gc	UCT 7Ca	UCT 8Gb	UCT 9MgM	UCT 10MgL	UCT 11Mgb	UCT 12Co	UCT 13MpB	UCT 14MgRe	UCT 15MgRo	UCT 16At	UCT 19Aq	UCT 20Ro	UCT 21Ac	UCT 22Cm	UCT 23Sf	
<b>Accession</b>																					
UCT1Ma	1																				
UCT2Lv		0,79	1																		
UCT3Cl		0,79	0,88	1																	
UCT5De		0,74	0,80	0,80	1																
UCT6Gc		0,79	0,82	0,79	0,74	1															
UCT7Ca		0,76	0,88	0,79	0,77	0,82	1														
UCT8Gb		0,74	0,77	0,83	0,73	0,71	0,71	1													
UCT9MgM		0,73	0,73	0,70	0,83	0,79	0,76	0,71	1												
UCT10MgL		0,67	0,76	0,73	0,89	0,73	0,76	0,74	0,85	1											
UCT11Mgb		0,65	0,74	0,77	0,79	0,77	0,80	0,82	0,80	0,83	1										
UCT12Co		0,70	0,73	0,70	0,77	0,76	0,79	0,68	0,79	0,79	0,77	1									
UCT13MpB		0,74	0,65	0,68	0,82	0,74	0,68	0,67	0,89	0,83	0,76	0,77	1								
UCT14MgRe		0,73	0,79	0,79	0,80	0,76	0,76	0,71	0,79	0,79	0,77	0,79	0,77	1							
UCT15MgRo		0,68	0,71	0,68	0,76	0,86	0,77	0,67	0,89	0,77	0,79	0,83	0,82	0,77	1						
UCT16At		0,77	0,86	0,80	0,76	0,77	0,86	0,76	0,71	0,74	0,73	0,74	0,64	0,77	0,73	1					
UCT19Aq		0,76	0,85	0,85	0,74	0,76	0,76	0,77	0,67	0,73	0,74	0,67	0,65	0,76	0,65	0,74	1				
UCT20Ro		0,76	0,82	0,76	0,74	0,76	0,88	0,71	0,73	0,76	0,77	0,76	0,71	0,73	0,71	0,77	0,76	1			
UCT21Ac		0,76	0,82	0,85	0,80	0,73	0,79	0,80	0,70	0,79	0,80	0,70	0,68	0,76	0,65	0,74	0,88	0,79	1		
UCT22Cm		0,74	0,83	0,83	0,73	0,80	0,83	0,76	0,74	0,77	0,79	0,77	0,70	0,77	0,73	0,82	0,83	0,83	0,86	1	
UCT23Sf		0,45	0,42	0,39	0,41	0,39	0,48	0,44	0,39	0,39	0,44	0,36	0,41	0,36	0,32	0,50	0,42	0,52	0,48	0,47	1



**Figure 1.** Dendograms of native germplasm potatoes using morphological data.



**Figura 2.-** A similarity tree-diagram produced by UPGMA analysis of AFLP data.

#### IV.- REFERENCES

- ARENS, P.; COOPS, H.; JANSEN, J. and VOSMAN, B. Molecular genetic analysis of black poplar (*Populus nigra* L.) along Dutch rivers. *Molecular Ecology* 1998, vol. 7, p. 11-18.
- ASHKENAZI, V.; CHANI, E.; LAVI, U.; LEVY, D.; HILLEL, J. and VEILLEUX, R. Development of microsatellite markers in potato and their use in phylogenetic and fingerprinting analyses. *Genome*, 2001, vol. 44, no. 1, p. 50-62.
- AVROVA, A.; IMANA, L.; TOTH, R. and TOTH, K. Application of amplified fragment length polymorphism fingerprinting for taxonomy and identification of the soft rot bacteria *Erwinia carotovora* and *Erwinia chrysanthemi*. *Applied and Environmental Microbiology*, 2002, vol. 68, no. 4, p. 1499-1508.
- BORNET, B.; GORAGUER, F.; JOLY, G. and BRANCHARD, M. Genetic diversity in European and Argentinian cultivated potatoes (*Solanum tuberosum* subsp. *tuberosum*) detected by inter-simple sequence repeats (ISSRs). *Genome*, 2002, vol. 45, no. 3, p. 481-484.
- CONTRERAS, A.; BANSE, J.; FUENTEALBA, J.; ARUTA, C. and MANQUIAN, N. Germoplasma chileno de papas (*Solanum tuberosum* L.). Valdivia. Universidad Austral de Chile. Facultad de Ciencias Agrarias. Instituto de Producción Vegetal. Informe final-1980. 1981, p. 33.
- FEDERICI, M.T.; VAUGHAN D.; TOMOOKA N. KAGA A.; WANG WANG X.; DOI K.; FRANCIS M.; ZORRILLA G. and SALDAIN N. Analysis of Uruguayan weedy rice genetic diversity using AFLP molecular markers. *Electronical Journal Biotechnology*, 2001, vol. 4, no. 3, p. 130-145.
- FURINI A. and WUNDER J. Analysis of eggplant (*Solanum melongena*)-related germplasm: morphological and AFLP data contribute to phylogenetic interpretations and germplasm utilization. *Theoretical Applied Genetics*, 2004, vol. 108, no. 2, p. 197-208.
- HIJMANS, R. J. and SPOONER, D. M. Geographic distribution of wild potato species. *American Journal of Botany*. 2001; vol. 88, no. 11, p. 2101-2112.
- HUAMÁN, Z.; WILLIAMS, J. T.; SALHUANA, W. and VINCENT, N. Descriptors for the cultivated potato and for the maintenance and distribution of germoplasm collections. Consultative Group on International Agricultural research. Rome. International Board for Plant genetic Resources (I.B.P.G.R.), 1977, p 47.

- ISENEGGER, D.A.; TAYLOR, P. W.; FORD, R.; FRANZ, P.; Mc GREGOR, G.R. and HUTCHINSON, J. DNA fingerprinting and genetic relationships of potato cultivars (*Solanum tuberosum* L.) commercially grown in Australia. Australian Journal of Agricultural Research, 2001, vol. 52, p. 911-918.
- KARDOLUS J.P; ECK HJ VAN and BERG R.G. VAN DEN. The potencial of AFLPs in biosystematics: a first application in *Solanum* taxonomy (*Solanaceae*). Plant Systematicd and Evolution, 1998, vol. 210, p. 87-103.
- KIM, J. H.; JOUNG, H.; KIM, H. Y. and LIM, Y. P. Estimation of genetic variation and relationship in potato (*Solanum tuberosum* L.) cultivars using AFLP markers. American Journal of Potato Research, 1998, vol. 75, p. 107-102.
- MAUGHAN, P. J. SAGHAI MAROOF, M. A. BUSS, G. R. and HUESTIS, G. M. Amplified fragment length polymorphism (AFLP) in soybean: species diversity, inheritance, and near-isogenic line analysis. Theoretical and Applied Genetics, 1996, vol . 93, no. 3, p. 392 - 401
- MARTINEZ, L.; CAVAGNARO, P.; MASUELLI, R. and RODRIGUEZ, J. Evaluation of diversity among Argentine grapevine (*Vitis vinifera* L.) varietes using morphological data and AFLP markers. Electronical Journal Biotechnology, 2003, vol. 6, no. 3, p. 241-250.
- McGREGOR, C.E.; GREYLING, M. M.; LAMBERT, C. A.; LOUW, J.H. and WARNICH, L. A comparative assessment of DNA fingerprinting techniques (RAPs, ISSR, AFLP and SSR) in tetraploid potato (*Solanum tuberosum* L.) germplasm. Euphytica, 2000, vol. 113, p. 135-144.
- McGREGOR C.E.; VAN TREUREN, R.; HOCKSTRA, R. and VAN HINTUM, TH. J. Analysis of the wild potato germplasm of the series *Acaulia* with AFLPs: implications for ex situ conservation. Theoretical Applied Genetics, 2002, vol. 104, p. 146-156.
- MILBOURNE, D.; MEYER, R.; BRADSHAW, J.E.; BAIRD, E.; BONAR, N.; PROVAN, J.; POWELL W. and WAUGH R. Comparison of PCR-based marker system for the analysis of genetic relationships in cultivated potato. Molecular Breeding, 1997, vol. 3, p. 127-136.
- MILBOURNE, D.; MEYER, R.; COLLINS, A.; RAMSAY, L.; GEBHARDT, C. and WAUGH, R. Isolation, characterisation and mapping of simple sequence repeat loci in potato. Molecular and General Genetics, 1998, vol. 259, no. 3, p. 233-245.
- MILLER, J. T. and SPOONER, D. M. Collapse of species boundaries in the wild potato *Solanum brevicaule* complex (Solanaceae, sp. Sect. Petota): molecular data. Plant Systematic and Evolution, 1999, vol. 214, p. 103-130.

- ORTIZ, R. and HUAMAN, Z. Inheritance of morphological and tuber characteristics. In: CAB International. Potato Genetics. Bradshaw, J. E. y Mackay, G. R. (Eds). 1994, p. 263-279.
- PAUL, S.; WACHIRA, F. N.; POWELL, W. and WAUGH, R. Diversity and genetic differentiation among populations of Indian and Kenyan tea (*Camellia sinensis* (L.) O. Kuntze) revealed by AFLP markers. Theoretical and Applied Genetics, 1997, vol. 94, p. 255-263.
- POWELL, W.; MORGANTE, M.; ANDRE, C.; HANAFY, M.; VOGEL, J.; TINGEY S. and RAFALSKI A. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding, 1996, vol. 2, p.225-238.
- RAKER, C. M. and SPOONER, D. M. Chilean tetraploid cultivated potato, *Solanum tuberosum*, is distinct from Andean populations: microsatellite data. Crop Science. 2002, vol. 42, p. 1451-1458.
- RITTER, A. Aplicación de la biotecnología a la mejora genética de la patata. In: PATATA 2000. Vitoria-Gasteiz, España. 2000, p. 8.
- RITTER, E.; LUCCA, F.; SÁNCHEZ, I.; RUIZ DE GALARRETA, J.; ARAGONÉS, A.; CASTAÑÓN, S.; BRYAN, G.; WAUGH, R.; LEFEBVRE, V.; ROUSSELLE-BOURGOISE, F.; GEBHARDT, C.; VAN ECK, H.; VAN OS, H.; TACO, J. and BAKKER, J. Recursos genómicos en la papa y posibilidades de su explotación. Suplemento Revista Latinoamericana de la Papa. 2004, p. 17.
- ROLDÁN-RUIZ, I.; VAN EEUWIJK, F. A.; GILLILAND, T.J.; DUBREIL, P.; DILLMAN, C.; LALLEMAND, J.; DE LOOSE, M. and BARIL, C.P. A comparative study of molecular and morphological methods of describing relationships between perennial ryegrass (*Lolium Perenne* L.) varieties. Theoretical and Applied Genetics, 2001, vol. 103, no. 8, p. 1138-1150.
- ROUPPE VAN DER VOORT, J.; VAN ECK, H.; DRAAIESTRA, J.; VAN ZANDVORRT, P.; JACOBSEN, E. and BAKKER, J. An online catalogue of AFLP markers covering the potato genome. Molecular Breeding, 1998, vol. 4, p. 73-77.
- SAVELKOUL, P.; AARTS, H.; DE HAAS, J.; DIJKSHOORN, L.; DUIM, B.; OTSEN, M.; MADEMAKER, J.; SCHOUWS, L. and LENSTRA, J. Minireview: Amplified-Fragment Length Polymorphism Analysis: the State of an Art. Journal of Clinical Microbiology, 1999, vol. 37, no10, p. 3083–3091
- SPOONER, D.M.; PERALTA, I. and KNAPP, S.. Comparison of AFLPs with Other Markers for Phylogenetic Inference in Wild Tomatoes (*Solanum* L. Section *Lycopersicon* [MILL.] Wettst). Taxon, 2005a, vol. 54, p. 43-61.
- SPOONER, D.; MCLEAN, K.; RAMSAY, G.; WAUGH, R. and BRYAN, G. A single domestication for potato based on multilocus amplified fragment length

- polymorphism genotyping. Proceeding of the National Academy of Sciences, 2005b, vol. 102, no. 41, p. 14694–14699.
- STEGEGEMANN, H. and LOESCHECKE, V. Index. Europäischer Kartoffelsorten; Bestimmung durch elecktrophoretische Spektren. Paul Parey, Berlín. 1976, p. 214.
- STRAADT, I. K. and RASMUSSEN O. S. AFLP analysis of *Solanum phureja* DNA introgressed into potato dihaploids. Plant Breeding, 2003, vol. 122, no. 4, p. 352-356.
- SUN, G.; WANG-PRUSKI, G.; MAYICH, M. and JONG, H. RAPD and pedigree-based genetic diversity estimates in cultivated diploid potato hybrids. Theoretical and Applied Genetics, 2003, vol. 107, no. 1, p. 110-115.
- VOS, P.; HOGERS, R.; BLEEKER, M.; REIJANS, M.; VAN DE LEE, T.; HORNES, M.; FRIJTERS, A.; POT, J.; PELEMAN, J.; KUIPER, M. and ZABEAU, M. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Research, 1995, vol. 23, no. 21, p. 4407-4414.
- XU, R. Q.; TOMOOKA, N. and VAUGHAN, D. A. AFLP markers for characterizing the azuki bean complex. Crop Science, 2000, vol. 40, p. 808-815.
- YEE, E.; KIDWELL, K.K.; SILLS, G.R. and LUMPKIN, T.A. Diversity among selected *Vigna angularis* (Azuki) accessions on the basis of RAPD and AFLP markers. Crop Science, 1999. vol. 39, 268–275.

## **Confrontation des deux études de diversité moléculaire**

Dix-neuf variétés natives, la variété améliorée Désirée et *S. fernandezianum* étaient communes aux deux études de diversité moléculaire, l'une basée sur les marqueurs microsatellites (SSRs), l'autre sur des marqueurs AFLP. Les indices de similarité ou de dissimilarité, ainsi que les méthodes de classification employées n'étaient pas les mêmes dans les deux études, mais on peut toutefois tirer quelques conclusions de la confrontation de ces deux études.

Dans le cas des marqueurs AFLP, *S. fernandezianum* apparaît nettement différentié du groupe des *S. tuberosum*, alors que cette différence est beaucoup moins nette dans l'étude basée sur les SSRs. Nous interprétons cette différence comme résultant de vitesses d'évolution différentes pour les deux types de marqueurs. L'évolution des marqueurs AFLP est due à des événements de mutations ponctuelles dans les sites de restriction et/ou à des insertions/délétions entre les sites de restriction, alors que les marqueurs microsatellites sont connus pour avoir des taux de mutation beaucoup plus élevés et présenter par conséquent des séries alléliques longues. C'est ce que nous avons constaté dans notre étude avec un nombre d'allèles par locus SSR qui varie de 5 à 12 et trois locus parmi les 7 qui révèlent 12 allèles. Le nombre moyen d'allèles par locus atteint 9,16 et les valeurs de PIC associées à chacun des locus présentent des valeurs élevées. Cette diversité liée à la rapidité d'évolution des marqueurs SSRs se traduit par l'existence au sein des *S. tuberosum* de dissimilarités importantes, dont certaines sont comparables à celles que l'on peut trouver entre *S. tuberosum* et *S. fernandezianum*. La vitesse d'évolution des marqueurs microsatellites aboutit donc à ce que la probabilité soit forte de trouver deux variétés de l'espèce *S. tuberosum* qui présentent entre elles des dissimilarités aussi grande qu'avec *S. fernandezianum*. En effet, les dissimilarités estimées entre *S. fernandezianum* et les différentes variétés de *S. tuberosum* sont comprises entre 0.9 et 1.0, et des valeurs du même ordre de grandeur existent au sein de *S. tuberosum*. Et nous trouvons 22 comparaisons entre variétés natives, ainsi que 24 comparaisons entre variétés natives et variétés améliorées pour lesquelles les indices de dissimilarité sont estimés entre 0.9 et 0.96. En ce qui concerne l'analyse de la diversité par les marqueurs AFLP, c'est un indice de similarité qui a été employé mais on remarque que cet indice varie au sein des *S. tuberosum* entre 0.64 (pour les couples les plus dissemblables) et 0.89 (pour les couples les plus ressemblants). Les comparaisons entre *S. tuberosum* et *S. fernandezianum* explorent quant à elle une gamme de similarité moindre et totalement disjointe, comprise entre 0.32 et 0.52. La vitesse d'évolution, c'est à dire d'accumulation de mutations, aux marqueurs est

donc un facteur important à prendre compte lors du choix des marqueurs en fonction des groupes génétiques que l'on souhaite différencier. Pour mettre en évidence des différences entre entités qui ont divergé depuis peu de temps il faut des marqueurs à évolution rapide, pour mettre en évidence des différences entre groupes ayant divergé depuis plus longtemps il faut des marqueurs à évolution plus lente, les marqueurs à évolution rapide ayant atteint un niveau saturant, tous les allèles possibles étant apparus dans chacun des groupes.

Un autre point apparaît lorsque l'on confronte les résultats des analyses réalisées avec les deux sortes de marqueurs, c'est que la diversité des *S. tuberosum* étudiés est assez peu structurée. Il n'est en effet pas facile de trouver un niveau statistiquement fondé auquel couper l'arbre de classification. L'étude basée sur les SSRs fournit des fréquences de bootstrap pour chaque noeud de regroupement et on constate que très peu prennent des valeurs élevées, à part pour quelques groupes terminaux. L'observation du dendrogramme basé sur les AFLP montre que la différentiation entre le groupe des *S. tuberosum* et *S. fernandezianum* mise à part, il n'y a pas ici non plus de coupure évidente au sein des *S. tuberosum*. Par ailleurs, les deux classifications de la diversité au sein des variétés natives, celle basée sur les SSRs et celle basées sur les AFLP, ne montrent pas de similitudes. Comme nous l'avons déjà évoqué les deux types de marqueurs n'évoluent vraisemblablement pas à la même vitesse, ils révèlent donc vraisemblablement une diversité différente. Malgré tout, si une structuration forte existait au sein des variétés natives, les deux types de marqueurs devraient la révéler. Une structuration forte aurait pu exister si la sélection des variétés natives avait suivi un scenario du type suivant : effet de fondation de quelques groupes, puis différenciation intra-groupe par sélection au sein de la diversité résiduelle d'origine et sélection de mutations apparues *de novo* au cours des générations. Ce scenario suppose très peu de flux géniques et de recombinaisons entre les sous-groupes et une grande proximité génétique entre variétés au sein de chaque sous-groupe. L'image que nous donnent les marqueurs SSRs et AFLP de la diversité entre variétés natives ne valide pas un tel scenario, et nous pouvons conclure que la différenciation phénotypique des variétés natives ne correspond pas à une différenciation génétique des marqueurs neutres. Cette hypothèse est aussi en accord avec les faibles valeurs de bootstrap associées à la classification sur les marqueurs SSRs. Ces faibles valeurs montrent que même en utilisant un seul type de marqueurs, aucune structuration nette n'apparaît. Cela nous oriente vers un scenario où la sélection des différentes formes phénotypiques se serait faite sur une base génétique commune au sein de laquelle des reproductions par voie sexuée entre différentes formes suivies de semis de graines et de sélection ont dû régulièrement exister.

Enfin, l'étude basée sur les SSRs montre une différenciation faible entre les pommes de terre natives et les variétés cultivées localement, ce qui laisse supposer une base génétique commune. L'analyse de la diversité des AFLPs ne contredit pas cette conclusion, car bien qu'elle n'inclue qu'une seule variété cultivée (*Désirée*), cette variété se retrouve classée parmi les variétés natives. La diversité phénotypique des variétés cultivées est beaucoup plus faible que celle des variétés natives mais cela ne se traduit donc pas par une base génétique beaucoup plus restreinte que celle des variétés natives. La diversité des pommes de terre de Chiloé (natives et cultivées) prises en compte dans notre étude nécessite maintenant d'être mieux positionnée vis-à-vis de la diversité d'une collection élargie de pommes de terre d'origine mondiale. Nous n'avons pas pu réaliser ce travail dans le cadre et le temps impartis pour cette thèse car nous avons dû dans un premier temps beaucoup investir dans l'acquisition des moyens et des compétences techniques, c'est toutefois une perspective vers laquelle nous souhaitons orienter nos futures études.

## **CHAPITRE VI. Field Characterization And In-Vitro Evaluation by Inoculation of Separated Leaflets of Resistance to late blight (*Phytophthora infestans* mont. De Bary.) In A Collection of potatoes of the genus Solanum native to Chile.**

### **I.- INTRODUCTION**

The potato is the third most important crop in Chile, with a production of 831,019.7 tons, an area planted of 53,779.51 hectares and a national average yield of 15.45 tons/ ha<sup>-1</sup>, cultivated by 58,551 farmers (National Institute of Statistics - INE, 2007). The main production areas are concentrated in southern Chile in the Araucanía and Los Lagos Regions, with 14,029 and 11,154 hectares and production of 212,351 tons and 213,268 tons respectively. Chile is considered a sub-centre of the origin of cultivated potatoes, with native and introduced genetic material co-existing in the country. This accounts for its rich heritage, which should be characterised, conserved and used. Native potatoes present a rich variety of colours, both of the pulp and the skin, opening up great possibilities for the development of novel gourmet products. However since the introduction of the Desireé variety in 1962, more than 50 varieties have been introduced, principally from Europe. On the other hand, late blight, caused by the fungus *Phytophthora infestans* Mont. De Bary, is one of the most important diseases of the crop (Hijmans *et al.* 2000), and can destroy complete plantations in a short space of time. It is therefore considered to be the most serious problem affecting production world wide. It affects plants at any stage of their development and early infection can produce losses of up to 100% of production. *P. infestans* is a very difficult enemy to fight due to its population diversity (Fry, 2008). Work has been done over many years to obtain resistant cultivars, although in the majority of cases this resistance is overcome within 2 or 3 years (Estrada *et al.* 1994), due to the emergence of ever more aggressive isolations. In this context, wild species are used as a source of resistance for potato improvement (Andrivon *et al.*, 2003).

#### **1.1.- Potato Late blight.**

Potato crops are subject to attacks by various pathogens, including viruses, bacteria, nematodes and fungi. The value of global lost production with the costs of crop protection are estimated at five billion dollars annually (Turkensteen and Flier, 2002). Late blight, a disease caused by the Oomycete *Phytophthora infestans* (Mont.) de Bary, is the most important limiting factor for potato cultivation all over the world, and is therefore one of the most studied pathologies world wide (Fry and Goodwin, 1997; Escalante and Armas, 2004,

Secor *et al.*, 2009). *Phytophthora infestans* originated in the Toluca Valley, in central Mexico. It appeared almost simultaneously in Europe and United States in the 1830s, causing severe damage to potato crops (Jaramillo, 2003). This disease has reached disastrous proportions on various occasions; in the 1840s it was the cause of the Potato Famine in Ireland, causing the death of more than one million people, and the emigration of another one and a half million to America, out of a population of eight million (Ríos, 1999). Torres (2002) indicates that the disease was first reported in Norway in 1841, then in United States in 1842 (in potato fields close to the ports of Philadelphia and New York), and in the same year it was reported in six European countries (Belgium, Denmark, England, Germany, Ireland and Scotland). Subsequently, between 1845 and 1847, all the potato fields, cultivated especially in Ireland, were destroyed by this devastating disease in that country as well as the others. In Chile, blight caused disastrous consequences in 1950, leading to concern over phytosanitary aspects among farmers, producers and public figures related with agriculture. The entry of this pathogen into Chile, and its negative consequences for potato production, were debated openly and publicly (Ciampi, 2001).

Late blight affects both the foliage (stems and leaves) and the tubers (Ríos, 1999). In the leaves, the disease first appears as small, irregular spots of light to dark green. A whitish mildew develops on the back of the leaf, coincident with the spots on the face, consisting of sporangiophores and sporangia. If inoculum pressure is high in a particular zone, various spots may appear on a single leaflet, caused by different points of infection. In the stems, the symptoms are present as dark, continuous lesions, generally located in the upper or middle third of the plant, reaching more than 10 cm in length in some cases. In the tubers, very superficial, irregular depressions are observed on the outer part, of variable size and hard consistency. If the affected tuber is cut open, an irregularly-shaped, purple necrosis of granular appearance is observed in the cut surface, advancing from the periphery towards the centre of the medulla (Torres, 2002).

Muiño (1999), cited by De la Vega *et al.* (2006), indicates that it is the principal disease of the crop, in economic terms. It causes annual losses estimated at around three billion dollars in developed countries, and is largely responsible for huge annual expense on the application of fungicides to potato crops.

## **1.2.- Genetic resistance; mechanisms and sources against late blight.**

In recent years, the search for genetic resistance to late blight has intensified, since it is a practical and economic way of combating the disease. Initially the search concentrated on specific resistance, where promising results were obtained. However the varieties of potato released with this type of resistance turned out to be susceptible to attack by *P. infestans* which evolved by selection in response to the host resistance. Today, emphasis is being placed on non specific resistance, which is considered a more lasting option against the many variants or races of this pathogen.

Two types of resistance to the disease may be distinguished: specific and non-specific. These two types of resistance are often called vertical and horizontal by plant breeders, we will often refer to this terminology in the following. The first is characterised by triggering a hyper-sensitive response in the form of small necrotic lesions, and is called specific, racial, monogenic, qualitative, or complete resistance. It is controlled by major genes (*R-genes*) of mendelian inheritance, expressed under conditions of dominance. The dominant and recessive alleles of a specific *R-gene* locus are noted R and r. Genes of this type are incorporated from *Solanum demissum* and *Solanum stoloniferum*. This type of resistance is not very long-lasting, since *P. infestans* develops different races of greater virulence which can overcome the resistance (Flier *et al.*, 1998, Flier *et al.*, 2003). The genes of the pathogen are called avirulence genes (*Avr*), they are recognized by specific R genes of the host plant in a gene by gene interaction. The only isolations able to attack a plant holding a specific R allele are those with the corresponding recessive non expressed avirulence allele. The dominant and recessive alleles of an *Avr* locus are noted Avr and avr (Flor, 1971, Staskawiez *et al.*, 1995, Díaz *et al.*, 2003).

Horizontal resistance, known also as quantitative or field resistance, is governed by a large number of minor genes (*r*), with small effects and additive in character; it apparently does not involve gene by gene interaction and therefore it is assumed to be uniformly distributed against all the races of the pathogen, delaying their development and thus allowing timely treatment to avoid their propagation. Its stability is attributed to its capacity to maintain a balance between all the races of *P. infestans* present in a location (Robinson, 1996). Horizontal resistance acts in different ways, either through the production of toxic exudates on the leaf surface, confinement of the fungal structures to the cell walls, low colonization of the mesophyll, slow collapse of the petioles or reduction of the reproductive range of the pathogen; in other words it raises physiological or chemical barriers in the tissues of the host which dilate the range and frequency of the penetration and reproduction of the oomycete

(Wastie, 1991; Colon *et al.*, 1995). Horizontal resistance interrupts or retards one or more stages of the life cycle of the pathogen. In addition, other factors come into play such as certain characteristics of the plant, for example the thickness of the leaf cuticle and/or the presence of substances which inhibit the development of the pathogen, duration of the latency period, etc. (Micheletto *et al.*, 2000). Plants which have this type of resistance are infected in the field, but the damage and the percentage of the crop infected are much lower than in susceptible plants. Horizontal-non-specific resistances are more stable over time and space compared with specific resistances driven by R-Genes (Umaerus and Umaerus, 1994; Goodwin *et al.*, 1995; Micheletto *et al.*, 1999; Micheletto *et al.*, 2000; Parlevliet, 2002; Forbes *et al.*, 2005). When a variety with horizontal resistance is exposed in the field to conditions which are very favourable for the pathogen, and to high inoculum pressure, resistance is lost and the variety behaves like a susceptible variety, however the resistance is maintained and the variety returns to resistant behaviour in less favourable environments for *P. infestans* (Torres, 2002). Pérez and Forbes (2008), similarly indicate that in varieties with horizontal resistance the start and development of the disease are much slower than in susceptible varieties. Zlesak and Thill (2004) indicate that the level of quantitative resistance in the existing germplasm banks of *S. tuberosum* appear to be insufficient to allow a significant reduction in the use of fungicides in the future. Secor *et al.* (2009) add that, due to the genetic complexity of the host-pathogen interaction and the plasticity of the genome of this pathogen, stable resistance has been difficult to introduce by traditional means of genetic improvement.

With respect to sources of resistance, there are hundreds of native cultivars belonging to various species which may assist: *Solanum stenotomum*, *S. goniocalyx*, *S. phureja*, *S. ajanhuiri*, *S. chaucha*, *S. juzepczukii*, *S. andigena* and *S. curtilobum*. Many of these are cultivated where blight is endemic, and it would appear that some have lost their tolerance to more aggressive races of the pathogen. Fortunately there is a huge variety in the gene pool of potatoes through these cultivars, which may serve as sources for genetic resistance (Navia *et al.*, 2001). At the same time Colon *et al.* (1995) indicate that resistance to late blight may be found in various wild species of *Solanum*, and to some degree in different cultivated potatoes. This resistance varies from low level partial resistance, as in some cultivated potatoes, to immunity in some wild species (Wastie, 1991). There are also different genes involved in foliar and tuber resistance to *P. infestans* in the Solanaceae family, and some of these genes might be associated with late maturity (Umaerus and Umaerus 1994; Colon *et al.*, 1995; Simko *et al.*, 2006). Recently, Naerstad *et al.* (2007) reported high horizontal resistance, both foliar and tuber, in improved clones, which has allowed the intervals between fungicide applications to be increased. The majority of the

genes identified are encoded for foliar resistance, while very little is known about the genes which affect resistance in the tubers (Simko *et al.*, 2006). Meanwhile Visker (2005) indicates a possible physiological association between resistance to late blight and the type of maturity of the foliage, related to the effects on resistance of the age of the plant and the age and position of the leaf. The position of the leaf turned out to be the most significant factor in combating the disease, with apical leaves being much more resistant to late blight than basal leaves, while the age of the plant and age of the leaf only have minor effects, with resistance being maintained unchanged throughout life (Visker *et al.*, 2003). Barquero *et al.* (2005b) indicate that the search for genetic resistance to late blight has intensified, and that currently emphasis is placed on non specific resistance, since this is considered a longer-lasting option against the many variants or races of this pathogen. Trujillo (2004) adds that horizontal resistance to late blight is controlled by QTLs, and is the type most used in conventional improvement programmes due to its greater durability.

With respect to the resistance components, those which are used permanently are: latency period, size of lesion, lesion growth rate, and relative sporulation area. In general, they all show considerable variation between cultivars and localities (Colon *et al.*, 1995; Al-Kherb *et al.*, 1995; Zúñiga *et al.*, 2000; Flier *et al.*, 2003; Barquero *et al.*, 2005b; Gabriel *et al.*, 2007a, Gabriel *et al.*, 2007b).

### **1.3.- Native potatoes resistance.**

Barandalla *et al.* (2008), evaluating a group of cultivated native Peru varieties of the genus Solanum with respect to the principal pathogens affecting the crop, among them *Phytophthora infestans*, indicate that great variability exists in terms of the levels of resistance in the various entries, with no correlation between infection of the leaf and the tuber. The native variety "Chimi Lucki" and the older "Kasta" and "Morasa" presented complete resistance to leaf infection, while the native varieties "Poluya" and "Camusa" presented partial resistance. None of the accessions presented total resistance to tuber infection (Table 1).

**Table 1.** Resistance to *Phytophthora infestans* observed in the collection of native Peru potatoes and old cultivars of the genus Solanum.

Code	Variety	Species	Leaf resistance	Tuber resistance
NK-136	Kasta	S. tuberosum	R	
NK-338	Morada	S. tuberosum	R	
NKD-128	Huagalina	S. andigena		PR
NKD-133	Chimi lucki	S. juzepczukii	R	
NKD-148	Cceccorani	S. stenotomum		PR
NKD-153	Unknown	S. goniocalix		PR
NKD-158	Poluya	S. stenotomum	PR	
NKD-159	Camusa	S. andigena	PR	

**R:** Resistant; **PR:** partially resistant.

Source: Adapted from Barandalla *et. al.* (2008).

Lucca *et al.* (2008) show that the variability existing in *S. tuberosum* ssp *andigena* (adg) and *S. tarinjense* have not yet been fully explored in the south east of Buenos Aires province in Argentina. The results of field trials conducted during the 2007/08 season, with 14 genotypes of *S. tarinjense*, 1 of *S. gourlayi*, 9 of *S. andigena* and 2 of *S. tuberosum*, indicate the existence of significant differences between the genotypes in the area under the curve for the partial and final progress of the disease. The genotypes of *S. tarinjense* which presented the greatest values were Oka 5880.22 and Hof 1717.10, with total areas of 1780.8 and 1749.0 respectively. Finally, according to the results of research done in Argentina, Bolivia, Colombia and Peru, it may be stated that there is high variability which may be used in improvement to obtain lasting resistance to late blight in Latin America, with sources of resistance which range from wild species to *S. tuberosum* (Huarte and Capezio, 2003).

#### 1.4.- Background information on late blight in Chile.

Historically, the first reports of *P. infestans* in Chile date from the 1950s, supposedly introduced from Argentina (Arentsen, 1994), since migrations have played an important role in the dispersion of this fungus (Ristaino, 2009). In southern Chile, there have been repeated years with highly aggressive blight attacks on plantations and production losses greater than 50%, with the majority of native varieties and commercial cultivars under cultivation being affected to a greater or lesser degree (Table 2). Government estimates for the 2007 season indicate losses in yield for potato crops of 28.9 % in the province of Valdivia, 18.2% in the province of Osorno, 17.2% in the province of Llanquihue and 49.9% in the province of Chiloé. The degrees of incidence and severity in some years reach 100% of the crop (Table 2).

The above may be explained by changes in the populations of the fungus towards more aggressive forms, resulting from strains resistant to metalaxyl, mutations, asexual recombinations (Pérez *et al.*, 2001; Paez *et al.*, 2005), incorrect application of fungicides or more favourable environmental conditions (McLeod *et al.*, 2001). Acuña *et al.* (2007) determined that the populations of *P. infestans* in Chile are only of A1 (asexual) mating type, no sexual (A2) mating types have been discovered in Chile yet. However they have experienced a genetic change to genotypes which are highly resistant to metalaxyl and complex pathotypes with up to 9 and 10 avr genes (recessive mutation at an Avr locus, not recognized by the corresponding R gene). With respect to the frequency of avr genes of *P. infestans* in isolations in southern Chile (Table 3), Acuña (2008) indicates the existence of complex isolations with presence of the majority of the avr genes, with the exception of avr9. During the 2006/2007 and 2007/08 seasons, a greater prevalence was observed of avr11, avr10, avr8, avr7, avr5 avr3 and avr1 .

**Table 2.** Incidence and severity of late blight in Chile.

Season	Incidence (%)	Severity (%)
2006/07	90	40-100
2007/08	50	Very variable
2009/10	100	5-100

**Table 3.** Frequency of avr genes of *Phytophthora infestans* in isolations in southern Chile.

avr genes	Isolations with the gene (%)			
	2003-04	2004-05	2006-07	2007-08
avr1	59	39	98	97
avr2	14	6	20	3
avr3	42	31	100	96
avr4	48	35	78	14
avr5	64	47	98	89
avr6	8	8	10	6
avr7	37	39	100	90
avr8	14	22	100	88
avr9	0	0	0	0
avr10	90	95	100	99
avr11	95	98	100	97

Source: Acuña. (2008).

In the Chiloe archipelago, the tubers of native potato varieties have been a staple food of the population for years. Many local varieties and ecotypes might have had high susceptibility to the disease before the arrival of late blight on the island. Nevertheless, since its arrival

selection for more resistant clones may have begun. Identification studies of some of the avr genes present in isolations recovered from native material (Table 4) show that they are complex, with the presence of various avr genes (Acuña, 2008).

**Table 4.** Identification of avirulence genes in isolations of *P. infestans* recovered from native potatoes (Chiloe).

Isolation	District of origin	Source from which it was recovered	Mating type	avr alleles
20	Castro	Native red potato	A1(*)	1-5-6-10
29	Castro	Native red potato	A1	1-3-4-5-6-7-10-11
38	Castro	Native white potato	A1	1-3-4-5-6-7-8-10-11
60	Quemchi	Cultivar Desirée	A1	1-5
61	Quemchi	Cultivar Romano	A1	1-4-5-7-10-11

**Source:** (Acuña, 2008; Personal communication)

(\*) A1: asexual mating type

The objective of this work was to evaluate the field resistance of native accessions of potato to natural infections of *Phytophthora infestans* and evaluate the resistance of 10 accessions, by *in-vitro* inoculation of separated leaflets with a complex isolation of the fungus. The specific objectives were to measure the effects of the disease on leaf damage and the area under the curve for the progress of the disease during three growing seasons.

## II.- MATERIALS AND METHODS

### 2.1.- Plant material.

The material evaluated in the different seasons is presented in Table 5. For the first season (2006/07), 18 accessions of native potato were studied, plus one commercial cultivar used as a control. During the second and third seasons, 30 native potato accessions were studied, with 2 commercial cultivars (controls). All this material originates from southern Chile, the majority from Chiloé Island. For the first season the control cultivar was Desirée, which is the most widely-planted variety in Chile. For the second and third seasons, the cultivar Karú was also included, which is characterised by the highest resistance to late blight of all the commercial material present in the country (Kalazich, 2006).

For *in-vitro* inoculation of separated leaflets were selected ten accession for their good behaviour against late blight observed in the field in previous years. This was UCT- 6Gc, UCT-18Mn, UCT-26Ach, UCT- 3Cl, UCT- 1Ma, UCT-30Ño, UCT-15MgRo, UCT-34Cor, UCT-35AzC and UCT- 9MgM. The differential Craig's Royal (R0) was used as a susceptible control, and as a resistant control the advanced line R8906384 belonging to the potato improvement programme of the Institute of Agricultural and Livestock Research (INIA-Remehue), which has proved to have excellent resistance in Chile and United States (Secor, 2009). These were included in all the repetitions.

## **2.2.- Farming seasons and treatment of the crop.**

The material was evaluated over three farming seasons, with field tests established in the village of Pillanlelbún ( $38^{\circ} 39' 2.21''$  Lat. South;  $72^{\circ} 27' 3.4''$  West; 107 masl), Araucanía Region, Chile. The farming seasons were 2006/07, 2008/09 and 2009/10. In each season the field trials were established between 15th and 20th October, with emergence occurring in the following month. The seed dose used was equivalent to  $2,500 \text{ kg ha}^{-1}$ . The sowing distances used were 0.7 metres between rows and 0.3 metres between plants in the row. The crop was fertilized with a potato mixture 11:30:11 (N:P:K) at a dose equivalent to  $1,200 \text{ kg ha}^{-1}$ . Under these conditions the completed vegetative stage occurred in mid-December. The crop was banked up 60 days after planting using a ploughshare drawn by horse or when the plants were between 20 an 30 cm in height. In all the seasons, weeds were controlled by one application of Sencor SC (Metribuzin), at a dose equivalent to 1.1 litres of commercial product per hectare.

## **2.3.-Crop evaluations.**

### 2.3.1.- Reaction to *Phytophthora infestans*.

The field evaluation of the reaction of the accessions to blight was done using a scale based on the percentage of foliage affected in various stages of development of the crop, presented in Table 6 (Henfling, 1982; 1987). Subsequently, in order to evaluate the progress of the disease over time, the area under the progress of disease curve (AUPDC) was estimated (Jeger and Viljanen-Rollinson, 2001, Yuen and Forbes, 2009) using Formula 1. After that, the relative area under the progress of disease curve (AUPDCr) was calculated; this value was obtained by dividing the AUPDC by the total number of days elapsed between the first and last evaluation of the diseased leaf area (Formula 2). An evaluation with 100% of the leaf area diseased with late blight since the first day of evaluation would have a value

of 1.0. All the AUPDCr values are expressed as a proportion of this value. Low AUPDCr values indicate low levels of infection during the evaluation period, corresponding to the more resistant genotypes (Pérez and Forbes, 2008). The evaluations started when the first symptoms of the disease were observed, it corresponded to 76, 75 and 70 days after planting in 2006-2007, 2008-2009 and 2009-2010 respectively. It stopped 55, 47 and 34 days later in 2006-2007, 2008-2009 and 2009-2010 respectively, which corresponded to 130, 121 and 103 days after planting for the different years.

**Table 5.** Accessions evaluated of native Chilean *Solanum*.

Nº	Accessión	Local name	Origin	Number of seasons in evaluation
1	UCT-11Mgb	Meca gato blanca	Isla grande de Chiloe	3
2	UCT-14MgRe	Redonda	Isla de grande Chiloe	3
3	UCT-17Br	Bruja	Isla de Quinchao.	3
4	UCT- 6Gc	Guadacho colorado	Chonchi, Isla grande de Chiloe	3
5	UCT-24Tn	Tonta	Castro, Isla grande de Chiloe	3
6	UCT-22Cm	Clavela morada	Castro, Isla grande de Chiloe	3
7	UCT-25Gñ	Guicoña	Quellón, Isla grande de Chiloe	3
8	UCT- 7Ca	Camota	Isla grande de Chiloe	3
9	UCT-18Mn	Michuñe negro	Isla grande de Chiloe	2
10	UCT-26Ach	Azul chañihue	Isla grande de Chiloe	2
11	UCT-27Mu	Murta	Quellón, Isla grande de Chiloe	2
12	UCT-28MiR	Michuñe rojo	Isla grande de Chiloe	2
13	UCT-29Mol	Molejona	Isla grande de Chiloe	2
14	UCT- 3Cl	Clavela	Los Muermos, Continente	3
15	UCT- 1Ma	Michuñe azul	Isla grande de Chiloe	3
16	UCT-16At	Azul tabla	Isla grande de Chiloe	3
17	UCT-30Ño	Ñocha	Isla grande de Chiloe	3
18	UCT-19Aq	Azul de quento	Castro, Isla grande de Chiloe	2
19	UCT- 2Lv	Lengua	Castro, Isla grande de Chiloe	2
20	UTC-31Ob	Ojitos blanco	Ancud, isla grande de Chiloe	2
21	UCT-32Ci	Cielito	Castro, Isla grande de Chiloe	2
22	UCT-20Ro	Rosada	Isla grande de Chiloe	3
23	UCT-33Cab	Cabrita	Isla grande de Chiloe	2
24	UCT-15MgRo	Meca gato rojo	Isla grandede Chiloe	3
25	UCT-21Ac	Azul cristalina	Isla grande de chiloe	3
26	UCT-34Cor	Cordillera	Castro,Isla grande de Chiloe	2
27	UCT-35AzC	Azul caucheque	Castro, isla grande de Chiloe	2
28	UCT- 8Gb	Guadacho blanco	Ancud, isla grande de Chiloe	3
29	UCT- 9MgM	Meca gato morada	Ancud, Isla grande de Chiloe	3
30	UCT-10MgL	Meca gato morada larga	Los Muermos, continente	3
31	Testigo	Cultivar: Desirée.	Europa	3
32	Testigo	Cultivar: Karú.	Chile	2

**Formula 1.**

$$AUDPC = \sum_{i=0}^{n-1} [(X_{i+1} + X_i)/2](T_{i+1} - T_i)$$

Where:  $AUDPC$  = Area Under Disease Progress Curve,  $X_i$  = Proportion of affected tissue in the observation expressed in %,  $(T_{i+1} - T_i)$  = Time elapsed between two readings,  $n$  = Total number of readings.

**Formula 2.**

$$AUDPCr = \frac{AUDPC / 100}{(\text{Time in days between the last evaluation and the first evaluation})}$$

Finally, based on the values observed for the relative area under the progress of the disease curve (AUPDCr), the accessions were classified as very resistant (0.0-0.15); resistant (0.15-0.30); moderately resistant (0.30-0.45); moderately susceptible (0.45-0.60); susceptible (0.60-0.75) and very susceptible (0.75-1).

**Table 6.** Value scale of damage due to late blight.

<b>Value scale</b>	<b>Average affected tissue (%)</b>	<b>Symptoms</b>
1	0	No blight observed.
2	2,5	Maximum 10 lesions/plant.
3	10	Plants appear healthy, leaf area affected (20 leaflets).
4	25	Majority of plants affected, 25% foliage destroyed (fd).
5	50	Plot appears green, lower leaves dead, 50% leaf area destroyed.
6	75	Plot appears green with brown patches, 75% of every plant affected.
7	90	Only upper leaves are green, stems with extensive lesions.
8	97,5	Plot appears brown, majority of stems affected or dead.
9	100	Stems and leaves dead.

Source: Henfling(1987).

### **2.3.2.- Experimental design and statistical analysis.**

In all the seasons, the experimental design used was of random complete blocks. In the first season there were 19 treatments and four repetitions. In the second and third seasons there were 32 treatments and four repetitions. The data expressed as a proportion were transformed by arcsin  $\sqrt{p}$ . The data were subjected to analysis of variance. The mean values were compared by Duncan's statistic ( $p \leq 0.05$ ). The statistical programme used was SPSS version 15.0 for Windows (SPSS Inc, 2006). Heritabilities were calculated for AUDPC and AUDPCr on the sample of 32 varieties experimented the second and third years. It was also calculated over years on the mean of AUDPCr of the different varieties calculated for each year separately, in that way the two different years were considered as replications.

### **2.4.-Protocol for the *in-vitro* inoculation of separated leaflets.**

Twenty tubers of each accession were planted in 1 lt. pots and cultivated under greenhouse conditions. The test was done using young leaflets which were collected early in the day, before 10:30 AM. These were taken from fully developed plants at age 8 weeks (Barquero et al., 2005a). The isolation used for inoculation was Pi287, a carrier of 10 avirulence factors identified in the country (avr1, avr2, avr3, avr4, avr5, avr6, avr7, avr8, avr10, avr11) (Acuña et al., 2007). The leaflets were inoculated following the protocol described by Colón, et al., (2004). The isolation was cultivated in rye agar for 10 to 12 days at 18°C. The plates were washed with 10 ml sterile distilled water to collect the sporangia. It was then incubated at 4°C for 3 hours. The humidity chambers were prepared by placing a paper towel inside the containers and a mesh on top, and sprinkling with 10 ml of sterile distilled water to humidify them. For each inoculation, one leaflet from each accession was selected. Completely developed leaves were selected from the top third of the plant. The leaflets were distributed inside the humidity chambers, including the susceptible and resistant controls. One additional leaflet of the susceptible control was taken for inoculation with water. The humidity chambers were duly identified, indicating the repetition, plant number and internal distribution of each accession (figure 3). Each plate wash was calibrated with the fungus at a concentration of  $2 \times 10^3$  zoospores  $\times \text{ml}^{-1}$  using a haemacytometer. Each leaflet was inoculated with 30  $\mu\text{l}$  of the inoculum, adjusted to the concentration indicated. The leaflets were inoculated in the abaxial zone. The specimens were then incubated in a growing chamber with artificial lighting, using white light, and temperature controlled at 18°C. After 12 hours the drop of inoculum deposited on the leaflet was dried with filter paper (Figure 1).



**Figure 1.** Laboratory: Inoculation protocol.

- a). Stem with late blight b). Plants established in greenhouse
- c). Inoculation of native accessions separate leaflets d). Incubation at 18°C.

## 2.5.- Resistance components evaluated.

The resistance components were evaluated at 72, 96 and 120 hours after inoculation. The components were latency period, leaf necrosis, size of lesion and sporulation level. All the evaluations were done on the abaxial zone of the leaflet.

### 2.5.1. Resistance components

-Percentage of infection success

Each leaflet was scored 1 or 0 depending on the development or not of an infection after inoculation.

- Latency period.

This corresponds to the period of time in hours between inoculation and the appearance of the sporangia. This period allows resistance to be classified and the development of the disease in the different accessions to be compared. The sporulation process was monitored as from 72 hours after inoculation.

- Leaf necrosis.

A percentage scale was used where: 0.1 % corresponds to a small, separated necrotic lesion; 1.0% represents necrosis in the inoculation area; 5-100% corresponds to the total percentage of the leaflet affected by necrosis (Colon *et al.*, 2004). This trait gives a measure relative to the leaflet area. A big leaflet with a big lesion can present the same value as a small leaflet with a smaller lesion.

- Size of lesion.

The size of the lesion affected by the pathogen was determined by measuring the length and width of the necrotic area. Assuming an elliptical development of the lesion, its size (SL) was estimated in square centimetres ( $\text{cm}^2$ ) by  $SL = (\pi * L_1 * L_2) / 4$ , where  $L_1$  and  $L_2$  correspond to the length and width of the lesion (Andrivon *et al.*, 2007). This trait informs on the absolute size of the necrotic area independently of the area of the leaflet. If all the leaflets had the same area whatever the variety this trait should be highly correlated with the previous one.

-Level of sporulation.

A scale of 1 to 3 was used, where: 1= represents absence of sporulation, 2= corresponds to slight to moderate sporulation (50% of necrosed area with sporangia), 3= corresponds to intense sporulation (100 % of necrosed area with sporangia) (Colon *et al.*, 2004).

### 2.5.2.- Statistical analysis.

The varieties were compared for the proportion of non infected leaflets using a Chi<sup>2</sup> test for homogeneity. The data for latency period, leaf necrosis, size of lesion and sporulation level were subjected to an analysis of variance (anova) in accordance with the experiment design. The mean values were compared using Duncan's statistic ( $p \leq 0.05$ ).

We estimated the heritabilities of the different components as  $H^2 = \text{VarG}/(\text{VarG} + \text{VarR})$  and  $H^2_{\text{mean}} = \text{VarG}/(\text{VarG} + \text{VarR}/N_{\text{rep}})$  with VarG the genetic variance between accessions, VarR the environmental variance and Nrep the number of repetitions. VarG was estimated as  $(\text{MSG} - \text{MSR})/N_{\text{rep}}$ , with MSG and MSR the genetic and residual mean squares. As the anovas were performed on the sole leaflets which developed an infection, Nrep was not the same for the different genotypes, consequently we used the harmonic mean among genotypes which value was 32.3 (close to the arithmetic mean 33.1).  $H^2_{\text{mean}}$  is a measure of the level of repeatability, its value is an estimate of the expected correlation between the genetic means estimated on two independent experiments of the same genotypes repeated Nrep times in each experiment. It is also an estimate of the coefficient of determination ( $R^2$ ) of the genetic value by the mean phenotypic value of the variety. Based on the estimated values of VarG and VarR we also calculated  $H^2_{\text{mean20}} = \text{VarG}/(\text{VarG} + 2 * \text{VarR}/N_{\text{rep}})$ . This

coefficient tells us about the repeatability and the coefficient of determination ( $R^2$  as previously defined) in an experiment with twenty repetitions. It allows to assess the possibility of reducing the number of repetitions per experiment in order to increase the number of varieties tested.

### **III.- RESULTS**

#### **3.1.- Field characterization of resistance to late blight.**

##### 3.1.1.- Results of the first season.

###### 3.1.1.1.- Evaluation of leaf damage.

Table 7 shows the results of the first evaluation season. Statistically significant differences are observed for leaf damage at all evaluation dates. At 76 days after planting the accession UCT-15MgRo presents no leaf damage, while UCT-14MgRe shows 2% leaf damage with approximately 10 lesions per plant. The other accessions and the cultivar Desirée occupy intermediate positions ranging between 0.5 and 1.75% foliage damaged. Subsequently, at 90 days after planting, the accession UCT-25Gñ showed the highest damage value, with a scale value of 5.37 points and 59.38% leaf damage. The lowest damage by contrast was found in accessions UCT-15MgRo and UCT-11Mgb with less than 5% damage. In the third evaluation, the accession UCT-25Gñ continued with the greatest leaf damage, with 85.63%. The accession UCT-15MgRo presented the least damage at only 11.8%. This was followed by accessions UCT-30Ño, UCT-6Gc and UCT-11Mgb with 17.5, 23.1 and 30.0% leaf damage respectively. At this date, the cultivar Desirée occupied an intermediate position with 31.2 % leaf damage. Finally, at the fourth evaluation (130 days after planting), the accession UCT-6Gc shows the lowest value with 38.75% leaf damage. This is followed by UCT-15MgRo with 46.8% leaf damage. The accession UCT-25Gñ closed the season as the most susceptible to the disease, with leaf damage affecting 92.8% of the foliage. In general terms, several of the accessions studied (UCT-15MgRo, UCT-6Gc, UCT-30Ño) maintained a damage level during most of the phenological cycle which was lower than that observed in the cultivar Desirée, the control which in Chile is described as moderately susceptible to the disease.

**Table 7.** Percentage of foliage damaged by late blight in native accessions of potato, during the first season (2006/07).

Accessions	Leaf damage (%)				
	76		90		108
	04-01-07	18-01-07	05-02-07	28-02-07(**)	
UCT 22Cm	1.62 ab	18.63 bcd	47.50 bcd	76.56 abcd	
UCT 17Br	0.75 bc	4.00 cd	29.37 cd	73.75 abcd	
UCT 21Ac	1.00 ab	34.38 b	45.00 bcd	64.38 bcde	
UCT 7Ca	0.75 abc	5.00 cd	36.88 bcd	66.88 abcde	
UCT 6Gc	1.25 ab	10.00 bcd	30.00 cd	38.75 e	
UCT 30Ño	1.00 ab	3.44 cd	17.50 cd	58.13 cde	
UCT 24Tn	1.75 ab	25.00 bc	50.00 bc	71.88 abc	
UCT 14Mg Re	2.00 a	22.25 bc	71.88 ab	90.00 ab	
UCT 25 Gñ	1.00 ab	59.38 a	85.63 a	92.80 a	
UCT 1Ma	1.25 ab	7.18 cd	46.88 bcd	86.25 abc	
UCT 9MgM	1.50 ab	17.80 bcd	43.75 bcd	79.38 abcd	
UCT 3Cl	1.75 ab	9.05 bcd	37.50 bcd	83.13 abc	
UCT 15MgRo	0.00 c	1.25 d	11.88 d	46.88 de	
UCT 11Mgb	0.50 bc	1.88 d	23.12 cd	75.63 abcd	
UCT 8Gb	0.50 bc	9.05 bcd	43.75 bcd	84.06 abc	
UCT 16At	1.25 ab	6.23 cd	40.63 bcd	82.50 abc	
UCT 10MgL	1.50 ab	9.98 bcd	50.00 bcd	90.00 abc	
UCT 20Ro	1.00 ab	17.8 bcd	33.75 bcd	82.19 abc	
Cultivar: Desirée	1.00 ab	21.2 bcd	31.25 bcd	62.50 bcde	

(\*): Days after planting.

(\*\*): Evaluation dates.

Values with the same letter do not differ significantly from one another according to Duncan's test ( $p>0.05$ ).

Figure 2 shows the different accessions in the field during the first season.



**Figure 2.** Field trials in the first season:

a) plots in the field; b) beginning of flowering; c) leaf damage; d) stem damage.

### 3.1.1.2.- Area Under Disease Progress Curve (AUDPC).

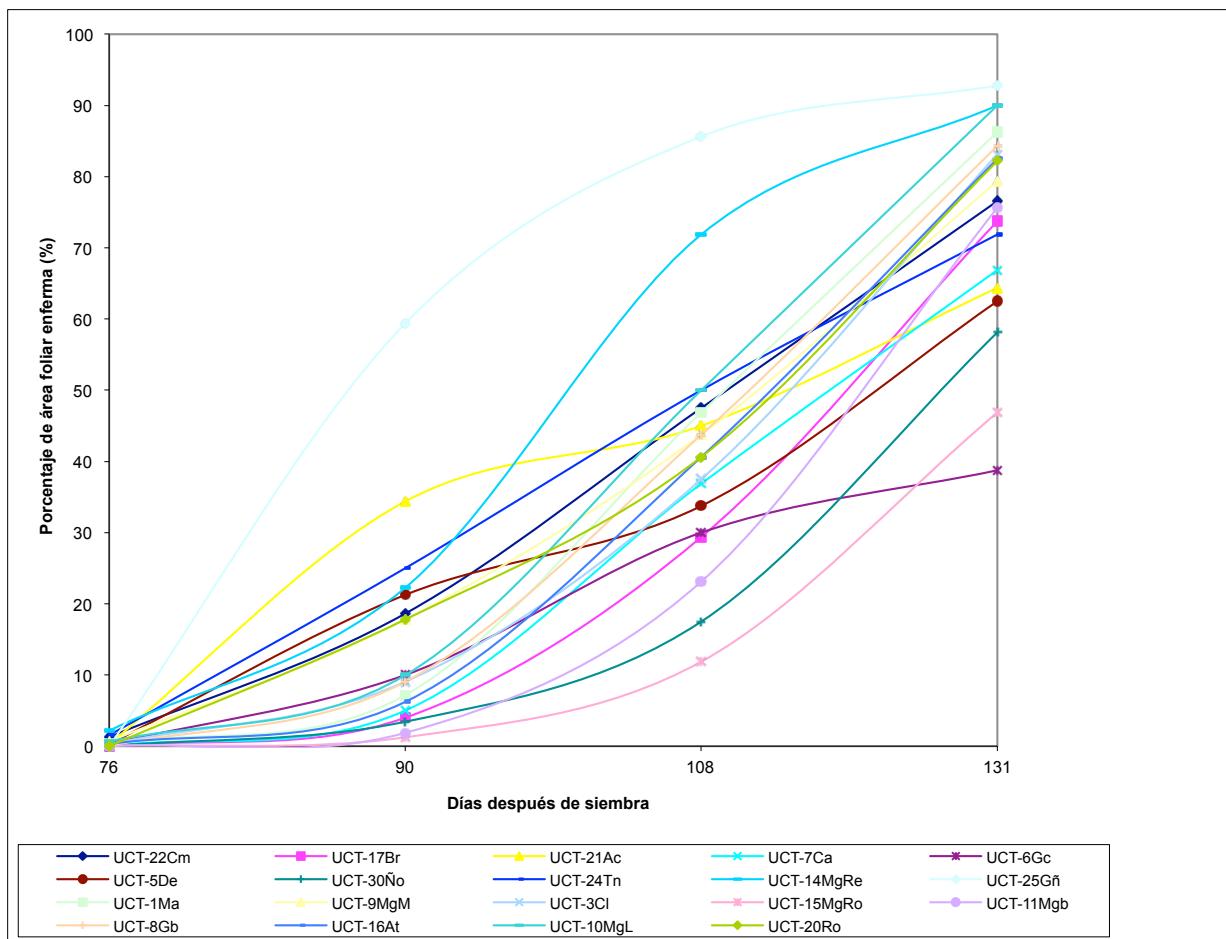
Table 10 presents the results of the analysis of the area under the disease progress curve (AUDPC) and the relative AUDPC (AUDPCr) for the various accessions evaluated in the first season.

The results show that significant differences existed in the area under the disease progress curve between the different accessions. Accession UCT-25Gñ presented the highest value for the area under the disease progress curve (AUDPC), with 3779.51, being the most affected by late blight. The lowest area under the disease progress curve was presented by accession UCT-15MgRo, with only 802.5. In general terms, a large part of the material was situated in an intermediate range between 1089 and 2878, including the cultivar Desirée, used as a control. Based on the foregoing, it may be said that in this season many of the

accessions had a moderately susceptible behaviour, with the exception of UCT-25Gñ and UCT-14MgRe which were very susceptible, with relative AUDPC values of 0.69 and 0.52 respectively. The accession UCT-15MgRo presented the best behaviour, with an AUDPCr of only 0.15. From this it may be concluded that the germoplasm of native potatoes contains interesting material for combating this disease directly or indirectly.

### 3.1.1.3.- Disease progress curve.

Figure 3 shows the evolution of leaf damage in the different accessions evaluated during the first season. It may be observed that for the first stage of growth, (76 to 90 days after planting), two groups appear with marked differences in the diseased leaf area. The first includes accessions UCT-10MgL, UCT-6Gc, UCT-1Ma, UCT-3Cl, UCT-16At, UCT-7Ca, UCT-8Gb, UCT-17Br, UCT-11MgB, UCT-30Ño and UCT-15MgRo, which are characterised by leaf damage not exceeding 10% in the evaluation scale. The other group consists of accessions UCT-20Ro, UCT-9MgM, UCT-22Cm, UCT-14MgRe, UCT-24Tn and the control Desirée, which are in the range between 17.8 and 25% leaf damage. Accessions UCT-21Ac and UCT-25Gñ stand out for their high level of leaf damage, 34.37 and 59.37% respectively. At 108 days after planting, the accessions show differentiated behaviour, with the least severity being observed in accession UCT-15MgRo. Accessions UCT-17Br, UCT-6Gc, UCT-11MgB and UCT-30Ño present levels of leaf damage in the range 17.5 to 30%; while the cultivar Desirée (control) and accessions UCT-7Ca, UCT-3Cl, UCT-20Ro, UCT-16At, UCT-8Gb, UCT-9MgM, UCT-1Ma, UCT-21Ac, UCT-10MgL, UCT-24Tn and UCT-22Cm present leaf damage in the range 33.7% to 50.0%. The accessions presenting the highest values were UCT-14MgRe and UCT-25Gñ, with 71.8% and 85.6% respectively. Finally, 131 days after planting, the accessions presenting the lowest values for leaf damage were UCT-6Gc with 38.7% and UCT-15MgRo with 46.8%. The highest values were observed in accessions UCT-14MgRe and UCT-10MgL, both 90%, and UCT-25Gñ with 92.8% leaf damage.



### 3.1.2.- Results of the second season.

#### 3.1.2.1.- Evaluation of leaf damage.

Table 8 shows the results of the second evaluation season. In general this season presented a low incidence of the disease, associated with a drier spring and summer (Figure 4). Nevertheless, significant differences were found in leaf damage due to late blight in all the evaluations. The accessions with the lowest percentage of leaf damage were UCT-34Cor, UCT-6Gc and UCT-26Ach, which at the sixth evaluation (121 days after planting) presented 4.37%, 10.0% and 11.8% damage respectively. It should be added that the leaf damage occurred later, especially in accessions UCT-34Cor, UCT-26Ach and UCT-30Ño. The accessions presenting the greatest leaf damage towards crop maturity were UCT-29Mol, UCT-16At, UCT-32Ci and UCT33-Cab, all with 62.5% leaf damage. The cultivars Desirée and Karú presented moderate levels of leaf damage at 53.75% and 31.25% respectively. Based on the above, at least eight accessions presented better behaviour than the better of the two cultivars used as controls.

#### 3.1.2.2.- Area Under Disease Progress Curve (AUDPC).

Table 10 presents the area under the disease progress curve (AUDPC) and the relative AUDPC obtained for the second farming season, with statistically significant differences observed in the AUDPC. The greatest area is presented by accession UCT-33Cab with 1162.36. High values are also found in accessions UCT-28MiR and UCT-24Tn, with 1062.5 and 1028.1 respectively. The lowest AUDPC value corresponded to accession UCT-34Cor with 56.91. This was followed by UCT-26Ach and UCT-30Ño with areas of 138.44 and 181.56 respectively. The control cultivars presented areas of 560.94 (Desirée) and 336.88 (Karú). Based on the above, the greatest relative area under the curve was found in accession UCT-33cab with a value of 0.25. This was followed by accessions UCT-28MiR and UCT-24Tn with relative values of 0.23 and 0.22 respectively. The lowest relative value corresponded to accession UCT-34Cor with 0.01. This was followed by UCT-26Ach and UCT-30Ño with areas of 0.03 and 0.04 respectively.

**Table 8.** Percentage of foliage damaged by late blight in native accessions of potato, during the second season (2008/09).

Accessions	Leaf damage (%)							121 (*)
	75	84	93	99	107	121 (*)		
	05-01-2009	14-01-2009	23-01-2009	29-01-2009	06-02-2009	20-02-2009 (**)		
UCT-11Mgb	0.00	c	7.81 a	13.75 bcd	17.50 abcd	27.50 ab	53.12 abc	
UCT-14MgRe	0.31	bc	1.87 bcdefg	8.12 cdef	10.00 abcde	10.00 bcdef	53.12 ab	
UCT-17Br	0.80	bc	0.62 efg	7.18 cdef	10.00 abcde	27.50 abc	43.75 abc	
UCT-6Gc	2.50 a		2.50 abcdef	1.87 efg	13.75 abcde	12.50 abcde	10.00 fg	
UCT-24Tn	0.00	c	5.62 abcd	10.00 bcde	27.50 ab	28.75 abcd	56.25 ab	
UCT-22Cm	0.62	bc	3.43 abcde	6.25 cdef	17.50 abcd	31.25 ab	50.00 ab	
UCT-25Gñ	1.25 b		4.06 abcde	18.12 abc	15.62 abcde	27.50 abc	50.00 ab	
UCT-7Ca	2.50 a		2.81 abcdefg	10.93 cde	8.12 abcde	16.56 abcde	26.25 bcdef	
UCT-18Mn	0.00	c	0.93 defg	10.62 cdef	10.00 abcde	22.81 abcd	46.87 abc	
UCT-26Ach	0.00	c	0.00	0.00 g	2.50 de	3.43 ef	11.80 efg	
UCT-27Mu	0.00	c	4.37 abcd	7.18 cdef	10.50 abcde	11.87 bcdef	35.62 abcde	
UCT-28MiR	0.00	c	6.25 ab	25.00 a	30.00 a	27.50 abc	43.75 abc	
UCT-29Mol	1.25 b		7.50 a	7.50 ab	10.00 abcde	19.37 abcde	62.50 a	
UCT-3Cl	0.00	c	2.18 abcdefg	6.25 cdef	23.75 abc	26.56 abc	50.00 ab	
UCT-1Ma	0.00	c	0.93 defg	3.75 defg	8.12 bcde	10.00 cdef	32.81 bcdef	
UCT-16At	0.00	c	2.50 cdefg	10.00 cdef	11.87 abcde	23.75 abcd	62.50 a	
UCT-30Ño	0.00	c	0.00 g	0.00 g	2.50 cde	4.37 def	16.56 defg	
UCT-19Aq	0.00	c	0.62 efg	3.75 defg	6.25 bcde	17.50 abcde	37.50 abcd	
UCT-2Lv	1.25 b		3.43 abcdef	9.06 cdef	16.25 abcd	27.50 abc	56.25 ab	
UTC-31Ob	0.62	bc	0.62 efg	6.25 cdef	4.37 cde	8.12 cdef	37.50 abcd	
UCT-32Ci	0.00	c	1.87 bcdefg	17.50 abc	21.87 abcd	21.25 abcd	62.50 a	
UCT-20Ro	0.00	c	3.43 abcdef	5.31 cdefg	11.87 abcde	20.00 abcde	46.87 abc	
UCT-33Cab	1.25 b		5.62 abcd	11.87 bcde	24.37 abcd	37.18 a	62.50 a	
UCT-15MgRo	2.50 a		5.00 abc	7.50 cdefg	8.12 bcde	12.50 abcde	22.81 cdefg	
UCT-21Ac	0.00	c	1.87 abcdefg	7.50 cdef	8.12 abcde	17.50 abcde	55.00 ab	
UCT-34Cor	0.00	c	0.00 g	0.00 g	1.30 e	1.56 f	4.37 g	
UCT-35AzC	0.00	c	1.25 cdefg	16.56 bcde	21.87 abcd	23.75 abcd	53.12 ab	
UCT-8Gb	0.00	c	3.75 abcdef	11.87 bcde	11.87 abcde	17.50 abcde	36.87 abcd	
UCT-9MgM	0.00	c	0.31 fg	1.25 fg	10.20 abcde	14.37 abcde	27.50 bcdef	
UCT-10MgL	0.00	c	0.93 cdefg	2.50 defg	14.37 abcde	17.18 abcde	30.62 bcdef	
Cult: Desirée	0.00	c	2.50 abcdef	2.50 defg	6.25 bcde	9.00 cdef	53.75 ab	
Cult: Karú	0.62	bc	0.62 efg	1.87 efg	5.31 cde	5.31 def	31.25 bcdef	

(\*): Days after planting. (\*\*): Evaluation dates.

Values with the same letter do not differ significantly from one another according to Duncan's test ( $p>0.05$ ).



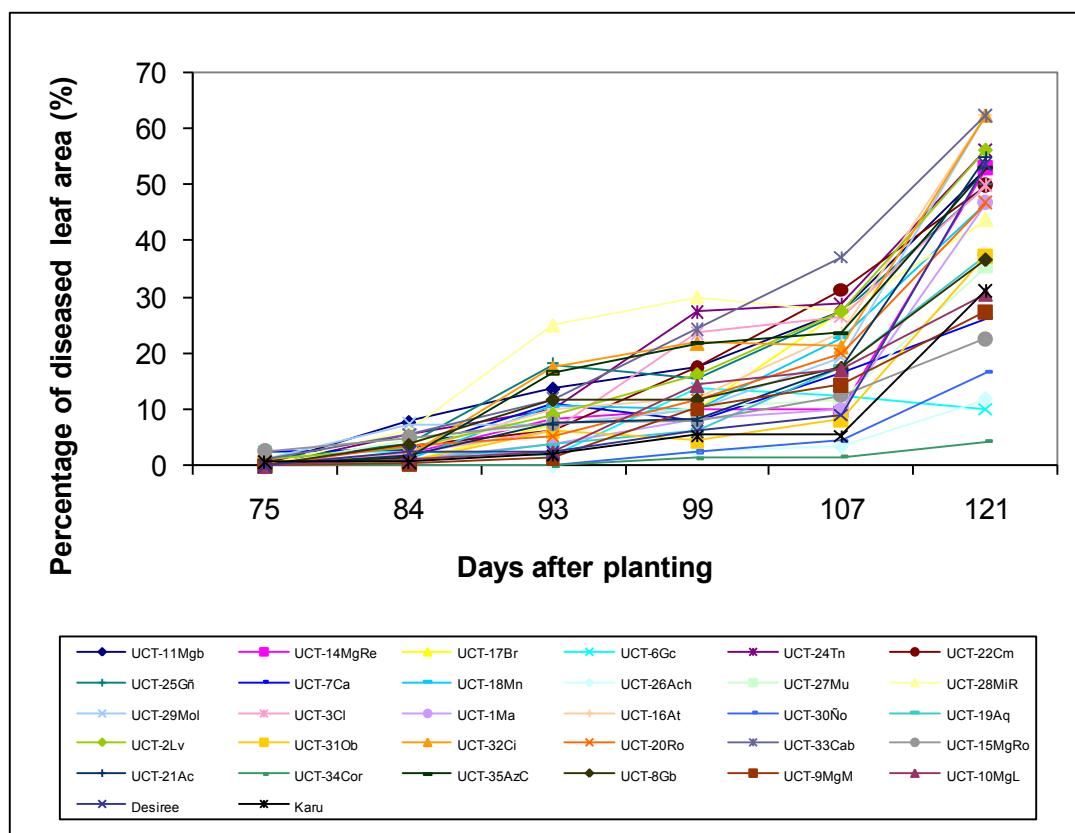
**Figure 4.** Field trials in the second season.

a) native potato accessions; b) start of flowering.

### 3.1.2.3.- Disease progress curve.

Figure 5 presents the disease progress curve for the various accessions evaluated during the 2008/2009 season. A wide range of variation is found in the response to the disease as expressed in the diseased leaf area. At 121 days after planting, this variation ranges between 4.37 and 62.5%. The former case corresponds to accession UCT-34Cor, while in the latter we find accessions UCT-29Mol, UCT16At and UCT-33Cab.

Figure 6 presents the leaf damage observed in two of the accessions included in this season.



**Figure 5.** Disease progress curves observed during the second season (2008/09).



**Figure 6.** Leaf damage in native potato accessions (Second season)

a) accession UCT-15MgRo; b) accession UCT-14MgRe.

### 3.1.3.- Results of the third season.

#### 3.1.3.1.- Evaluation of leaf damage.

Table 9 shows the results of the third evaluation season. In general this season suffered high incidence of the disease, associated with a rainy spring and summer and a high level of precipitation in January. This led to rapid progress of the disease, with accession UCT-33Cab for example presenting 100% leaf damage at 103 days after planting. On the other hand the results show that significant differences exist in leaf damage due to late blight in all the evaluations. In this season, the accessions with the lowest percentage of leaf damage were UCT-34Cor, UCT-26Ach and UCT-27Mu, which at 103 days after planting presented 28.0%, 28.75% and 30.0% damage respectively. During the first 90 days after planting accessions UCT-34Cor, UCT-26Ach and UCT-27Mu presented very low levels of leaf damage, not exceeding 10%. By contrast several accessions reached high levels of leaf damage due to late blight early in the season, for example UCT-33Cab, UCT-25Gñ and UCT-14MgRe with 100.0%, 96.75% and 94.25% leaf destroyed respectively. Meanwhile the cultivars Desirée and Karú presented similar levels to the 2008/09 season, with 52.50 and 46.25% leaf damage respectively.

#### 3.1.3.2.- Area Under Disease Progress Curve (AUDPC).

Table 10 presents the area under the disease progress curve (AUDPC) and the relative AUDPC obtained for the third farming season. Statistically significant differences were observed in the AUDPC, with the greatest area presented by accession UCT-25Gñ at 1,601.05. High values were also recorded for accessions UCT-32Ci and UCT-33Cab, with 1508.68 and 1507.55 respectively. As in the previous season, the lowest value for the

AUDPC corresponded to accession UCT-34Cor with 290.50. This was followed by accessions UCT-27Mu and UCT-26Ach with 292.38 and 307.38 respectively. The control cultivars presented areas of 650.50 (Desirée) and 574.50 (Karú). Based on the above, the greatest relative area under the curve was found in accession UCT-25Gñ with a value of 0.48. This was followed by accessions UCT-32Ci and UCT-33cab, both with relative AUDPC values of 0.45. The lowest relative value was again presented by accession UCT-34Cor with 0.09. In general terms, it may be said that the relative AUDPC values observed were comparatively higher than in the second season, for all the accessions.

**Table 9.** Percentage of foliage damaged by late blight in native accessions of potato, during the third season (2009/10).

Accessions	Leaf damage (%)												
	70	75	78	85	91	96	103 (*)						
	30-12-200	4-01-10	07-01-10	14-01-2010	20-01-10	25-01-10	01-02-10 (**)						
UCT-11Mgb	0.00 a	1.50	bcd e	2.12	cde	4.75	cde	21.25	defgh	41.25	ghijk	62.50	fghi
UCT-14MgRe	0.00 a	2.25	bcd e	3.75	bcd e	22.50	abc	45.00	abcd	75.50	abcdef	94.25	abc
UCT-17Br	0.00 a	4.75	abcde	5.25	abcde	8.25	bcd e	17.00	fgh	38.75	ghijk	63.75	fghi
UCT-6Gc	3.75 ab	7.50	abcde	7.50	abcd	9.50	bcd e	17.50	fgh	31.25	hijk	50.00	hij
UCT-24Tn	0.00 a	1.25	cde	2.12	cde	4.50	cde	12.50	gh	35.00	hijk	68.75	fghi
UCT-22Cm	0.00 a	10.00	abde	5.50	cde	7.25	bcd e	18.75	fgh	42.50	ghijk	70.0	efghi
UCT-25Gñ	3.50 ab	12.75	abcd	24.00 a		36.25 a		60.00 a		86.17	ab	96.75	ab
UCT-7Ca	2.00 ab	9.50	abcde	14.50	abc	16.25	abcde	33.75	bcd efg	56.25	cdefgh	79.25	cdef
UCT-18Mn	1.50 a	15.00	ab	12.50	abc	19.00	abcd	37.50	abcd e f	53.75	cdefgh	82.50	bcdef
UCT-26Ach	0.00 a	0.25	e	0.25	e	2.75	de	10.75	gh	21.25	ijk	28.75	j
UCT-27Mu	0.25 a	0.50	e	0.75	de	3.25	de	7.25	h	20.00	k	30.00	j
UCT-28MiR	2.00 ab	8.75	abcde	8.75	abcd	11.50	bcd e	26.25	cdefg	47.50	fghijk	73.75	defgh
UCT-29Mol	6.25 b	12.00	abc	7.00	abcde	11.50	bcd e	23.75	defgh	55.00	cdefgh	75.00	defgh
UCT-3Cl	0.00 a	1.25	de	1.50	de	6.00	bcd e	21.25	defgh	62.50	abcd e f g h	81.25	cdef
UCT-1Ma	2.75 ab	3.00	abcde	1.75	cde	10.75	bcd e	25.00	defgh	50.00	efghij	72.50	efgh
UCT-16At	1.25 a	2.00	bcde	3.25	cde	7.00	bcd e	17.00	fgh	46.25	ghijk	67.50	fghi
UCT-30No	1.25 a	5.00	abcde	6.75	abcde	12.25	bcd e	31.25	cdefg	57.50	cdefgh	85.00	bcdef
UCT-19Aq	1.25 a	5.00	abcde	5.00	bcd e	16.25	abcd e	52.50	abc	75.67	abcd	88.50	bcde
UCT-2Llv	5.00 ab	9.25	abcde	9.25	abcd	11.25	bcd e	18.75	efgh	38.75	ghijk	63.75	fghi
UTC-31Ob	1.75 a	5.25	abcde	8.00	abcde	13.75	bcd e	34.25	bcd efg	57.50	cdefgh	78.00	def
UCT-32Ci	0.50 a	17.75	abc	15.62	abc	38.75 a		57.50	ab	78.17	abc	91.75	bcdef
UCT-20Ro	0.25 a	1.25	bcde	6.12	abde	10.00	bcd e	25.00	defgh	51.25	defgh	62.50	fghi
UCT-33Cab	3.75 ab	17.75 a	19.37	ab	25.00	ab	52.50	abc	88.17 a		100.00 a		
UCT-25ab	2.50 ab	6.25	abcde	7.50	abcd	12.75	bcd e	27.50	cdefgh	50.00	defgh	60.50	fghi
15MgRo													
UCT-21Ac	0.25 a	6.00	abcde	7.75	abcd	21.75	abc	45.00	abcde	78.75	abcde	89.75	bcde
UCT-34Cor	0.00 a	0.25	e	1.25	de	2.75	e	7.25	h	21.25	jk	28.00	j
UCT-35AzC	0.00 a	2.50	abcde	3.12	cde	10.00	bcd e	30.00	cdefg	68.75	abcdefg	90.75	bcde
UCT-8Gb	1.00 a	1.75	abcde	2.25	cde	5.75	bcd e	21.25	defgh	41.25	ghijk	63.75	fghi
UCT-9MgM	0.25 a	1.75	bcde	2.25	cde	10.00	bcd e	26.25	defgh	52.50	defgh	77.50	defg
UCT-10MgL	0.25 a	2.25	abcde	3.00	cde	12.50	bcd e	33.75	bcdefg	51.25	defghi	75.00	defgh
Cult: Desirée	2.50 a	3.50	abcde	4.37	bcd e	7.25	bcd e	25.00	defgh	40.00	ghijk	52.50	ghij
Cult: Karú	0.00 a	4.00	abcde	4.00	bcd e	6.50	bcd e	21.25	defgh	36.25	hijk	46.25	ij

(\*): Days after planting.

(\*\*): Evaluation dates.

Values with the same letter do not differ significantly from one another according to Duncan's test ( $p>0.05$ ).

Figure 7 presents the leaf damage observed in two of the accessions included in this season.



**Figure 7.** Leaf damage during the third season.

a) Accession UCT-25Gñ; b) Accession UCT-34Cor.

### 3.1.3.3.- Disease progress curve.

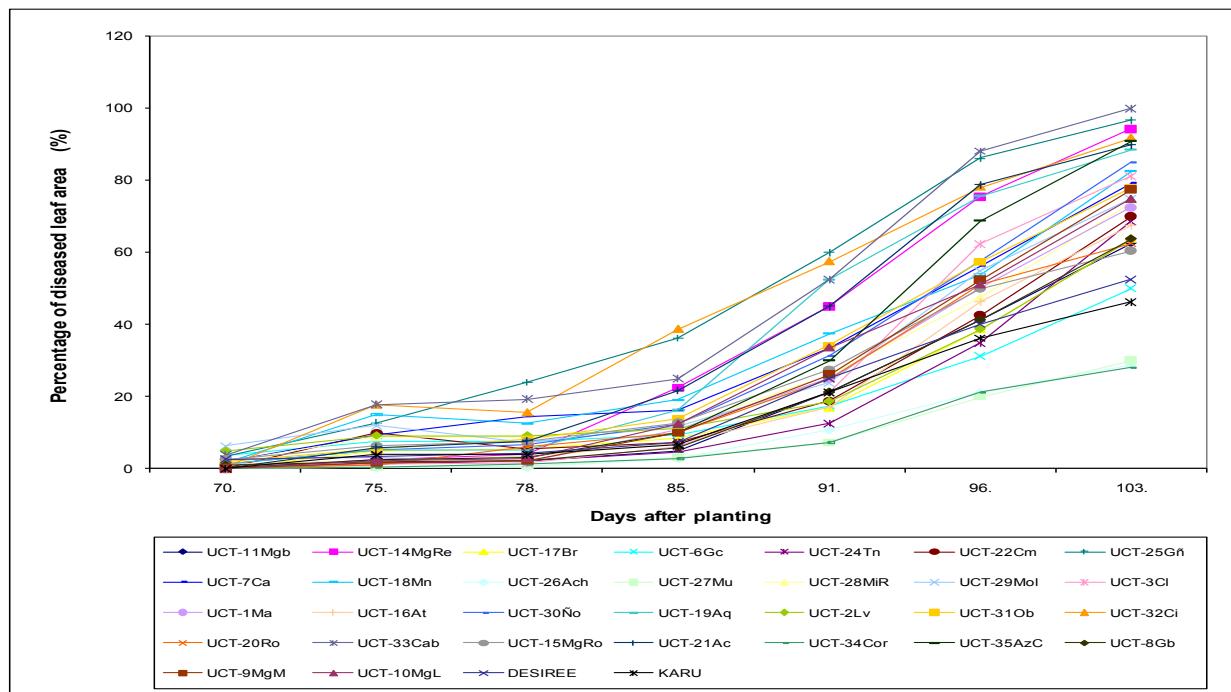
Figure 8 (below) presents the disease progress curve for the various accessions evaluated during the 2009/2010 season. As in the previous season, a wide range of variation is found in the response to the disease as expressed in the diseased leaf area. The values observed at 103 days after planting were higher, and ranged between 28.0% and 100.0% diseased leaf area. The former case corresponds to accession UCT-34Cor, while in the latter we find accession UCT-33Cab.

**Table 10.** AUDPC and AUDPCr of native potato accessions during the first, second and third season.

Accessions	AUDPC 2006/07		AUDPCr 2006/07		AUDPC 2008/09 $H^2=0.47$		AUDPCr 2008/09 $H^2=0.47$		AUDPC 2009/10 $H^2=0.44$		AUDPCr 2009/10 $H^2=0.44$	
UCT-11Mgb	1,377.25	ab	0,25	970.31	abc	0,21	630.63	efg	0,19			
UCT-14MgRe	2,878.44	cd	0,52	631.09	bcd	0,14	1,204.38	abcd	0,36			
UCT-17Br	1,519.56	ab	0,28	819.84	abcde	0,17	648.00	defg	0,19			
UCT-6Gc	1,227.63	ab	0,22	351.56	fgh	0,07	597.38	efg	0,18			
UCT-24Tn	2,263.81	bc	0,41	1,028.13	ab	0,22	564.25	efg	0,17			
UCT-22Cm	2,163.59	bc	0,39	896.88	abcd	0,19	717.75	cdefg	0,21			
UCT-25Gñ	3,779.51	d	0,69	940.00	abc	0,20	1,601.05	a	0,48			
UCT-7Ca	1,610.25	ab	0,29	541.41	cdefg	0,12	1,021.63	bcde	0,31			
UCT-18Mn	-	-	-	737.19	abcdef	0,16	1,067.25	bcde	0,32			
UCT-26Ach	-	-	-	138.44	gh	0,03	307.38	fg	0,09			
UCT-27Mu	-	-	-	546.78	cdefg	0,12	292.38	g	0,08			
UCT-28MiR	-	-	-	1,062.50	ab	0,23	846.00	cdefg	0,25			
UCT-29Mol	-	-	-	850.00	abcde	0,18	896.50	cde	0,27			
UCT-3Cl	1,881.74	abc	0,34	875.00	abcde	0,19	827.75	cdefg	0,25			
UCT-1Ma	2,076.36	bc	0,38	531.56	cdefg	0,11	788.75	cdefg	0,23			
UCT-16At	1,889.91	abc	0,34	879.38	abcde	0,19	680.13	cdefg	0,20			
UCT-30Ño	1,089.19	ab	0,20	181.56	gh	0,04	950.88	cde	0,28			
UCT-19Aq	-	-	-	532.50	cdefg	0,11	1,206.30	abcd	0,36			
UCT-2Lv	-	-	-	914.13	abcd	0,19	727.63	cdefg	0,22			
UTC-31Ob	-	-	-	437.81	efgh	0,09	961.13	cde	0,29			
UCT-32Ci	-	-	-	972.50	abc	0,21	1,508.68	ab	0,45			
UCT-20Ro	2,069.77	bc	0,38	702.03	bcd	0,15	765.00	cdefg	0,23			
UCT-33Cab	-	-	-	1,162.36	a	0,25	1,507.55	ab	0,45			
UCT-15MgRo	802.50	a	0,15	446.56	defgh	0,10	814.63	cdefg	0,24			
UCT-21Ac	2,219.81	bc	0,40	707.50	bcd	0,15	1,238.88	abc	0,37			
UCT-34Cor	-	-	-	56.91	h	0,01	290.50	g	0,09			
UCT-35AzC	-	-	-	921.72	abcd	0,19	985.75	bcde	0,29			
UCT-8Gb	2,011.89	abc	0,37	656.56	bcd	0,14	645.63	defg	0,19			
UCT-9MgM	2,104.99	bc	0,38	434.21	efgh	0,09	814.50	cdefg	0,24			
UCT-10MgL	2,230.10	bc	0,41	531.25	cdefg	0,11	861.50	cdef	0,26			
Cul: Desirée	1,759.38	abc	0,32	560.94	cdefg	0,12	650.50	defg	0,19			
Cul: Karú				336.88	fgh	0,07	574.50	efg	0,17			

$H^2$  broad sense heritability = VarG/(VarG+VarE)

Values with the same letter do not differ significantly from one another according to Duncan's test ( $p>0.05$ ).



**Figure 8.** Disease progress curves observed during the third season (2009/10).

### 3.1.4.- AUDPCr an over years assessment.

19 varieties were common to the three different years of evaluation and 32 were common to years 2008-2009 and 2009-2010. In the above, the results were presented for each year independently. Table 11 show noticeable differences between years for the mean levels of attacks and for the relative behavior of the varieties.

**Table 11.** AUDPCr calculated for the varieties common.

Accessions	AUDPCr for 19 varieties common			AUDPCr for 32 varieties common	
	2006/07	2008/09	2009/10	2008/09	2009/10
UCT-11Mgb	0.25	0.21	0.19	0.21	0.19
UCT-14MgRe	0.52	0.14	0.36	0.14	0.36
UCT-17Br	0.28	0.17	0.19	0.17	0.19
UCT- 6Gc	0.22	0.07	0.18	0.07	0.18
UCT-24Tn	0.41	0.22	0.17	0.22	0.17
UCT-22Cm	0.39	0.19	0.21	0.19	0.21
UCT-25Gr	0.69	0.20	0.48	0.2	0.48
UCT- 7Ca	0.29	0.12	0.31	0.12	0.31
UCT-18Mn	-	-	-	0.16	0.32
UCT-26Ach	-	-	-	0.03	0.09
UCT-27Mu	-	-	-	0.12	0.08
UCT-28MiR	-	-	-	0.23	0.25
UCT-29Mol	-	-	-	0.18	0.27
UCT- 3Cl	0.34	0.19	0.25	0.19	0.25
UCT- 1Ma	0.38	0.11	0.23	0.11	0.23
UCT-16At	0.34	0.19	0.20	0.19	0.2
UCT-30Ño	0.20	0.04	0.28	0.04	0.28
UCT-19Aq	-	-	-	0.11	0.36
UCT- 2Lv	-	-	-	0.19	0.22
UTC-31Ob	-	-	-	0.09	0.29
UCT-32Ci	-	-	-	0.21	0.45
UCT-20Ro	0.38	0.15	0.23	0.15	0.23
UCT-33Cab	-			0.25	0.45
UCT-15MgRo	0.15	0.10	0.24	0.1	0.24
UCT-21Ac	0.14	0.15	0.37	0.15	0.37
UCT-34Cor	-	-	-	0.01	0.09
UCT-35AzC	-	-	-	0.19	0.29
UCT- 8Gb	0.37	0.14	0.19	0.14	0.19
UCT- 9MgM	0.38	0.09	0.24	0.09	0.24
UCT-10MgL	0.41	0.11	0.26	0.11	0.26
Desirée	0.32	0.12	0.19	0.12	0.19
Karú				0.07	0.17
Means	0.35	0.14	0.25	0.14	0.25
Min	0.15	0.04	0.17	0.01	0.08
Max	0.69	0.22	0.48	0.25	0.48

The level of attacks was the lowest in year 2008-2009 and the highest in 2006-2007. 2008-2009 was also the less discriminatory between varieties with an AUDPCr ranging from 0.04 to 0.22 compared to 0.15 to 0.69 in 2006-2007 and 0.17 to 0.48 in 2009-2010 (table11a). Table 11 based on the whole set of 32 varieties confirms the lower level of attacks and the smaller range of variation for year 2008-2009 compared with 2009-2010. As a consequence the correlations between years were the lower with 2008-2009 (table 12a) and based on the restricted sample of 19 varieties it was null between 2008-2009 and 2009-2010. Nevertheless year 2008-2009 was not so badly correlated with year 2006-2007 (table12a) and we were surprised to find a high correlation between AUDPCr measured on year 2006-

2007 and the average values over years 2008-2009 and 2009-2010 ( $R=0.77$ ,  $P_{value} = 0.00006$ ).

**Table 12 :** Correlations between years for AUDPCr :

a) estimations based on 19 varieties common to the three years of experiments

19 varieties	2006/07	2008/09	2009/10
2006/07	1	$R^2=0.194$	$R^2=0.408$
2008/09	$R=0.441$ $P = 0.030$	1	$R^2=0.0002$
2009/10	$R=0.639$ $P=0.002$	$R=0.013$ $P=0.479$	1

b) estimations based on 32 varieties common to years 2008-2009 and 2009-2010

32 varieties	2008/09	2009/10
2008/09	1	$R^2=0.192$
2009/10	$R=0.438$ $P=0.006$	1

An additional argument in favour of interesting information brought by year 2008-2009 is that the correlation with 2009-2010 was estimated significant and positive when the extended set of 32 varieties was taken into account (table 12b). This result also indicated that the supplementary set of 13 varieties enhanced the genetic variability present in the collection.

**Table 13.** Mean AUDPCr comparisons between 32 varieties tested two different years (2008-2009 and 2009-2010).

		Groupe	AUDPCr $H^2=0.39$
UCT-34Cor		A	0.05
UCT-26Ach		AB	0.06
UCT-27Mu		ABC	0.10
Karú		ABC	0.12
(*) UCT- 6Gc	(ab)	ABC	0.13
(*) Desirée	(abc)	ABC	0.16
(*) UCT-30Ño	(ab)	ABC	0.16
(*) UCT- 9MgM	(bc)	ABC	0.17
(*) UCT- 8Gb	(abc)	ABC	0.17
(*) UCT- 1Ma	(bc)	ABC	0.17
(*) UCT-15MgRo	(a)	ABC	0.17
(*) UCT-17Br	(ab)	ABC	0.18
(*) UCT-10MgL	(bc)	ABC	0.19
(*) UCT-20Ro	(bc)	ABC	0.19
UTC-31Ob		ABC	0.19
(*) UCT-24Tn	(bc)	ABC	0.20
(*) UCT-16At	(abc)	ABC	0.20
(*) UCT-22Cm	(bc)	ABC	0.20
(*) UCT-11Mgb	(ab)	ABC	0.20
UCT- 2Lv		ABC	0.21
(*) UCT- 7Ca	(ab)	ABC	0.22
(*) UCT- 3Cl	(abc)	ABC	0.22
UCT-29Mol		ABC	0.23
UCT-19Aq		ABC	0.24
UCT-28MiR		ABC	0.24
UCT-18Mn		ABC	0.24
UCT-35AzC		ABC	0.24
(*) UCT-14MgRe	(cd)	ABC	0.25
(*) UCT-21Ac	(bc)	ABC	0.26
UCT-32Ci		ABC	0.33
(*) UCT-25Gñ	(d)	BC	0.34
UCT-33Cab		C	0.35

Values with the same letter do not differ significantly from one another according to Duncan's test ( $p>0.05$ ).

$H^2$ =Broad sense heritability based on the mean of the 4 repetitions, years being considered as replications

(\*) Varieties belonging to the restricted set of 19 varieties

(abcd) Classification based on the results of year 2006-2007 only

To confirm this result we carried out an ANOVA on the 32 varieties over both years (2008-2009 and 2009-2010). A complete two factors (Year, Variety and Year x Variety) model was used, the Year by Variety interaction was considered random and used as residual to test the main effects and to compare the means of the different varieties. The main effect of the varieties was found significant over years ( $F_{\text{test}}=2.28$ ,  $P_{\text{value}}= 0.01$ ). The comparisons of

means are presented table13. Based on the mean of the four replications each year, the within year environmental variance of the Variety and of the trait AUDPCr was estimated at 0.0015, where the variances of the Variety and of the Year by Variety effects were estimated having equal values (values 0.0026 for both components). The over year heritability was then estimated at 0.39.

As expected, the 13 additional varieties broadened the available genetic variability within the collection. Table 13 shows they were particularly frequent within the two tails of the distribution. Interestingly three native potatoes appeared the more resistant.

### **3.2.- In-vitro evaluation by inoculation of separated leaflets of resistance to late blight.**

#### 3.2.1.-Percentage of leaflets infected (%infection) by *P . infestans*.

When leaflets of 10 accessions of native potato *Solanum*, an advanced line and the cultivar Craig's Royal (R0) were inoculated with zoospores from isolation *Pi* 287, clear differences were observed in the development of the disease (Figure 9). Table 14 shows the proportions of infected and uninfected leaflets for each variety. These differences were highly significant ( $\text{Chi2}_{(11 \text{ dof})} = 42.81$ ,  $\text{Pvalue} = 1.2 \cdot 10^{-5}$ ). The highest contributions to the Chi2 were those of R-8906384 and UCT-34Cor (14.42 each) both presented 40% of non infected leaflets (16 non infected vs 24 infected). UCT-18Mn and UCT-9MgM, which presented 95% of leaflets with sporulation (38 infected vs 2 non infected) were also important contributors to the Chi2 (4.23 each). Other genotypes had a low contribution to the Chi2. This test revealed three groups (Table 16), one group with the lower probability of infection success (24 infected out of 40), one group with a very high probability of infection success (38 infected out of 40) and a group with medium to high probability of infection success (31 to 36 out of 40, equivalent to 77.5% to 90%). This group included the susceptible control Craig's Royal and the accession UCT-35AzC, both with 36 infected.

In the following, the evaluations of the different resistance components have been carried out on the infected leaflets only. In this way it was possible to compare the behaviours of the varieties once the infection was established. No significant differences were found for any trait measured 72 hours after infection. For all the traits 96h and 120h after inoculation we can note a general trend toward low or very low broad sense heritabilities (Table 15). This is consistent with the results of a previous experiment (data not shown) whose results were

unusable because the number of repetitions were too limited. It was these preliminary results that have led us here to greatly increase the number of repetitions.

### 3.2.- Latency period.

No significant differences were found among varieties for this trait, once a leaflet was infected the pathogen underwent its cycle at the same pace whatever the genotype. The mean duration between the inoculation and the appearance of the first sporangia was about 4 days.



**Figure 9.** Sporulacion on susceptible control: " Craigs Royal " (R0)

**Table 14.** Percentage of infected leaflets (120 hours after inoculation).

Accession	Infected leaflets (%)	Non- infected leaflets (%)
UCT-6Gc	77,5	22,5
UCT-18Mn	95,0	5,0
UCT-26Ach	85,0	15,0
UCT-3Cl	80,0	20,0
UCT-1Ma	85,0	15,0
UCT-30Ño	87,5	12,5
UCT-15MgRo	87,5	12,5
UCT-34Cor	60,0	40,0
UCT-35AzC	90,0	10,0
UCT-9MgM	95,0	5,0
Craig's Royal	90,0	10,0
Line R-8906384	60,0	40,0

### 3.2.3.- Leaf necrosis.

The results for leaf necrosis show highly significant differences among the accessions in the evaluations at 96 (Necr-96h) and 120 hours (Necr-120h) after inoculation (Table 15 and 16). The differences in the presence of leaf necrosis became apparent from 96 hours, reaching their maximum expression at 120 hours after inoculation. Nevertheless the anova showed a slightly better discrimination power at 96h after inoculation, the F values for Necr-96h and Necr-120h were 7.81 and 5.38 respectively. The heritabilities of the mean values ( $H^2_{\text{mean}}$ ) were very high and a reduction of the number of repetitions from 40 to 20 would be quite possible for these traits. UCT-15MgRo expressed the worst behaviour for Necr-96h, followed by UCT-34Cor and UCT-6Gc. The resistant and the susceptible controls were scored equivalent 96 and 120 hours after inoculation, Interestingly the susceptible control "Craig's royal" exhibited the best value for Necr-120h. UCT-15MgRo remained the worst 120 hours after inoculation but UCT-34Cor exhibited an average value. This might indicate a late onset of a resistance process which contributed to the slowdown of the progression of the disease. Four native varieties (UCT -26Ach, -3CI, -1Ma and -30No) behaved quite well 120h after inoculation.

### 3.2.4.- Size of lesion.

The sizes of lesion show significant differences among the potato accessions in the evaluations at 96 ( $\text{Cm}^2$ -96h) and 120 hours ( $\text{Cm}^2$ -120h) after inoculation. The differences in the size of the lesions became clearly apparent from 96 hours, reaching their greatest size at 120 hours after inoculation (Table 15 and 16). The measure after 120h appeared the most discriminant between genotypes (higher F value and higher heritability). The heritability of the means 120h after inoculation was high enough to consider a reduction to 20 repetitions of the experimental design. Some of the native varieties (UCT -3CI, -1Ma and -34Cor) showed a good behaviour, as good as the resistant control. The susceptible control Craig's Royal exhibited the worst behavior for this trait at the two period of scoring.

### 3.2.5.- Level of sporulation.

The levels of sporulation show significant differences among accessions in the evaluations at 96h (Spor-96h) and 120h (Spor-120h) after inoculation (Table 15). Although significant, differences revealed by the test at 96 hours were not very discriminating. For Spor-96h the F test was significant at the 2% level only and the Duncan multiple range test at the 5% level ranked all varieties in a single group. Therefore, in Table 6 groupings are made for this trait

on the basis of Duncan multiple range test at the 10% level and in the following we won't comment anymore the differences between accessions. Concerning Spor-120h it appeared very discriminatory. This shows that sporulation was not yet sufficiently developed 96 hours after inoculation. The F statistic for Spor-120h was the highest of all those observed on all the traits. Consequently Spor-120h also exhibited the highest heritabilities and a reduction in the number of repetitions would be possible while maintaining a strong discriminatory power for this trait. Four accessions (UCT -3CI, -1Ma, -6Gc and -9MgM) expressed the higher levels of sporulation (E, DE or CDE Duncan grouping). Two native accessions (UCT -34Cor, -26Ach) and the susceptible control "Craig's Royal" expressed the lower levels (A and AB Duncan grouping)

### 3.2.6. Overall summary of results on the different traits.

All notations made 72 hours after inoculation showed no significant difference, we can conclude that it is possible to make the economies of these notations. Scoring the percentage of necrosis was found more discriminating at 96 compared to 120 hours after inoculation. The reason was that it was more difficult to differentiate the varieties for which the percentage of necrosis was very high, the mean values among all varieties for Necr-96h and Necr-120h were 31% and 61% respectively. For this character scoring four days after inoculation was optimal when the necrosis occupied about 50% of the leaflet of the more susceptible varieties. Nevertheless, the observed rankings were almost the same from one scoring to another. For the sizes of the lesions the later scoring was more discriminatory but the rankings were broadly equivalent between the two notations. The levels of sporulation were much more discriminatory between varieties only 120h after inoculation.

**Table 15.** Global statistics based on the anovas

Trait	F <sub>(11;396)</sub>	P <sub>value</sub>	H <sup>2</sup>	H <sup>2</sup> <sub>mean</sub>	H <sup>2</sup> <sub>mean20</sub>
Necr-96h	7.81	<10 <sup>-4</sup>	0.17	0.87	0.77
Necr-120h	5.38	<10 <sup>-4</sup>	0.12	0.81	0.69
Cm <sup>2</sup> -96h	3.07	0.0006	0.06	0.67	0.51
Cm <sup>2</sup> -120h	6.36	<10 <sup>-4</sup>	0.14	0.84	0.73
Spor-96h	2.09	0.0204	0.03	0.52	0.35
Spor-120h	10.4	<10 <sup>-4</sup>	0.23	0.9	0.82

H<sup>2</sup>: broad sense heritability = VarG/(VarG+VarR)

H<sup>2</sup><sub>mean</sub> : VarG/(VarG+VarR/N<sub>rep</sub>)

H<sup>2</sup><sub>mean20</sub> : VarG/(VarG+2\*VarR/N<sub>rep</sub>)

**Table 16.** Comparison of means for the different traits

Accession	%	Infection (*)	Necr-96h (**)	Necr-120h (**)	Cm2-96h (**)	Cm2-120h (**)	Spor-96h (#)	Spor-120h (**)						
UCT-6Gc	0.78	B	39.2	CDE	70.3	BC	3.1	AB	6.4	AB	2.0	AB	2.6	CDE
UCT-18Mn	0.95	C	30.8	ABCD	69.8	BC	3.7	AB	7.7	ABC	1.9	AB	2.2	ABC
UCT-26Ach	0.85	B	24.3	ABC	51.6	AB	3.4	AB	8.1	BC	1.8	A	2.0	AB
UCT-3Cl	0.80	B	27.3	ABCD	57.9	AB	2.6	A	6.4	AB	2.2	B	2.8	E
UCT-1Ma	0.85	B	28.0	ABCD	52.6	AB	2.8	A	5.8	AB	2.2	B	2.6	DE
UCT-30Ño	0.88	B	<b>28.6</b>	ABCD	53.4	AB	3.8	AB	8.3	BC	2.0	AB	2.3	ABCD
UCT-15MgRo	0.88	B	52.9	E	80.5	C	3.5	AB	5.6	AB	2.2	B	2.4	BCD
UCT-34Cor	0.60	A	41.5	DE	59.8	ABC	2.7	A	4.9	A	1.9	AB	2.0	A
UCT-35AzC	0.90	B	31.5	ABCD	68.9	ABC	3.8	AB	7.8	BC	2.1	AB	2.3	ABCD
UCT-9MgM	0.95	C	35.1	BCD	67.1	ABC	4.3	AB	8.3	BC	2.1	AB	2.5	CDE
Craig's Royal	0.90	B	18.6	AB	48.0	A	4.9	B	10.3	C	2.0	AB	2.1	AB
Line R-8906384	0.60	A	17.5	A	50.4	AB	2.5	A	6.9	AB	1.8	A	2.2	ABC

(\*) The grouping for % infection is based on the Chi2 test

(\*\*) Duncan grouping at the 5% level, varieties with the same letter are not statistically different

(#) Duncan grouping at the 10% level, varieties with the same letter are not statistically different

### 3.2.7. Correlations between *in vitro* components and field resistance

We wanted to correlate the field evaluation with the *in vitro* components of the resistance. We selected the average value of the character AUDPCr (*cf.* chapter VI) over two years of field experiments to characterize the field resistance of the ten native varieties. Unfortunately, the controls Craig's Royal and Line R-8906384 were absent from the field experiments so that the correlation could only be estimated among ten varieties. Consequently the power to detect nonzero correlations with only eight degrees of freedom was low and extrapolations of the findings to larger collections of genotypes should be considered with caution and may not be reliable. Nevertheless we found some interesting correlations (Table 17). We recall here that for AUDPCr the best two accessions of the sample of 32 experimented in the field were UCT -34Cor and -26Ach with respective values of 0.05 and 0.06. The accessions UCT -35AzC and -18Mn had the worst AUDPCr among the ten accessions experimented *in vitro*. They were 26<sup>th</sup> and 27<sup>th</sup> in the sample of 32 varieties with values of 0.24 for both.

**Table. 17.** Correlations between traits estimated on the sample of 10 native accessions

Traits	AUDPCr	%infection	Necr-96h	Necr-120h	Cm2-96h	Cm2-120h	Spor-96h
%infection	<b>0.65*</b>						
Necr-96h	-0.15	-0.25					
Necr-120h	0.35	0.24	<b>0.77**</b>				
Cm2-96h	0.31	<b>0.76**</b>	0.03	0.36			
Cm2-120h	0.28	<b>0.73*</b>	<b>-0.56#</b>	-0.15	<b>0.78**</b>		
Spor-96h	<b>0.58#</b>	0.19	0.21	0.25	-0.17	-0.36	
Spor-120h	<b>0.55#</b>	0.23	-0.12	0.04	-0.16	-0.08	<b>0.81**</b>

Values in bold are significantly different from zero at the thresholds \* 0.1, \* 0.05, and \*\* 0.01

As expected all the pair of successive notations of the same trait were highly correlated. The percentage of success of infection and the size of the lesions were the only two *in vitro* components correlated together. A negative correlation, only significant at the 10% level, was found between Necr-96h and Cm2-120h, this might have happened by chance, the three other correlations between the leaf necrosis measurements and the sizes of the lesions were not significant. There is no obvious explanation to this negative correlation, as we have already mentioned if a correlation were to exist between those two traits it would be expected positive.

Regarding the correlation between traits measured *in vitro* and AUDPCr only the correlation between the percentage of *in vitro* infection success and the AUDPCr measured in the field were significantly correlated at the 5% level. Another correlation, only significant at the 10% level, was found between AUDBPCr and the levels of sporulation.

## **IV.- DISCUSSION**

### **4.1.- Field characterization of resistance to late blight.**

The prevailing climatic conditions during the three farming seasons led to development of the disease in varying degrees of incidence and severity. The results show that native potatoes contain material with widely varying response and behaviour with respect to late blight. In a comparative analysis of the relative AUDPC of the different materials (Table 10) at least three accessions may be distinguished with high resistance to the disease, presenting low relative AUDPC values, namely UCT-34Cor, UCT-26Ach and UCT-27Mu. Accession UCT-34Cor presented relative AUDPC values of 0.01 and 0.09 for the second and third seasons respectively. The results obtained show that accession UCT-25Gñ was always among the accessions with the highest AUDPC, translating into high relative AUDPC indices with values of 0.69, 0.20 and 0.48 for the three successive seasons. A similar tendency is observed for accessions UCT-32Ci and UCT.33cab.

Wide variation in response to this disease has been reported by various authors, including Colon (1994); Micheletto *et al.*, (2000); Flier *et al.* (2003); Jenkins and Jones (2003); Barquero *et al.* (2005); Lucca *et al.* (2008); Andreu *et al.* (2009) and Mendoza (2010). In this respect Barquero *et al.* (2005) report the existence of important differences between genotypes resulting from crosses with different wild species. They also indicate that the level of resistance present within groups of genotypes varies according to the locality, suggesting that the presence of resistance is influenced by the effect of major genes, the expression of which is dependent on the variation in the avirulence genes in the population of *P. infestans* present in the locations where the crop is cultivated. Likewise genotypes resulting from somatic hybridizations or crosses with the wild species *S. bulbocastanum*, *S. circaeifolium* and *S. okadae* were those which presented the lowest values for the area under the disease progress curve with values of 60, 80 and 79 respectively compared with the varieties Alpha, Waych'a, Pimpernell and Granola, used as controls, which presented the highest values of 477, 474, 466 and 427 respectively. They show that the resistance level may be defined under two categories: one as a high level of partial resistance and the other as complete resistance. In situations where there is a wide range of levels of infection, these suggest an incomplete, high-level, non-specific resistance. In this context, Colon (1994) adds that a wide expression of resistance in different accessions of a single genotype suggests the presence of numerous minor genes with additive effects scattered in the different genotypes. On the other hand an evaluation of uniform resistance may indicate complete or vertical resistance due to the presence of major genes.

The results of field experiments conducted by Flier *et al.* (2003) indicate differences between cultivars in terms of the stability of partial resistance. The level of resistance to late blight varies widely from very susceptible to moderately resistant in terms of AUDPC and percentage of blight in tubers. They report significant values for the effects of cultivar, isolation, years, cultivar by isolation and cultivar by years. The presence of a differential interaction which is independent of R-genes indicates some degree of adaptation to partial resistance of *P. infestans*, with the consequent effects on the stability and durability of the resistance. They add that the cultivar Bintje was the most susceptible, with AUDPCr of 52.1%. The cultivars Santé and Pimpernel presented intermediate levels of resistance with AUDPC values of 21.9% and 24.9% respectively. Finally, the most resistant cultivar was Karnico, with AUDPC of 1.9%. Other evaluations of quantitative resistance under field and greenhouse conditions, done by Micheletto *et al.* (2000) on wild diploid genotypes from Argentina of the species *Solanum chacoense*, *Solanum commersonii*, *Solanum microdontum* and *Solanum maglia*, were able to identify both highly resistant and susceptible genotypes. The AUDPC results show a high degree of variation at the level of wild species, and within each species. The author adds that there were also important differences between genotypes within each accession. Therefore the specificity of the accessions for the proportion of resistance is an important factor in projecting horizontal resistance. The AUDPC showed a high degree of variability between and within species, which confirms the value of the Argentinean species in improving resistance. These reports agree with the findings of Lucca *et al.* (2008), who state that the variability observed in field trials with *S. tuberosum* ssp. *andigena* (adg) and *S. tarinjense* reveal significant differences between the genotypes in the partial and final area under the disease progress curve. Genotypes of *S. tarinjense* which presented high AUDPC values were Oka 5880.22 and Hof 1717.10. Likewise Andreu *et al.* (2009), evaluating the susceptibility of ten cultivars in Argentina, report that the cultivar Shepody was the most susceptible with a value of 8 (on a scale of 1-9, where 9 is highly susceptible) and the highest AUDPC values. The cultivar Ranger Russet presented values close to 2.5, with resistance which may not be race-specific. Finally, the AUDPCr values observed in the present study are lower than those reported by Mendoza (2010) in Cameroon, who reports AUDPCr values of 0.39 and 0.49 for resistant and moderately resistant material respectively. Clones (free of R-genes) resistant to the disease presented AUDPCr values of 0.23.

Andrivon *et al.* (2003), after evaluating partial resistance of potato cultivars to natural epidemics of *P. infestans* over three years, indicate that the severity of the disease was significantly lower in susceptible cultivars when they were planted in rows alternating with

cultivars which had partial resistance. Mixing cultivars produced a large reduction in the rate of progress of the disease. Furthermore, cultivars provided with partial resistance showed similar behaviour whether planted alone or in a mixture. Mixing cultivars significantly reduced polycyclical natural epidemics, caused by pathogens which produce very rapid lesions in the foliage. The author indicates that the reduction in the area under the disease progress curve in mixed plantations results from the cumulative action of lesser effects. Likewise, Andrivon *et al.* (2007) indicate that the high susceptibility of the cultivar Desirée detected in plantations in Morocco may be a diagnosis of the erosion of partial resistance in this cultivar. Partial resistance is subject to erosion generated by long term exposure to aggressiveness.

Huarte and Capezio ( 2003) indicate that genotype by environment (GxE) interactions in resistance to late blight have not yet been clearly determined. Studies with families of tubers would seem to indicate important interactions between genotypes and locations with different day lengths. The absence of genotype by environment interactions has also been noted by Andrivon *et al.* (2007) for different varieties in different locations in Morocco. In general terms, the significance of the interaction does not appear to be greater than the variation due to genotype and location. This is in accordance with our results that show an interesting level of repeatability over years. .

#### **4.2.- In-vitro evaluation by inoculation of separated leaflets of resistance to late blight.**

The inoculation and incubation conditions of separated leaflets allowed optimum environments to be generated for the characterisation of the native potato accessions under the effects of the disease. Our results suggest that it is possible to reduce the complexity of the *in vitro* experiment in order to test more genotype for the same experimental investment. Measures realized 72 hours after inoculation have no interest since they are not discriminatory and a reduction of the number of repetitions from 40 to 20 would not affect greatly the discriminatory power of the different traits when they are measured at the optimum stage. The heritabilities based on the means of the traits measured 120h (leaf necrosis should preferentially be measured at 96h) after inoculation remained high (> 0.7) when dividing by two the number of repetitions.

Inoculation with a complex isolation of a fungus which carries 10 avr genes identified in the country demonstrates the presence of a wide range of expression of partial resistance to late blight, coinciding with the findings reported by Micheletto *et al.*, (2000); Barquero *et al.*

(2005b) and Lozoya *et al.* (2006). The resistant control R-8906384 showed an excellent behavior for each component of the resistance. He is always in the best group for each measurement. It is even consistently classified as first for the various measurements made 96 hours after inoculation. No native accession so well behaved, however UCT-34Cor ranked consistently among the very best regardless of the measured component except for Necr96h. UCT -26Ach performed also quite well for the different components except for the size of the lesion 120h after inoculation. No accession was found the worst for all the components, for example UCT-3CI and UCT-1Ma which were among the worst for the level of sporulation behaved very well for the size of the lesion. Even the variety described as susceptible showed good performance for some traits, such as leaf necrosis or the level of sporulation. These results suggest that resistance factors are present in different varieties, even those considered susceptible. It is only the combination of these different components that leads to a good level of resistance. In accordance to the results of Colon *et al.* (1995) which consider that the most important resistance components in *Solanum tuberosum* are efficiency of infection (equivalent to the probability of infection success), growth rate of the lesion and sporulation capacity, R-8906384 the resistant control and UCT-34Cor which had the best performance in the field trials revealed the highest performance for these three components. We also found a positive correlation between the probability of *in vitro* infection and the AUDPCr measured in the field. A lower correlation (only significant at the 10% level) was found between the AUDPCr and the level of *in vitro* sporulation and that for both measurements made 96h or 120h after inoculation. Results from Flier *et al.* (2003) show that the sporulation density and the growth rate of the lesion are not correlated with the leaf resistance measured by the AUDPC. Barquero *et al.* (2005b) report low correlation between the field AUDPC data and evaluations of separated leaflets in the laboratory. The possible causes of this low correlation are the influence exercised by environmental and genetic factors which determine resistance and the impossibility of finding an individual offering all the genetic factors which determine the virulence of the pathogen (Al-Kherb *et al.*, 1995). Zúñiga *et al.* (2000) report that under field conditions the correlation between the time of appearance of symptoms and the AUDPC was negative. Nevertheless, the correlation between the AUDPC and the development rate of the disease was positive.

In the present study, although resistance factors were present in different varieties and accumulated in some more than others, we did not observed completely immune varieties. In the last decade the populations of *P. infestans* in southern Chile have undergone a genetic change (Acuña *et al.*, 2007), which is related to the severity of the attacks in recent seasons. Changes in aggressiveness during the course of an epidemic suggest selection for more aggressive isolations. This selection may affect the durability of partial resistance and

may explain the progressive reduction in the partial resistance of the materials. Although only the asexual phase of *P. infestans* is known in Chile, it is possible that specificity for stability to late blight may exist in the Chilean germplasm, stimulated by genetic modification of the populations or immigration of new pathotypes. In this respect, Swiezynski *et al.* (2000) mention that symptoms of field resistance are difficult to separate from the incomplete resistance provided by the R-genes. Zúñiga *et al.* (2000) add that the quantitative expression of resistance is in itself insufficient proof of the effective absence of R-genes. Nevertheless, the resistance observed in the *Solanum* accessions is probably due to the action of minor genes, since the isolation Pi287 is carrier of all the avr factors identified in Chile (avr1, avr2, avr3, avr4, avr5, avr6, avr7, avr8, avr10, avr11), a situation which would stimulate the expression of real field resistance, uniformly distributed against all the races of the pathogen, emphasising the differences between the varieties when they grow in the same environment in Chiloe. It is also the case that the use of fungicides to control the disease is not usual in Chiloe Island. The above may explain in part the frequent strategy of establishing the crop with a mixture of varieties for protection against attacks of late blight.

Flier *et al.* (2003), evaluating the resistance of potato cultivars to aggressive races of *P. infestans* in the field and in the laboratory, report the presence of interaction effects between the cultivar and the isolation on the growth rate of the lesion and the sporulation density. This differential interaction is independent of the resistance based on R-genes, and indicates some degree of adaptation of *P. infestans* to partial resistance, with the consequent adverse effects on the stability and durability of the partial resistance. The results indicate specificity between isolations of *P. infestans* and the partial resistance of potato cultivars. Furthermore, compatibility studies using separated leaflets do not report incompatibility between cultivars and isolations in field conditions. These results also agree with those of Colon *et al.* (1995), who evaluated 22 cultivars exempt from R-genes in Holland (1929-1954) and found that the quantitative resistance was effective even after 40 years, evidence of its high durability although the complexity of the pathogen population has evolved due to the presence of both mating types (A1 and A2).

## V.- CONCLUSIONS

In general for field characterization of resistance to late blight, it may be concluded that in the native potato studied there exists a wide variation in response to and behaviour under attack of late potato blight. The presence of resistant material offers great possibilities for exploiting and making use of this material, either directly by integrated crop management or indirectly by incorporation into breeding programmes. Genetic resistance to late blight is a practical and economic method of combating the disease. Today, emphasis is being placed on non specific resistance, which is considered a more lasting option against the many variants or races of this pathogen.

In the comparative analysis of the AUDPCr at least three accessions were distinguished with high resistance to the disease and low AUDPCr values. These were UCT-34Cor, UCT-26Ach and UCT-27Mu. The majority of the native potato accessions fall into the range moderately resistant to moderately susceptible. This might be explained on the one hand by the cultivation of potatoes in Chiloe without the use of fungicides to control blight, and on the other by the traditional establishment of mixtures of varieties in order to reduce damage caused by the disease.

For in-vitro evaluation, the results allowed to propose a less onerous *in vitro* protocols in order to test more genotypes for the same level of experimental investment. The different resistance components demonstrated that within the material of Chilean native *Solanum*, there is a wide range of variation to late blight. The resistant control (R-8906384) and the accessions UCT-34-Cor presented the lowest infection efficiency, small sizes of the lesions and low levels of sporulation classifying them as the most resistant. This suggests low efficiency of the infection and high resistance to penetration in these materials. The more susceptible accessions often presented a good level of resistance for one or few components. It would be of great interest to go into the genetic control of the different resistance components to know if the same level of resistance expressed for a component by different accessions is under the same genetic control or not. Deciphering the genetic architecture of the non specific resistance is a challenge for the development of long lasting resistant varieties.

In the context of low input traditional production, the cultivation in mixture of genetically diverse varieties exhibiting different levels and mechanisms of resistance present an insurance against crop losses. The knowledge of the resistance components present in the different varieties may help to give scientific based advices and may also help to drive the

evolution of the mixture of varieties in response to the evolution of the pathogenic populations.

## VI.- LITERATURE CITED

- Acuña, I., B. Sagredo., R. Bravo., M. Gutiérrez., I. Maldonado., N. Gaete., J. Inostroza., G. Secor, V. Rivera., J. Kalazich., J. Solano, y J. Rojas. 2007. Using a forecasting system to develop integrated pest management strategies for control of late blight in southern Chile. Proceeding of the tenth workshop of an European network for development of an integrated control strategy of potato late blight. Bologna. Italia. 237-249.
- Acuña, I., R. Bravo., J. Inostroza., N. Gaete., I. Maldonado., J. Solano., C. Barrientos y M. Vargas. 2008. El tizón tardío en Chile. En : XXIII Congreso de la Asociación Latinoamericana de la papa y VI Seminario latinoamericano de Uso y Comercialización de la Papa: Memorias. 1<sup>a</sup> ed. Mar del Plata,: Universidad Nacional de Mar del Plata. Argentina. 30 Noviembre al 6 de Diciembre. 516p.
- .Andreu, A., D. Caldiz and G. Forbes. 2009. Phenotypic expression of resistance to *Phytophthora infestans* in processing potatoes in Argentina. American Journal of Potato Research. 87(2):177-187.
- Andrivon, D., Pilet, F., Montarry, J., Hafidi, M., Corbiere, R., Achbani, E., Pelle, R. y D. Ellisache. 2007. Adaptation of *Phytophthora infestans* to partial resistance in potato: evidence from French and Moroccan populations. Phytopathology. March 2007 Vol 97 (3): 338-343.
- Andrivon, D., Lucas, J. and Ellisache. 2003. Development of natural late blight epidemics in pure and mixed plots potato cultivars with different levels of partial resistance Plant Pathology 52(5):586-594.
- Al-kherb, S.M., Fininsa, C., Shattock, R.C., and D. S. Shaw. 1995. The inheritance of virulence of *Phytophthora infestans* to potato. Plant Pathology 44:552-562.
- Arentsen, S. 1994. Fitopatología Chilena, Historia y Desarrollo. Conference at VII Meeting of the Latinoamerican Phytopathological Society. Santiago. Chile.
- Barandalla, L., R. López., J. Ruiz de Galarreta, y E. Ritter. 2008. Evaluación frente a diferentes patógenos de una colección de cultivares antiguos y germoplasma nativo de papa. En: III Congreso Iberoamericano de Investigación y Desarrollo en Patata. Avances en Ciencia y Desarrollo de la Patata para una Agricultura Sostenible. Edicion: Administración de la Comunidad Autónoma del País Vasco. Departamento de Agricultura, Pesca y Alimentación. 5-10 de Octubre, 2008. Pp 59-62.

- Barquero, M., L. Gómez. y A. Brenes. 2005a. Resistencia al tizón tardío (*Phytophthora infestans*) en clones promisorios de papa en Costa Rica. *Agronomía Costarricense*. 29(3):31-45.
- Barquero, M., L. Gómez. and A. Brenes. 2005b. Resistencia al tizón tardío (*Phytophthora infestans*) en clones promisorios de papa en Costa Rica. *Agronomía Costarricense*. 29(3):31-45.
- Ciampi, L. 2001. La patología vegetal en Chile. *Revista Asociación Chilena de la Papa*. 3(9):1-2.
- Colon, L., Budding, D., Keizer, L., and Pieters, M. 1995. Components of resistance to late blight (*Phytophthora infestans*) in eight South American *Solanum* species. *European Journal of Plant Pathology* 101(4):441-456.
- Colon, L., Nielsen. B., and U. Darsow. 2004. EUCLIGHT-Detached leaf test for foliage blight resistance. EUCLIGHT.
- Díaz, M., D. Fajardo., J. Dilmer., C. García y V. Núñez. 2003. Identificación de genes R1 y R2 que confieren resistencia a *Phytophthora infestans* en genotipos colombianos de papa. *Revista Colombiana de Biotecnología* 5(2):40-50.
- Escalante, M., y C. Armas. 2004. Evaluación epidemiológica y económica en el manejo del tizón tardío de la papa en las Playitas, Estado Táchira, Venezuela. *Bioagro*. 16(3):189-196.
- Estrada, R.N., E. Fernández-Northcote, E, Carrasco y O. Navia. 1994. Mejoramiento genético para resistencia a enfermedades y plagas de la papa en Bolivia. En Broers LHM (ed.). *Memorias del 1<sup>er</sup> Taller Sobre Resistencia Duradera en Cultivos Alto Andinos de Bolivia, Colombia, Ecuador y Perú*. 30 de mayo a 03 de junio de 1994. INIAP-WAU-DGIS. Quito, Ecuador. pp 86-89.
- Flier, W., L. Turkensteen and A. Mulder. 1998. Variation in tuber pathogenecity of *Phytophthora infestans* in the Netherlands. *Potato Research* 41, 345-354.
- Flier, W., G. van den Bosch and L. Turkensteen. 2003. Stability of partial resistance in potato cultivars exposed to aggressive strain of *Phytophthora infestans*. *Plant Pathology*. 52, 326-337.
- Flor, H. 1971. Current status of the gene-for-gene concept. *Annual Review of Phytopathology* 9:275-296.
- Forbes, G., M. Chacón., H. Kirk.,M. Huarte., M. Van Damme., S. Distel., G.Mackay., H. Stewart., R. Lowe., J. Duncan., H. Mayton., W. Fry., D. Andrivon., D. Ellisséche., R. Pellé., H. Platt., G. Mackenzie., T. Tarn., L. Colon., D. Budding., H. Lozoya-Saldaña., A. Hernandez-Vilchis. and S. Capezio. 2005. Stability of resistance to *Phytophthora infestans* in potato: an international evaluation. *Plant pathology*. 54:364-372.

- Fry, W. 2008. *Phytophthora infestans*: the plant (and R gene) destroyer. *Mol. Plant Pathol.* 9(3): 385-402.
- Fry, W., and S. Goodwin. 1997. Re-emergence of potato and tomato late blight in the United States. *Plant Disease* 81:1349-1357.
- Gabriel, J., F. Forqueda., G. Plat y E. Fernández-Northcote. 2007a. Caracterización de genotipos de papa de Europa y Latinoamérica por resistencia a tizón y propiedades culinarias. *Revista latinoamericana de la Papa*. 14(1):10-23.
- Gabriel, A., G. Coca, J. Plata., and J. Parlevliet. 2007b. Characterization of the resistance to *Phytophthora infestans* in local potato cultivars in Bolivia. *Euphytica* 153: 321-328.
- Goodwin, S., L. Sujkowski and W. Fry. 1995. Rapid evolution of pathogenicity within clonal lineales of the potato late blight disease fungus. *Phytopathology* 85: 669-676.
- Henfling, J., W. 1982. Field screening procedures to evaluate resistance to late blight. CIP Technology Evaluation Series No. 1982-5. Available at <http://www.cipotato.org/library/pdfdocs/TechEval21147.pdf>. Accessed 15 April 2009.
- Henfling, W. 1987. El tizón tardío de la papa. 415 p. CIP Centro internacional de la papa. Boletines de información técnica. Capacitaciones y comunicaciones CIP. Lima, Perú.
- Hijmans, R., G. Forbes y T. Walter. 2000. Estimating the global severity of potato late blight with GIS-linked disease forecast models. *Plant Pathology*. 49: 697.705.
- Huarte, M y S. Capezio. 2003. Niveles disponibles de resistencia al tizón tardío. idiaXXI. Revista de información sobre investigación y desarrollo agropecuario. Vol 3(4):101-107.
- I.N.E. 2007. VII Censo Nacional Agropecuario. Instituto Nacional de Estadística. Disponible en <http://www.censoagropecuario.cl/noticias/08/6/10062008.html>. Leído el 03 mayo de 2010.
- Jaramillo, S. 2003. Monografía sobre tizón tardío. Universidad Nacional de Colombia. Disponible en <http://www.reuna.educo/temporales/memorias/Monografia.pdf>. Leído el 24 de Abril de 2007.
- Jenkins, J.C. and R. K. Jones. 2003. Classifying the relative host reaction in potato cultivars and breeding lines to the US-8 strain of *Phytophthora infestans* in Minnesota. *Plant. Dis.* 87:983-990.
- Kalazich, J. 2006. Variedades de papa. p. 49-64. In Rojas, J. S., y S. Orena (Eds.). Manual de producción de papa para la agricultura familiar campesina (AFC). Boletín N°147. Instituto de investigaciones agropecuarias (INIA). Osorno, Chile.
- Lozoya-Saldaña, H., Guzman-Galindo, L., Fernández-Pavia, S., Grünwald and E, McElhinny. 2006. *Phytophthora infestans* (Mont.) de Bary. I. Host-pathogen specificity and resistance components. *Agrociencia* 40 (2): 205-217.

- Lucca, F., C. Bedogni., P. Cicori., M. Huarte y S. Capezio. 2008. Comportamiento de genotipos de *Solanum tarjense*, *S. Gourlayi* y *S. tuberosum* ssp. *andigena* frente al tizón tardío en el se bonaerense argentino. En: III Congreso Iberoamericano de Investigación y Desarrollo en Patata. Avances en Ciencia y Desarrollo de la Patata para una Agricultura Sostenible. Edicion: Administración de la Comunidad Autónoma del País Vasco. Departamento de Agricultura, Pesca y Alimentación. 5-10 de Octubre, 2008. Pp: 49-51.
- McLeod. A., S. Denman., A. Sadie., and F. Denner. 2001. Characterization of South African isolates of *Phytophthora infestans*. *Plant Disease* 85(3): 287-291.
- Mendoza, H. 2010. Aspectos de la evaluación de la resistencia a la rancha, *Phytophthora infestans*. Memorias del XXIV Congreso Latinoamericano de la Papa. Asociación Latinoamericana de la papa ALAP y I Simposium Internacional de Recursos genéticos de la Papa. Instituto Nacional de Innovación Agraria - INIA. Cusco, Perú. Pp:219-220.
- Micheletto, S., Boland, R. y M. Huarte. 2000. Argentinian wild diploid *Solanum* species as sources of quantitative late blight resistance. *Theor. Appl. Genet.* 101:902-906.
- Micheletto, S., M. Andreoni and M. Huarte. 1999. Vertical resistance to late blight in wild potato species from Argentina. *Euphytica* 110: 133-138.
- Navia, O., Naerstad. R., Hermansen, A. and T. Bjor. 2001. Exploiting host resistance to reduce the use of fungicides to control potato late blight. *Plant Pathology*. 56: 156-166.
- Naerstad, R., A. Hermansen and T. Bjor. 2007. Exploiting host resistance to reduce the use of fungicides to control potato late blight. *Plant Pathology* 56: 156-166.
- Ríos, L. 1999. El tizón tardío. Una amenaza permanente. *Revista Asociación Chilena de la Papa* N° 3 p. 13-14.
- Parlevliet, J.E. 2002. Durability of resistance against fungal, bacterial and viral pathogens; present situation. *Euphytica* 124:147-156.
- Páez, O., R. Valverde., L. Gómez., A. y Brenes. 2005. Diversidad genética de aislamientos de *Phytophthora infestans* en plantaciones de papa en Costa Rica con el uso de RAPDS. *Agronomía Costarricense* 29(1): 41-55.
- Pérez, W., y G. Forbes. 2008. El tizón tardío de la papa (manual técnico). Disponible en <http://www.cipotato.org/publications/pdf/004271.pdf>. Leído el 03 de marzo de 2009.
- Pérez, W., S. Gamboa., y. Falcon., M. Coca., R. Raymundo and R, Nelson. 2001. Genetic structure of Peruvian populations of *Phytophthora infestans*. *Phytopathol.* 91(10): 956-965.
- Ristaino, J. B. 2009. Historical and Current Population Biology of late blight pathogen: from the Andes to the Irish Potato famine. En XV Congreso Latinoamericano de

Fitopatología. Asociación Latinoamericana de Fitopatología. Santiago, Chile. 12-16 de enero de 2009.

Robinson, R. 1996. Return to resistance: breeding crops to reduce pesticide dependence. David California (USA) Ag Access. 480 pp.

Secor, G., V. Rivera., A. Thomson y J. Rodriguez. 2009. The continuing battle for late blight resistance potato cultivars. En XV Congreso Latinoamericano de Fitopatología. Santiago, Chile. 12-16 de enero de 2009. Secor, G., V. Rivera., A. Thomson and J. Rodriguez. 2009. The continuing battle for late blight resistance potato cultivars. En XV Congreso Latinoamericano de Fitopatología. Santiago, Chile. 12-16 de enero de 2009.

Simko, I., S. Costanzo., V. Ramanjulu., B. Christ., and K. Haynes. 2006. Mapping polygenes for tuber resistance to late blight in a diploid *Solanum phureja* X *S. stenotomum* hybrid population. Plant Breeding 125:385-389.

SPSS Inc. 2006. SPSS Base 15.0 user's guide. Chicago, IL, USA. 591 pp.

Staskawiez, B.J., Ausubel, F.M., Baker, B.J., Ellis, J.G and J.D.G. Jones. 1995. Molecular genetics of plant disease resistance. Science 268: 661-667.

Swiezynski, K., L. Domanski., H. Zarzycka., E. Zimnoch-Guzowska. 2000. The reaction of potato differentials to *Phytophthora infestans* isolates collected in nature. Plant Breeding 119:119-126.

Torres, H. 2002. Manual de las enfermedades más importantes de la papa en el Perú. Centro Internacional de la papa. Lima, Perú. 59 p.

Trujillo, G. 2004. Desarrollo de marcadores SCAR y CAPS en un QTL con efecto importante sobre la resistencia al tizón tardío de la papa. Tesis de Grado en Biología. Facultad de Ciencias Biológicas, E.A.P. de Ciencias Biológicas. Universidad Nacional Mayor de San Marcos. Lima, Perú. 89p.

Turkensteen, L. J. y W. G. Flier, 2002. Late blight: Its global status in 2002 and beyond. Pp.1-9 Proc.Global Initiative on Late Blight Conf., 11-13 July 2002. Hamburg. Umaerus, V. and M. Umaerus, 1994. Inheritance of resistance to late blight. In N Bradshaw JE., Mackay GR (eds). Potato Genetics. CAB International, Wallingford, Oxford, UK, pp365-401.

Visker, M. 2005. Association between late blight resistance and foliage maturity type in potato. Physiological and genetic studies. Wageningen University, the Netherlands. 160 p.

Visker, M., Keizer, L., Budding, D., Van Loon, L., Colon, L., and Struik, P. 2003. Leaf position prevails over plant age and leaf age in reflecting resistance to late blight in potato. Phytopatology 93:666-674.

- Wastie , R. L. 1991. Breeding for resistance. In: Ingram DS and Wiliams DS (eds) .*Phytophthora infestans*, the cause of late blight of potato. Advances in Plant Pathology, Vol 7) pp 193-224) Academic Press Ltd, London.
- Yuen, J. E. and G. A. Forbes. 2009. Estimating the level of susceptibility to Phytophthora infestans in potato genotypes. *Phytopatology*. 99:782-786.
- Zlesak, D. and C. Thill. 2004. Foliar Resistance to *Phytophthora infestans* (Mont.) de Bary (US-8) in 13 Mexican and South American *Solanum Species* Having EBNs of 1, 2, and 4 and Implications for Breeding. *American Journal of potato Research*. 81(6):421-429.
- Zúñiga, L., J. Molina., M. Cadena y A. Rivera. 2000. Resistencia al tizón tardío de la papa (*Phytophthora infestans*) en cruzamientos de cultivares y clones de papa (*Solanum tuberosum* L.). *Revista Mexicana de Fitopatología*. 18(1):1-9.

## **GENERAL DISCUSSION.**

The so-called "native potatoes" are non-commercial varieties of potato, but have the ability to produce tubers and grow under very limited conditions and agronomic management in very adverse environment. For many years these "native potatoes" have been selected by local farmers in South America, Peru, Bolivia and Chile. These farmers were able to select and maintain a high diversity of germplasm with a wide panel of organoleptic qualities and culinary uses and various levels of resistance to diseases and biotic stress. Therefore, this material reaches a value as a genetic resource of incalculable value which requires countries that possess them to characterize, evaluate, store and finally to use them. Chile is considered a sub-center of origin of the cultivated potato. Thus, many existing native varieties show a rich diversity of shapes, colors and flavors, making it worth not only for direct use as food but also as a source of genes for crop improvement programs worldwide. Recent research has confirmed that the Chilean germplasm has contributed to the improvement of the potato in Europe from selections made before the late blight epidemic in 1840. Studies based on molecular markers diversity has shown that 99% of potato varieties in the world have Chilean potato genes, thereby making it an invaluable source for genetic improvement programs, as they correspond to clones that have been for centuries a kind of natural geographic isolation, without being subjected to gene introgression from other sources. The use of this material requires achieving the tasks of collection, conservation (*in-situ* and *ex situ*), characterization and evaluation to determine their characteristics.

This thesis falls within the general framework for the study of genetic diversity of native potatoes in Chile. The work focuses on conservation and characterization. Finally, it makes a specific assessment of the native material for resistance to late blight (*Phytophthora infestans*), the main crop disease in Chile and the world. The overall objective was to assess the genetic diversity of a collection of native potato varieties originating from the island of Chiloe (made by us from 2000 to 2007), to characterize the resistance of these varieties to late blight (*Phytophthora infestans*) and to inquire about the status of *in-situ* conservation. For this we have developed the following specific topics: a) conservation *in-situ* of native Solanum within the great isle of Chiloe and its impact on diversity, b) evaluation of the morphological diversity of the native potatoes that we have collected c) assessment of genetic diversity of the collection by the mean of microsatellite and AFLP markers, d) characterization of field and *in-vitro* resistances to late blight (*Phytophthora infestans*) of some of the accessions of the collection.

Based on the results of the surveys, we can conclude that *in-situ* maintenance of native potato diversity is not well preserved, due to strong social and economic changes on the island of Chiloe. Native varieties were part of a traditional culture but social relations and agriculture are changing. On the one hand, young people prefer activities in the business of the city, while other farmers who have farms of sufficient size are turning to products whose markets are guaranteed beyond subsistence farming and local markets. There is a clear process of market penetration of commercial cultivars in response to market conditions which imposed defined types of tubers, produced on a regular basis at competitive prices. This favors the replacing of native varieties for commercial cultivars. The lack of profitable opportunities and capacities to absorb sufficient amounts of added values limits the production of native potatoes. To maintain interest in the production of native potato it is necessary to develop a market lucrative enough that, given the specificity of the product, will occupy a niche market. The product "native potatoes of Chiloe" could be valued for its authenticity, its diversity, its history, the tradition it represents. For some varieties it would be necessary ensure their availability, a high level of quality and provide appropriate recipe to give them a competitive advantage and generate higher market demand. However, it is unlikely that these niche markets offer opportunities to all native varieties. So, beyond the few farmers which maintain diversity spontaneously, the maintenance of many varieties should be considered creating a larg network of *in-situ* maintenance connected with the *ex-situ* conservation. It was established that farms in Chiloe where they grow potatoes are small (less than 10 ha) and that they spend only 0.22 hectares on average for the cultivation of native potatoes, thereby generating very little surplus to offer the market. Native potatoes are established simultaneously in the garden with vegetable species such as beets, carrots, peas, beans, lettuce, pepper and garlic. They are set in mixtures with the aim of improving health protection against late blight (47% of farms). Native varieties are present in 80.5% of the farms of Chiloe, but only three varieties are currently cultivated in each farm in Chiloé. The main causes contributing to the loss of native potato varieties are: 1) the replacement of potato cultivation by other crops (grassland), 2) the replacement of native varieties by introduced cultivars, 3) the presence of disease development, such as late blight (*Phytophthora infestans*) with losses exceeding 50% of production, 4) migration processes of the permanent population from rural to urban areas in search of better living conditions and 5) change of the principal economic activities of the rural families, upon which the most important has been the business of salmon farming. A hundred different names were listed in the past, we have found that 47 in this survey. However, we did not do an exhaustive survey of all the farmers and the relationship between genetic diversity and diversity of names is not direct. Finally, there will be no mid-term total abandonment of peasant agriculture in Chiloé, some farmers are interested in the diversity of native potatoes

(example: a farmer survey, which maintains 28 varieties = possibility of a network), maintaining the garden is an opportunity because many people continue to maintain a vegetable garden even if their work is not "farmer". Additionally, there are already associations for the maintenance of native varieties.

In relation to studies of morphological diversity of the collection, the results obtained show the formation of groups under the popular name and local attributes assigned by the farmers themselves to the varieties and especially those characters related to the characteristics of the tuber and plant morphology. Tuber forms were from common oval forms to rare spindle forms. The shapes of the sprouts were ovoid, spherical, cylindrical and conical and the growth habits of the sprouts were very heterogeneous. The flower color ranges from deep purple to white. The ripening period ranged from 100 days for the earliest accessions to 150/160 for the latest accessions.

Molecular evaluation of the collection (SSRs and AFLP) reveals a high degree of genetic diversity. Both markers were consistent in classifying *Solanum fernandezianum* as the more distant genotype compared to all the others. This was an expected result, the species *Solanum fernandezianum* having been chosen as outgroup. The SSRs allowed the estimation of the polymorphic information content (PIC) for seven loci which values ranged between 0.63 to 0.89. This indicates that the native material of Chiloe represents a wide genetic diversity. Both types of markers, SSRs and AFLP, did not provide the same groupings among the accessions. That said, the high diversity revealed by SSR markers resulted in a classification poorly supported by re-sampling methods and low bootstrap probabilities associated to the majority of nodes of the classification tree. This is also an indication that the native material of Chiloe is loosely related., Nevertheless 5 native accessions have always been part of the same group when using morphological data and SSRs. These were the UCT-10MgL, -9MgM, -31Ob, -1Ma and -15MgRo accessions. They share in common a rare form of tuber, being typically fusiform and high content of pigment in skin and meat. The SSR-based study showed a low differentiation between native potatoes and improved cultivated varieties. The analysis of diversity based on the AFLP was not inconsistent with this result because despite containing only one cultivar (Desiree), the variety was grouped with the native varieties. This may reflect the presence of ancestral genes of Chilean origin in the most popular varieties currently cultivated in Chiloe and although the phenotypic diversity of the cultivated varieties is much smaller than that of the native varieties, this does not seem to result in a much narrower genetic base.

In relation to resistance to late blight, there is wide variation of the response and performance of the native material under the attack of *Phytophthora infestans*. Most accessions of native potato fall into the ranks moderately resistant to moderately susceptible. The comparative analysis of the AUDPCr distinguished at least three accessions with high field resistance to disease : UCT-34Cor, UCT-26Ach and UCT-27Mu. Among these three accessions the two first, UCT-34Cor and UCT-26Ach, have also been tested *in vitro* and both varieties have shown excellent performances for different components of the resistance.

Finally, this thesis has allowed the consolidation of our academic training. We acquired methods and developed protocols. The work allowed the use of different softwares for the analysis of genetic diversity. We have set targets for research and selected materials by the mean of field and laboratory work. We have consulted a lot of scientific literature and have widely discussed the results we obtained. The laboratory of molecular biology during this period was equipped with thermal cycler, analytical balance, refrigerated centrifuge and extractor. Protocols were implemented as SSRs molecular analysis and *in-vitro* resistance tests. On the other hand, we have visited a research center in Chile and France. We have established a network in Chile consisting of colleagues in molecular biology and pathology and institutes such as the Regional Research Center (Remehue) of the INIA (Instituto de Investigaciones Agropecuarias; Chile). In the future it would be of great interest to develop collaborations abroad, especially with the CIP (Centro Internacional de la Papa; Perú).

## **ANNEXES**

**ANNEXE 1.. Enquête.**

**UNIVERSIDAD CATOLICA DE TEMUCO**  
**ENCUESTA DE PRODUCCIÓN DE VARIEDADES NATIVAS DE PAPA**

\* Productor: .....

Sector o localidad:.....

*Ubicación	Comuna:	Localidad:
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Coordenadas: (DATUM 69):      UTM      HUSO

Superficie total del predio:      \_\_\_\_\_ Hectáreas      Rubro principal: \_\_\_\_\_

Vive en el predio: Si\_\_\_\_\_ No\_\_\_\_\_ Sexo\_\_\_\_\_ Edad:\_\_\_\_\_ Escolaridad:\_\_\_\_\_  
Etnia\_\_\_\_\_

\* La fuerza de trabajo predial es ¿?.

- |  |  |
|--|--|
| 1.- Un aporte familiar no remunerado.                                      |  |
| 2.- Un aporte comunitario tipo “minga”,                                    |  |
| 3.- realizado por trabajadores y obreros agrícolas contratados con salario |  |
| 4.- Otro tipo. (Indicar cual.....).  |  |

\* Se dedica a otra actividad productiva aparte de la agrícola.

1.-Si       2.-No

\* A que otra actividad productiva se dedica aparte de la agrícola.

- |  |  |
|--|--|
| 1.- Pesca artesanal.   |  |
| 2.- Actividad acuícola (salmonicultura).                           |  |
| 3.- El cultivo y/recolección de algas marinas.                     |  |
| 4.- Elaboración de productos artesanales (Tejedoras)               |  |
| 5.- Actividad forestal.  |  |
| 6.- Actividad turística.   |  |
| 7.- Migración temporal a otras regiones del país o fuera del país. |  |
| 8.- Otra.  |  |

\* A que organización campesina pertenece y participa (Indicar el o los nombres de las organizaciones).

- |                 |
|-----------------|
| Organización 1: |
| Organización 2: |
| Organización 3: |

\* Maneja el cultivo de papa como parte de una rotación.  1.-Si  2.-No

\* Cada cuento año planta papa en un mismo potrero. (Largo de la rotación de cultivos).

1.- Monocultivo de papas	
2.- Papa cada 2 años	
3.- Papa cada 3 años	
4.- Papa cada 4 años	
5.- Papa cada 5 años	

\* Especies integrantes de la rotación de cultivos del predio.

1.- Algún cereal.	
2.- Otro cultivo anual.	
3.- Pradera degradada.	
4.- Pradera artificial establecida.	
5.- Otros (indicar.....)	

\* Forma de aprovisionarse de semilla (ORIGEN) de papa de variedades nativas para la plantación.

1.-Adquisición (semilla corriente) en proveedores de la ciudad.	
2.-Adquisición (semilla corriente) a otros campesinos o agricultores de la comunidad..	
3.-Intercambio de semilla (semilla corriente)	
4.-Adquisición en comercio establecido (semilla corriente)	
5.-Adquisición en ferias locales (semilla corriente).	
6.-Adquisición desde un programa de asistencia técnica (semilla corriente).	
7.-Semilla propia producida y cosechada en el propio predio.	

\* La Semilla utilizada de variedades nativas de papa es

<input type="checkbox"/>	1.-Propia	<input type="checkbox"/>	3.-Certificada	<input type="checkbox"/>	5.-No Legal (corriente)
<input type="checkbox"/>	2.-Comprada	<input type="checkbox"/>	4.-Corriente legal	<input type="checkbox"/>	<b>6.-Usa Semilla botánico</b>

\* Tasa de renovación de la “semilla tubérculo” de variedades de papa de nativas es...

1.- Renovación anual.	
2.- Cada dos años.	
3.- Cada tres años	
4.- Cada cuatro años.	
5.- Cada cinco años.	
6.- Nunca renueva la semilla de las variedades mejoradas o modernas.	

\* Principales problemas de gestión y tecnológicos que puedan afectar el establecimiento de cultivos de papas.

Ítems	SI	NO
1.- Falta de suelo disponible en calidad y cantidad.		
2.- Mala calidad de los tubérculos semillas empleados.		
3.- Daños y pérdidas causadas por enfermedades. (Fungosas, Virales, Bacteriana)		
4.- Daños y pérdidas causadas por plagas. (Gusanos de suelo, Pilme, Cuncunillas)		
5.- Falta de mecanización del cultivo en todas las etapas de producción, recolección, transporte, selección y almacenamiento.		
6.- Déficit en equipamiento de Riego.		
7.- Falta de recursos para adquirir fertilizantes.		
8.- Falta de recursos para adquirir agroquímicos.		
9.- Carencia de infraestructura y tecnología de almacenamiento.		
10.- Falta de recursos para contratación de mano de obra.		
11.- No hay disponibilidad de mano obra en el sector.		
12.- No hay disponibilidad de maquinaria que preste servicio en el sector.		
13.- Difícil acceder a fuentes de financieras para gastos operacionales.		

\* ¿Planta variedades nativas de papa?.

<input type="checkbox"/>	1.-Si	<input type="checkbox"/>	2.No
--------------------------	-------	--------------------------	------

\* ¿Planta variedades introducidas de papa?.

<input type="checkbox"/>	1.-Si	<input type="checkbox"/>	2.No
--------------------------	-------	--------------------------	------

Cuales ?

Variedad 1:	Variedad 11:	Variedad 21:
Variedad 2	Variedad 12:	Variedad 22:
Variedad 3:	Variedad 13:	Variedad 23:
Variedad 4:	Variedad 14:	Variedad 24:
Variedad 5:	Variedad 15:	Variedad 25:
Variedad 6:	Variedad 16:	Variedad 26:
Variedad 7:	Variedad 17:	Variedad 27:
Variedad 8:	Variedad 18:	Variedad 28:
Variedad 9:	Variedad 19:	Variedad 29:
Variedad 10:	Variedad 20:	Variedad 30:

\*PUEDE INDICAR EL NOMBRE DE LA VARIANDE NATIVAS DE PAPA QUE HA PLANTADO EN LOS ULTIMOS CUATRO AÑOS.

TEMPORADA 2003/2004	TEMPORADA 2004/2005	TEMPORADA 2005/2006	TEMPORADA 2006/2007

\*).INDIQUE COMO HA EVOLUCIONADO LA SUPERFICIE (hectáreas) DE VARIEDADES NATIVAS DE PAPA QUE HA PLANTADO EN LOS ULTIMOS CUATRO AÑOS.

TEMPORADA 2003/2004	TEMPORADA 2004/2005	TEMPORADA 2005/2006	TEMPORADA 2006/2007

\*Temporada 2006/2007 ¿Cuántos sacos plantó de variedades nativas de papa\_?

<input type="checkbox"/>	Nº de sacos	<input type="checkbox"/>
--------------------------	-------------	--------------------------

\* Planta papas nativas en forme de “CHAHUEN”, es decir, MEZCLA DE VARIEDADES?.

<input type="checkbox"/>	1.Si	<input type="checkbox"/>	2.No
--------------------------	------	--------------------------	------

\* Planta variedades nativas de papas en la “huerta” de su predio.

<input type="checkbox"/>	1.Si	<input type="checkbox"/>	2.No
--------------------------	------	--------------------------	------

\* Estimación del rendimiento de su cultivo de variedades nativas de papa.

1.- el 5 X 1.	
2.- el 6 X 1.	
3.- el 7 X 1.	
4.- el 8 X 1.	
5.- el 9 X 1.	
6.- el 10 X 1	
7.- el 15 X 1.	
8.- el 20 X 1.	
9.- Otro valor	

\* Vende sus diferentes variedades de papas nativas.

Si

No

\* Sus volúmenes de venta anual de papas nativas alcanzan a:

\* Indicar el precio alcanzado en la venta de variedades nativas de papa es:

(\$/kg): \_\_\_\_\_

(\$/saco de 50 kg): \_\_\_\_\_

\* Indique cual de las siguientes enfermedades se ha presentado y/o declarado epifitia.

1.- Tizón tardío.	
2.- Tizón temprano.	
3.- Rhizoctoniosis.	
4.- Otra	

\* Que variedades son más resistentes y a qué?

#### DE LAS VARIEADADES INTRODUCIDAS DE PAPA.

\* Temporada 2006/2007 ¿Cuántos sacos plantó de variedades introducidas?

Nº de sacos  Tamaño del saco

\*Temporada 2006/2007 ¿Cuántos sacos cosechó de variedades introducidas ?

Nº de sacos  Tamaño del saco

Cuáles ?

TEMPORADA 2003/2004	TEMPORADA 2004/2005	TEMPORADA 2005/2006	TEMPORADA 2006/2007

\*INDIQUE COMO HA EVOLUCIONADO LA SUPERFICIE (hectáreas) DE VARIEDADES INTRODUCIDAS DE PAPA QUE HA PLANTADO EN LOS ULTIMOS CUATRO AÑOS.

TEMPORADA 2003/2004	TEMPORADA 2004/2005	TEMPORADA 2005/2006	TEMPORADA 2006/2007

Superficie total plantada con variedades introducidas en el predio el último año.

Hectáreas

\*) Seleccione las variedades de papas mejoradas que utiliza en su predio:

<input type="checkbox"/> DESIREE	<input type="checkbox"/> CARDINAL	<input type="checkbox"/> KARU INIA	<input type="checkbox"/> ROSARA
<input type="checkbox"/> ATICA	<input type="checkbox"/> CORNADO	<input type="checkbox"/> KENNEBEC	<input type="checkbox"/> SHEPODY
<input type="checkbox"/> AMADEUS	<input type="checkbox"/> AGATA.	<input type="checkbox"/> MONALISA	<input type="checkbox"/> SYMFONIA
<input type="checkbox"/> ASTERIX	<input type="checkbox"/> FL – 1833	<input type="checkbox"/> ONA INIA	<input type="checkbox"/> VIVALDI
<input type="checkbox"/> ATLANTIC	<input type="checkbox"/> FL – 1867	<input type="checkbox"/> PUKARA	<input type="checkbox"/> YAGANA
<input type="checkbox"/> BARAKA	<input type="checkbox"/> FL – 1879	<input type="checkbox"/> RED SCARLETT	<input type="checkbox"/> Otra
<input type="checkbox"/> BERBER	<input type="checkbox"/> GRANOLA	<input type="checkbox"/> RODEO	
<input type="checkbox"/> BINTJE	<input type="checkbox"/> INNOVATOR	<input type="checkbox"/> ROMANO	
<input type="checkbox"/> CAESAR			

\*Apreciación personal del nivel de rendimiento del cultivo de papas Introducidas.

1.- Rendimiento muy alto.	<input type="checkbox"/>
2.- Rendimiento alto.	<input type="checkbox"/>
3.- Rendimiento medio.	<input type="checkbox"/>
4.- Rendimiento bajo	<input type="checkbox"/>
5.- Rendimiento muy bajo a malo.	<input type="checkbox"/>

\* Estimación del rendimiento de su cultivo de variedades e introducidas de papa.

1.- 5 X 1.	<input type="checkbox"/>
2.- 6 X 1.	<input type="checkbox"/>
3.- 7 X 1.	<input type="checkbox"/>
4.- 8 X 1.	<input type="checkbox"/>
5.- 9 X 1.	<input type="checkbox"/>
6.- 10 X 1	<input type="checkbox"/>
7.- 15 X 1.	<input type="checkbox"/>
8.- 20 X 1.	<input type="checkbox"/>
9.- Otro valor	<input type="checkbox"/>

\* Cuál es la forma de aprovisionarse de semilla de papa de variedades introducida para la plantación. (El origen de la semilla).

1.-Adquisición (semilla corriente) en proveedores de la ciudad.	<input type="checkbox"/>
2.-Adquisición (semilla corriente) a otros campesinos o agricultores de la comunidad..	<input type="checkbox"/>
3.-Intercambio de semilla (semilla corriente)	<input type="checkbox"/>
4.-Adquisición en comercio establecido (semilla corriente)	<input type="checkbox"/>
5.-Adquisición en ferias locales (semilla corriente).	<input type="checkbox"/>
6.-Adquisición desde un programa de asistencia técnica (semilla corriente).	<input type="checkbox"/>
7.-Semilla propia producida y cosechada en el propio predio.	<input type="checkbox"/>

\* Cuál es la semilla utilizada de variedades introducidas de papa.

- 1.-Propia  
2.-Comprada

- 3.-Certificada  
4.-Corriente legal

- 5.-No Legal (corriente)  
6.-Usa Semilla  
botánica

\* Cuál es la tasa de renovación de la “semilla tubérculo” de variedades de papa introducidas.

1.- Renovación anual.	<input type="checkbox"/>
2.- Cada dos años.	<input type="checkbox"/>
3.- Cada tres años	<input type="checkbox"/>
4.- Cada cuatro años.	<input type="checkbox"/>
5.- Cada cinco años.	<input type="checkbox"/>
6.- Nunca renueva la semilla de las variedades mejoradas o modernas.	<input type="checkbox"/>

\* Vende sus diferentes variedades de papas mejoradas.

 Si No

\* Sus volúmenes de venta anual de papas mejoradas alcanzan a:

\* Indicar el precio alcanzado en la venta de papa mejorada es:

(\$/kg): \_\_\_\_\_  
(\$/saco de 50 kg): \_\_\_\_\_

## **ANNEXE 2.. Experience of ex-situ conservation of *Solanum Fernandezianum* (solanaceae)**

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### **Resumen**

*Solanum fernandezianum* is an endemic species in danger of extinction the Robinson Crusoe Island, Chile. The fruit was collected during March and April of 2002. The seeds were storaged for a year to 4°C. The percentage of germination in laboratory conditions was 14.3 %. After 22 weeks plants initiated flowering and fruiting. The conservation strategy considered germination of seeds, production of plants in incubation chamber, transplantation to pots and pots arranged in a greenhouse.

### **Introducción**

Los recursos fitogenéticos constituyen la base biológica de la seguridad alimentaria mundial. Estos recursos, a su vez, están formados por la rica diversidad de material genético que contienen las variedades tradicionales y los cultivares modernos, así como las plantas silvestres afines a las cultivadas por el hombre. Además, constituyen un depósito de adaptabilidad genética que sirve de garantía ante el peligro potencial presentado por los cambios medioambientales y económicos (FAO 1996).

Uno de los cultivos alimenticios mundiales más demandados es la papa (*Solanum tuberosum* L.), por su alto contenido de almidón, con una fuente diversa de germoplasma utilizada para su fitomejoramiento. En este contexto, se puede cruzar con la mayoría de sus parientes silvestres, incorporando resistencia a estrés (Hijmans & Spooner 2001). Entre estas papas silvestres se encuentra *Solanum fernandezianum* Phil., serie *Etuberosum*, siendo una planta diploide y perenne rizomatosa (Contreras & Spooner 1999), caracterizada morfológicamente por ser de tipo herbácea, con tallos y hojas glabras y flores de color azul violeta. Es originaria de la Isla Robinson Crusoe en el Archipiélago de Juan Fernández, Parque Nacional y Reserva de la Biosfera de Chile, es una especie endémica con una distribución restringida sólo a esta isla en áreas semidespejadas con abundante presencia

de helechos arborescentes y herbáceos, la vegetación asociada coincide con la reportada por Marticorena *et al.* (1998). Esta especie es considerada en peligro de extinción (Benoit 1989). También ha sido clasificada en peligro por Stuessy *et al.* (1998), Danton *et al.* (1999), Ricci (2006); y vulnerable por Ricci (1989, 1990, 1992).

La flora vascular del Archipiélago posee un alto grado de endemismo, de 211 especies nativas, 132 (63%) son endémicas (Marticorena *et al.* 1998, Ricci 2006), siendo uno de los números de especies endémicas por unidad de área más altos del mundo (Roger *et al.* 1983, Stuessy *et al.* 1992).

El material silvestre de esta especie presenta un alto valor con respecto de la resistencia a plagas y enfermedades: al nemátodo dorado (*Globodera rostochiensis* (Wollenweber) Behrens) (Hanneman & Bamberg 1986), resistencia al virus del enrollamiento de la hoja de la papa (PLRV), al virus del mosaico rugoso (PVY) y al virus del mosaico suave (PVA) (Valkonen *et al.* 1992). En híbridos en los que participa *S. fernandezianum* se encontró resistencia a heladas, hipersensibilidad al virus Y, tolerancia al virus del enrollamiento de la hoja y resistencia a marchitez bacteriana, siendo una especie muy susceptible al tizón tardío causado por el hongo *Phytophthora infestans* (Montagne) de Bary (Hijmans *et al.* 2003). Por otra parte, estudios sobre potencial de flujo génico entre especies de papas cultivadas y silvestres mostraron que al usar en los cruzamientos accesiones de *S. fernandezianum*, ellas fueron fértiles para la totalidad de las semillas producidas (Jackson & Hanneman 1996).

La mayor amenaza actual de esta especie en su hábitat es su consumo por animales exóticos, tales como la cabra (*Capra hircus* L., 1758) y el conejo europeo (*Oryctolagus cuniculus* L., 1758), considerados plagas para la isla, lo cual aumenta el riesgo de extinción (Ricci 2006).

Considerando que aún existe escasa información relacionada con la conservación *ex-situ* de esta especie en Chile, salvo los estudios de Ricci (1998, 2006), se realizaron ensayos de germinación, establecimiento y aclimatación que permitan la conservación de esta papa silvestre.

## Materiales y métodos

Entre los meses de marzo y abril de 2002 se realizó una expedición de colecta al Archipiélago. El principal sitio de colecta correspondió al camino a Plazoleta Yunque en Isla Robinson Crusoe, situado a 257 m de altitud (33°39'9,03``S, 78°50'45,9``W).

Para la obtención del germoplasma se recolectaron bayas maduras, las que se limpian y guardaron en bolsas de papel con su correspondiente codificación y almacenadas transitoriamente en neveras de icopor. Posteriormente fueron secadas en

laboratorio a temperatura ambiente por 30 días. Finalizado el período de secado, se extrajeron las semillas de los frutos y luego se almacenaron a 4 °C por el período de un año en tubos de polipropileno estériles. Para el análisis de germinación, las semillas se incubaron en una cámara de germinación a 22 °C. El análisis se basó en las normas (ISTA 2006), para esto se dispuso de una muestra de 400 semillas, que fueron sembradas con 4 repeticiones de 100 semillas cada una en placas petri con sustrato TP (Fig.1).

Las plántulas obtenidas fueron transplantadas a contenedores de poliestireno expandido y luego a maceteros de 1 y 10 L de capacidad, todas provistas de turba estéril y bajo condiciones controladas de aclimatación hasta la obtención de plantas adultas. Se consideró un fotoperíodo de 16 horas y una temperatura constante de 22 ± 2 °C en cámara de incubación, seguido de un período en invernadero para un mejor establecimiento. Durante esta etapa, las plantas recibieron abundante riego con aplicaciones semanales de fertilizantes foliares (Fig. 2).

## Resultados

Del total de bayas maduras colectadas se extrajeron 12.674 semillas, de ellas 7.000 fueron incorporadas al Banco de Germoplasma del CRI-Carillanca para su conservación a largo plazo, y 5.674 para los trabajos de laboratorio e invernadero.

*Solanum fernandezianum* presentó un porcentaje promedio de germinación de un 14,3%, y aunque este porcentaje fue bajo, los valores son muy similares a los observados por Towill (1983), quien reporta que en semillas de *S. fernandezianum*, almacenadas bajo condiciones herméticas durante 14 años a temperaturas de 1 a 3 °C con 4 a 5% de humedad, los porcentajes de germinación alcanzaron un 14,0%. Por el contrario, Ricci (1998) obtuvo un 90% de germinación, utilizando para ello semillas no almacenadas y estratificadas por 7 días a -5 °C. Esto indicaría que el almacenaje y la no estratificación de las semillas disminuyen fuertemente su capacidad germinativa, ya que las semillas fueron extraídas directamente de los frutos y luego se almacenaron a 4 °C por el período de un año bajo condiciones estériles, lo cual en alguna medida asegura su calidad inicial. Además, en los ensayos no se apreciaron semillas muertas o dañadas.

A partir de la semilla colectada y germinada se inició una estrategia de cultivo en ambiente controlado, hasta la obtención de plantas adultas que permitieran la reproducción de la especie. Posteriormente, éstas fueron transplantadas a macetas plásticas, donde las plantas iniciaron sus ramificaciones y desplegaron sus primeros foliolos alcanzando una altura de 20 a 25 cm después de 4 semanas de crecimiento. Luego, bajo condiciones de invernadero y transcurridas 8 a 10 semanas, se obtuvo la producción de plantas adultas

cuya altura osciló entre 70 y 80 cm, las que iniciaron el proceso de floración y fructificación al cabo de 22 semanas de iniciada la germinación (Fig.3).

De este material, se obtuvo un promedio de 20 frutos por planta, y la producción de semillas por fruto estuvo dentro del rango de 40 a 70 semillas. Estos valores son similares a los reportados por Jackson & Hanneman (1996) en estudios de cruzamiento con esta especie. En base a lo anterior, se puede concluir que a pesar de los bajos porcentajes de germinación obtenidos, la semilla de *S. fernandezianum* permitió la propagación de la especie. La semilla fue viable después de su almacenamiento y conservación por un año, a temperaturas de 4 °C.

### Conclusión

Estos resultados sirven de base para futuras estrategias de conservación que contribuyan en forma importante a reducir el peligro de extinción que afecta a esta especie, respondiendo de esta forma al “Plan de acción mundial para la conservación y utilización sostenible de los recursos fitogenéticos para la alimentación y la agricultura” impulsado por la FAO (1996).

### Agradecimientos

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### Bibliografía

- BENOIT, I.L. Ed. 1989. Libro rojo de la flora terrestre de Chile. CONAF, Santiago de Chile. 157 pp.
- CONTRERAS, A. & D.M. SPOONER. 1999. Revision of *Solanum* section *Etuberosum* (subgenus *Potatoe*). In: (Nee, M., D.E. Symon, R.N. Lester & J.P. Jessop. (eds). Solanaceae IV, pp. 227-245. Royal Botanic Gardens, Kew.
- DANTON, P., E. BRETEAU & M. BRAFFAY. 1999. Les îles de Robinson. Trésor vivant des mers du Sud. Entre légende et réalité. Yves Rocher, Nathan, France. 144 pp.
- FAO. 1996. Conservación y utilización sostenible de los recursos fitogenéticos para la alimentación y la agricultura: Plan de acción mundial e informe sobre el estado de los recursos fitogenéticos en el mundo. Organización de las Naciones Unidas para la Agricultura y la Alimentación. Italia. 10 pp.
- HANNEMAN, R.E. & J.B. BAMBERG. 1986. Inventory of tuber-bearing *Solanum* species. Bulletin Agricultural Experiment Station, University of Wisconsin. USA. 533 pp.

- HIJMANS, R.J. & D.M. SPOONER. 2001. Geographic distribution of wild potato species. American Journal of Botany 88: 2101-2112.
- HIJMANS, R.J., M. MACOBS, J. BAMBERG & D. SPOONER. 2003. Frost tolerance in wild potato species: Assessing the predictivity of taxonomic, geographic and ecological factors. Euphytica 130: 47-59.
- ISTA. 2006. International rules for seed testing. Published by: The International Seed Testing Association (ISTA). 388 pp.
- JACKSON, S.A. & R.E. HANNEMAN. 1996. Potential gene flow between cultivated potato and its wild tuber-bearing relatives: implications for risk assessment of transgenic potatoes. (<http://www.nbiap.vt.edu;brarg/brasym96/jackson96.htm>).
- MARTICORENA, C., T. STUESSY & C. BAEZA. 1998. Catálogo de la flora vascular de la Isla Robinson Crusoe o Juan Fernández, Chile. Gayana Botánica 55(2): 187-211.
- RICCI, M. 1989. Programa de conservación y recuperación de plantas amenazadas de Juan Fernández. Informe Final, 1 etapa, Proyecto CONAF – WWF – US 3313, Chile, 63 pp.
- RICCI, M. 1990. Programa de conservación y recuperación de plantas amenazadas de Juan Fernández. Informe Final, 2 etapa, Proyecto CONAF – WWF – US 3313, Chile, 53 pp.
- RICCI, M. 1992. Programa de conservación y recuperación de plantas amenazadas de Juan Fernández. Informe Final, 3 etapa, Proyecto CONAF – WWF – US 3313, Chile, 55 pp.
- RICCI, M. 1998. Técnicas de propagación y viverización de algunas especies de la flora vascular endémica del Archipiélago de Juan Fernández. Países Bajos – CONAF, Chile. 229 pp.
- RICCI, M. 2006. Conservation status and *ex situ* cultivation efforts of endemic flora of the Juan Fernández Archipelago. Biodiversity and Conservation 15: 3111-3130.
- ROGER, S., T. STUESSY & R. RODRÍGUEZ. 1983. Chromosome numbers from the flora of the Juan Fernández Islands. American Journal of Botany 70(6): 799-810.
- STUESSY, T., C. MARTICORENA, R. RODRÍGUEZ, D. CRAWFORD & O. SILVA. 1992. Endemism in the vascular flora of the Juan Fernández Islands. Aliso 13: 297-307.
- STUESSY, T.F., U. SWENSON, D.J. CRAWFORD, G. ANDERSON & M. SILVA. 1998. Plant conservation in the Juan Fernandez Archipelago, Chile. Aliso 16: 89–98.
- TOWILL, L.E. 1983. Longevity of true seed from tuber-bearing and closely related non-tuber-bearing *Solanum* species. American Journal Potato Research 60: 75-83.
- VALKONEN, J.P., G. BRIGNETI, F. SALAZAR, E. PEHUE & R.W. GIBSON. 1992. Interaction of the *Solanum* spp. of the Etuberosa group and nine potato-infecting viruses and viroid. Annual Applied Biology 120: 301-313.

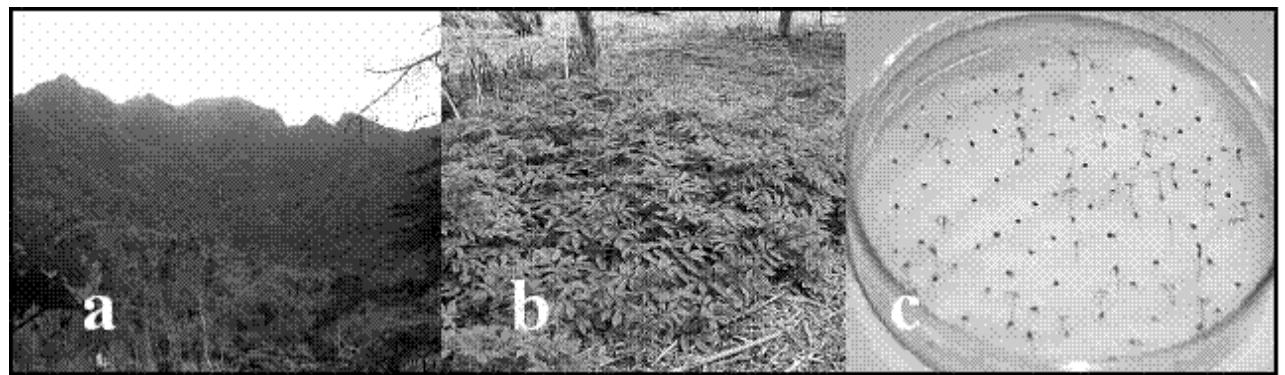


Figura 1. a) Isla Robinson Crusoe, b) plantas de *Solanum fernandezianum*, c) germinación de semillas (Fotos: de J. Solano).

Figure 1. a) Island Robinson Crusoe, b) plants of *Solanum fernandezianum*, c) germination of seeds (Photographs: from J. Solano).

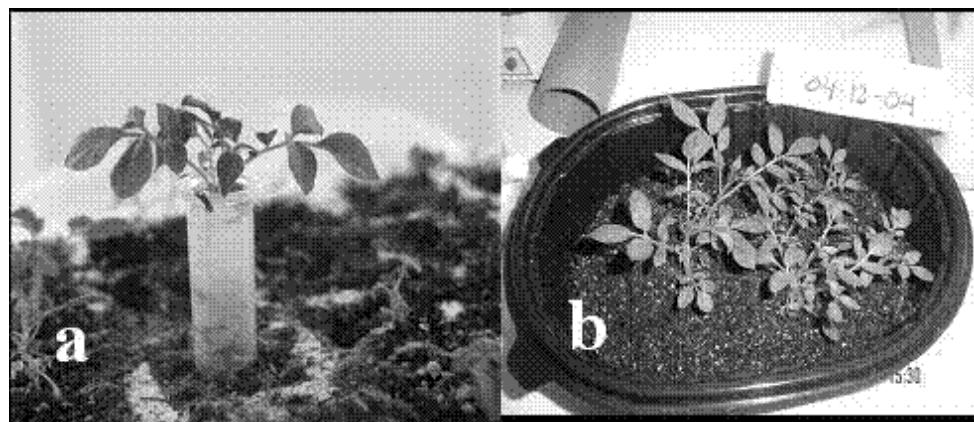


Figura 2. a) Cámara de incubación: producción de plántulas en contenedores de poliestireno expandido, b) Cámara de incubación: producción de plantas en macetas (Fotos: de J. Solano).

Figure 2. a) Camera of incubation: production of plant in speedling, b) Incubation camera: production of plants in pot (Photographs: from J. Solano).

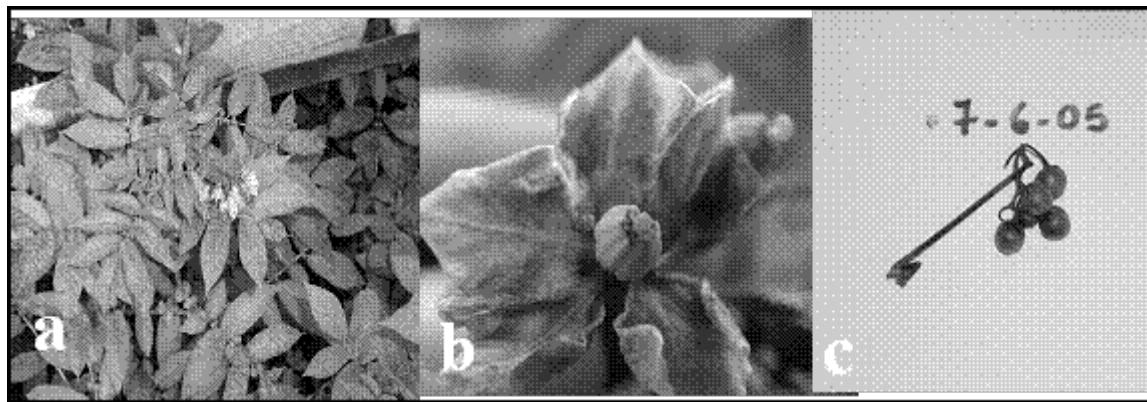


Figura 3. a) Invernadero de policarbonato: producción de plantas adultas en maceteros, b) producción de flores, c) frutos en invernadero (Fotos: de J. Solano).

Figure 3. a) Polycarbonate greenhouse: production of adult plants en flowerpots, b) production of flowers, c) fruits in greenhouse (Photographs: from J. Solano).

### **ANNEXE 3.- Description of native potato collections in Chiloe.**

Huaman. Z., J. Williams., W. Salhuana., and N. Vincent. 1977. Descriptors for the cultivated potato and for the maintenance and distribution of germoplasm collections. Consultative Group on International Agricultural Research. Rome. International Board for Plant Genetic Resources (I.B.P.G.R.). p 47.

ACCESSION: UCT-11Mgb

NAME: Meca gato blanco

TUBER

Shape: elongate

Unusual shape: absent

Predominant skin colour: white-cream

Secondary skin colour: absent

Distribution of secondary skin colour: absent

Tuber skin type: smooth

Predominant flesh colour: cream

Secondary flesh colour: absent

Distribution of secondary flesh colour: absent

Note of eyes per tuber: intermediate

Average number of eyes: 7,0

Depth of tuber eyes: shallow

Distribution of tuber eyes: evenly distributed

Presence of eyebrows: inconspicuous

SPROUT

Shape: wide cylindrical

Growth habit of spout: intermediate

Length of lateral branches: short

Predominant colour: red

Pubescence: glabrescent

Lenticels pigmentation of sprout: absent

STEM

Stem colour: green with purple knots

Stem cross section: angular

Stem wings: straight

Stem anthocyanic coloration: absent or very weak

Average main stem: 3, 9

PLANT

Foliage structure: intermediate type

Growth habit: semi-upright

Height: medium

LEAF

Openness: intermediate

Presence of secondary leaflet: strong

Abaxial leaf pubescence: pubescence

Adaxial leaf pubescence: pubescence

Green colour: light

Anthocyanic coloration on midrib of upper side: absent or very weak

Terminal and lateral leaflets frequency of coalescence: absent or very low

Size of terminal leaflet: medium size

Size of lateral leaflets: medium size

Second pair of lateral leaflets (width in relation length): narrow

Leaflet: waviness of margin: absent or very weak

Leaflet: depth of veins: medium

Leaflet: glossiness of the upper side: medium

Leaf dissection: weakly dissected

FLOWER

Calyx colour: green with some red

Predominant colour of corolla: intense red

Secondary colour of corolla: white



ACCESSION: UCT-14MgRe

NAME: Redonda

TUBER

Shape: round

Unusual shape: absent

Predominant skin colour: dark purple

Secondary skin colour: absent

Distribution of secondary skin colour: absent

Tuber skin type: smooth

Predominant flesh colour: white

Secondary flesh colour: violet

Distribution of secondary flesh colour: vascular ring medulla

Note of eyes per tuber: intermediate

Average number of eyes: 7,4

Depth of tuber eyes: very deep

Distribution of tuber eyes: predominantly apical

Presence of eyebrows: inconspicuous

SPROUT

Shape: ovoid

Growth habit of spout: open

Length of lateral branches: long

Predominant colour: fuchsia

Pubescence: glabrescent

Lenticels pigmentation of sprout: absent

STEM

Stem colour: purple

Stem section: angular

Stem wings: straight

Stem anthocyanic coloration: very strong

Average main stem: 3,15

PLANT

Foliage structure: branching type

Growth habit: upright

Height: high

LEAF

Openness: intermediate

Presence of secondary leaflet: medium

Abaxial leaf pubescence: pubescence

Adaxial leaf pubescence: pubescence

Green colour: light

Anthocyanic coloration on midrib of upper side: very strong

Terminal and lateral leaflets frequency of coalescence: medium

Size of terminal leaflet: medium

Size of lateral leaflets: medium

Second pair of lateral leaflets (width in relation length): medium

Leaflet: waviness of margin: absent or very weak

Leaflet: depth of veins: shallow

Leaflet: glossiness of upperside: medium

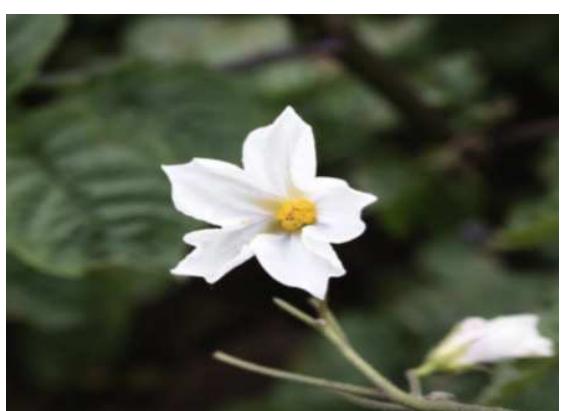
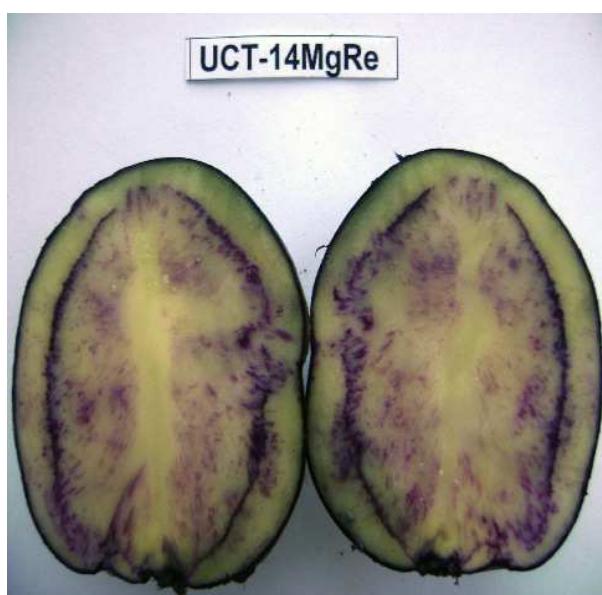
Leaf dissection: weakly dissected

FLOWER

Calyx colour: green with some purple

Predominant colour of corolla: yellow

Secondary colour of corolla: light blue



ACCESSION: UCT-17Br

NAME: Bruja

TUBER

Shape: oblong

Unusual shape: absent

Predominant skin colour: purple

Secondary skin colour: absent

Distribution of secondary skin colour: absent

Tuber skin type: smooth

Predominant flesh colour: white

Secondary flesh colour: violet

Distribution of secondary flesh colour: vascular ring and medulla

Note of eyes per tuber: intermediate

Average number of eyes: 8,5

Depth of tuber eyes: deep

Distribution of tuber eyes: evenly distributed

Presence of eyebrows: markedly

SPROUT

Shape: wide cylindrical

Growth habit of spout: closed

Length of lateral branches: medium

Predominant colour: fuchsia

Pubescence: strongly pubescent

Lenticels pigmentation of sprout: present

STEM

Stem colour: green with purple knots

Stem section: angular

Stem wings: straight

Stem anthocyanic coloration: weak

Average main stem: 5,4

PLANT

Foliage structure: intermediate type

Growth habit: semi-upright

Height: medium

LEAF

Openness: intermediate

Presence of secondary leaflet: weak

Abaxial leaf pubescence: pubescence

Adaxial leaf pubescence: pubescence

Green colour: dark

Anthocyanic coloration on midrib of upper side: slightly pigmented

Terminal and lateral leaflets frequency of coalescence: absent or very weak

Size of terminal leaflet: medium

Size of lateral leaflets: medium

Second pair of lateral leaflets (width in relation length): close

Leaflet: waviness of margin: absent or very weak

Leaflet: depth of veins: shallow

Leaflet: glossiness of upperside: dull

Leaf dissection: weakly dissected

FLOWER

Calyx colour: green with some purple

Predominant colour of corolla: yellow

Secondary colour of corolla: absent



ACCESSION: UTC-6Gc

NAME: Guadacho colorado

TUBER

Shape: long

Unusual shape: absent

Predominant skin colour: red

Secondary skin colour: absent

Distribution of secondary skin colour: absent

Tuber skin type: smooth

Predominant flesh colour: cream

Secondary flesh colour: red

Distribution of secondary flesh colour: vascular ring and medulla

Note of eyes per tuber: intermediate

Average number of eyes: 10,2

Depth of tuber eyes: medium

Distribution of tuber eyes: evenly distributed

Presence of eyebrows: inconspicuous

SPROUT

Shape: wide cylindrical

Growth habit of spout: close

Length of lateral branches: short

Predominant colour: pink

Pubescence: slightly pubescent

Lenticels pigmentation of sprout: absent

STEM

Stem colour: green with purple knots

Stem section: angular

Stem wings: straight

Stem anthocyanic coloration: weak

Average main stem: 2,8

PLANT

Foliage structure: branched type

Growth habit: upright

Height: low

LEAF

Openness: intermediate

Presence of secondary leaflet: weak

Abaxial leaf pubescence: pubescence

Adaxial leaf pubescence: pubescence

Green colour: dark

Anthocyanic coloration on midrib of upper sides lightly pigmented

Terminal and lateral leaflets frequency of coalescence: absent or very low

Size of terminal leaflet: medium

Size of lateral leaflets: small size

Second pair of lateral leaflets (width in relation length): close

Leaflet: waviness of margin: weak

Leaflet: depth of veins: shallow

Leaflet: glossiness of upperside: average (medium)

Leaf dissection: moderately dissected

FLOWER

Calyx colour: purple with some green

Predominant colour of corolla: deep red

Secondary colour of corolla: white



ACCESSION: UCT-24Tn

NAME: Tonta

TUBER

Shape: round

Unusual shape: absent

Predominant skin colour: yellow

Secondary skin colour: absent

Distribution of secondary skin colour: absent

Tuber skin type: smooth

Predominant flesh colour: cream

Secondary flesh colour: absent

Distribution of secondary flesh colour: absent

Note of eyes per tuber: few

Average number of eyes: 5,0

Depth of tuber eyes: deep

Distribution of tuber eyes: evenly distributed

Presence of eyebrows: marked

SPROUT

Shape: conical ovoid

Growth habit of spout: open

Length of lateral branches: short

Predominant colour: pink

Pubescence: strongly pubescent

Lenticels pigmentation of sprout: absent

STEM

Stem colour: green

Stem section: angular

Stem wings: straight

Stem anthocyanic coloration: absent or very weak

Average main stem: 3,45

PLANT

Foliage structure: intermediate type

Growth habit: semi-upright

Height: short

LEAF

Openness: intermediate

Presence of secondary leaflet: medium

Abaxial leaf pubescence: pubescence

Adaxial leaf pubescence: pubescence

Green colour: light

Anthocyanic coloration on midrib of upper side: absent or very weak

Terminal and lateral leaflets frequency of coalescence: high

Size of terminal leaflet: big size

Size of lateral leaflets: medium

Second pair of lateral leaflets (width in relation length): wide

Leaflet: waviness of margin: absent or very weak

Leaflet: depth of veins: medium

Leaflet: glossiness of upperside: medium

Leaf dissection: weakly dissected

FLOWER

Calyx colour:

Predominant colour of corolla:

Secondary colour of corolla:



ACCESSION: UCT-22Cm

NAME: Clavela morada

TUBER

Shape: oval

Unusual shape: absent

Predominant skin colour: yellow

Secondary skin colour: purple

Distribution of secondary skin colour: scattered, disseminated

Tuber skin type: smooth

Predominant flesh colour: cream

Secondary flesh colour: absent

Distribution of secondary flesh colour: absent

Note of eyes per tuber: intermediate

Average number of eyes: 7,0

Depth of tuber eyes: shallow

Distribution of tuber eyes: evenly distributed

Presence of eyebrows: inconspicuous

SPROUT

Shape: conical

Growth habit of spout: open

Length of lateral branches: short

Predominant colour: fuchsia

Pubescence: shallow

Lenticels pigmentation of sprout: absent

STEM

Stem colour: green with some red knots

Stem section: angular

Stem wings: wavy

Stem anthocyanic coloration: absent or very weak

Average main stem: 3,85

PLANT

Foliage structure: intermediate type

Growth habit: semi-upright

Height: slow

LEAF

Openness: intermediate

Presence of secondary leaflet: weak

Abaxial leaf pubescence: pubescence

Adaxial leaf pubescence: pubescence

Green colour: light

Anthocyanic coloration on midrib of upper side: absent or weak

Terminal and lateral leaflets frequency of coalescence: medium

Size of terminal leaflet: medium

Size of lateral leaflets: medium

Second pair of lateral leaflets (width in relation length): close

Leaflet: waviness of margin: medium

Leaflet: depth of veins: medium

Leaflet: glossiness of upperside: medium

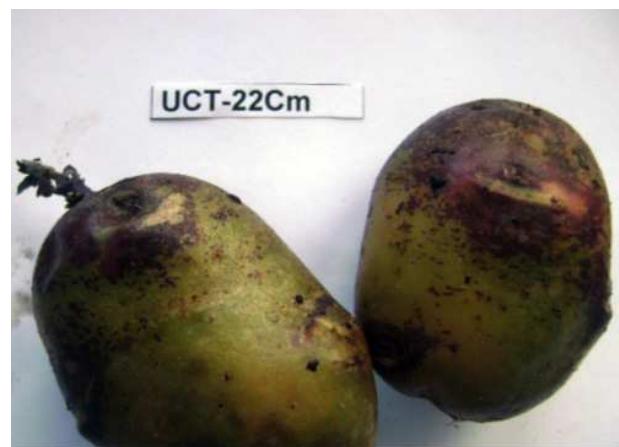
Leaf dissection: weakly dissected

FLOWER

Calyx colour:

Predominant colour of corolla:

Secondary colour of corolla



ACCESSION: UCT-25Gñ

NAME: Guicoña

TUBER

Shape: round

Unusual shape: absent

Predominant skin colour: yellow

Secondary skin colour: purple-black

Distribution of secondary skin colour: scattered areas

Tuber skin type: smooth

Predominant flesh colour: cream

Secondary flesh colour: violet

Distribution of secondary flesh colour: vascular ring and medulla

Note of eyes per tuber: intermediate

Average number of eyes: 7,7

Depth of tuber eyes: medium

Distribution of tuber eyes: evenly distributed

Presence of eyebrows: inconspicuous

SPROUT

Shape: wide cylindrical

Growth habit of spout: intermediate

Length of lateral branches: short

Predominant colour: fuchsia

Pubescence: glabrescent (slightly pubescent)

Lenticels pigmentation of sprout: absent

STEM

Stem colour: green

Stem section: angular

Stem wings: straight

Stem anthocyanic coloration: very weak

Average main stem: 7,7

PLANT

Foliage structure: intermediate type

Growth habit: semi-upright

Height: slow

LEAF

Openness: intermediate

Presence of secondary leaflet: medium

Abaxial leaf pubescence: glabrous

Adaxial leaf pubescence: glabrous

Green colour: light

Anthocyanic coloration on midrib of upper side: absent or very weak

Terminal and lateral leaflets frequency of coalescence: low frequency

Size of terminal leaflet: medium

Size of lateral leaflets: medium

Second pair of lateral leaflets (width in relation length): close

Leaflet: waviness of margin: weak

Leaflet: depth of veins: shallow

Leaflet: glossiness of upperside: dull

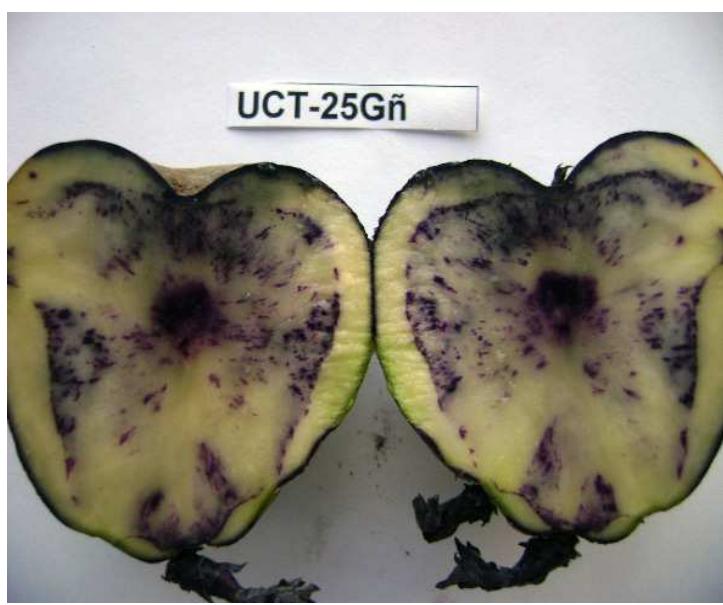
Leaf dissection: weakly dissected

FLOWER

Calyx colour:

Predominant colour of corolla:

Secondary colour of corolla



ACCESSION: UCT-7Ca

NAME: Camota

TUBER

Shape: oval

Unusual shape: absent

Predominant skin colour: purple-red

Secondary skin colour: yellow

Distribution of secondary skin colour: scattered spot

Tuber skin type: smooth

Predominant flesh colour: cream

Secondary flesh colour: absent

Distribution of secondary flesh colour: absent

Note of eyes per tuber: intermediate

Average number of eyes: 6,0

Depth of tuber eyes: very deep

Distribution of tuber eyes: evenly distributed

Presence of eyebrows: inconspicuous

SPROUT

Shape: spherical

Growth habit of spout: intermediate

Length of lateral branches: short

Predominant colour: purple

Pubescence: glabrous

Lenticels pigmentation of sprout: absent

STEM

Stem colour: green with some purple knots

Stem section: angular

Stem wings: straight

Stem anthocyanic coloration: medium

Average main stem: 4,1

PLANT

Foliage structure: branched type

Growth habit: upright

Height: medium

LEAF

Openness: intermediate

Presence of secondary leaflet: weak

Abaxial leaf pubescence: pubescent

Adaxial leaf pubescence: pubescent

Green colour: light

Anthocyanic coloration on midrib of upper side: slightly pigmented

Terminal and lateral leaflets frequency of coalescence: absent or very weak

Size of terminal leaflet: medium

Size of lateral leaflets: small size

Second pair of lateral leaflets (width in relation length): close

Leaflet: waviness of margin: absent o very weak

Leaflet: depth of veins: shallow

Leaflet: glossiness of upperside: medium

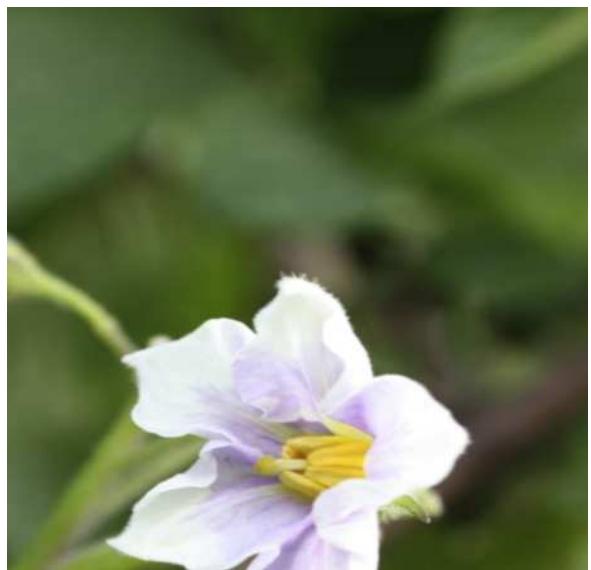
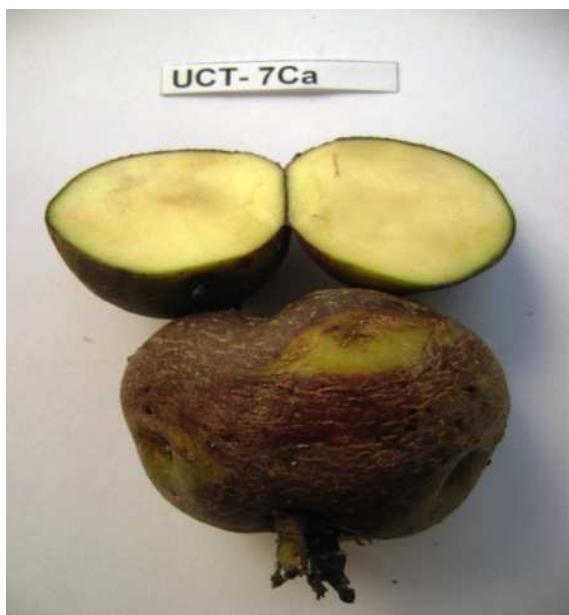
Leaf dissection: weakly dissected

FLOWER

Calyx colour: green with some purple

Predominant colour of corolla: light purple

Secondary colour of corolla: absent



ACCESSION: UCT-18Mn

NAME: Michuñe negro

TUBER

Shape:

Unusual shape: fusiform constrained

Predominant skin colour: dark purple-black

Secondary skin colour: absent

Distribution of secondary skin colour: absent

Tuber skin type: smooth

Predominant flesh colour: white

Secondary flesh colour: violet

Distribution of secondary flesh colour: vascular ring and medulla

Note of eyes per tuber: intermediate

Average number of eyes: 10,1

Depth of tuber eyes: very deep

Distribution of tuber eyes: evenly distributed

Presence of eyebrows: marked

SPROUT

Shape: conical

Growth habit of spout: intermediate

Length of lateral branches: long

Predominant colour: violet

Pubescence: strongly pubescent

Lenticels pigmentation of sprout:

STEM

Stem colour: green with some purple knots

Stem section: angular

Stem wings: straight

Stem anthocyanic coloration: medium

Average main stem: 3,30

PLANT

Foliage structure: intermediate type

Growth habit: semi-upright

Height: medium

LEAF

Openness: intermediate

Presence of secondary leaflet: medium

Abaxial leaf pubescence: strongly pubescent

Adaxial leaf pubescence: strongly pubescent

Green colour: dark

Anthocyanic coloration on midrib of upper side: slightly pigmented

Terminal and lateral leaflets frequency of coalescence: absent or low

Size of terminal leaflet: medium

Size of lateral leaflets: small size

Second pair of lateral leaflets (width in relation length): wide

Leaflet: waviness of margin: weak

Leaflet: depth of veins: medium

Leaflet: glossiness of upperside: dull

Leaf dissection: dissected just

FLOWER

Calyx colour: green with some purple

Predominant colour of corolla: yellow

Secondary colour of corolla: absent



ACCESSION: UCT-26Ach

NAME: Azul chañihue

TUBER

Shape: ovate

Unusual shape: absent

Predominant skin colour: purple

Secondary skin colour: absent

Distribution of secondary skin colour: absent

Tuber skin type: smooth

Predominant flesh colour: cream

Secondary flesh colour: absent

Distribution of secondary flesh colour: absent

Note of eyes per tuber: intermediate

Average number of eyes: 6.7

Depth of tuber eyes: deep

Distribution of tuber eyes: evenly distributed

Presence of eyebrows: markedly

SPROUT

Shape: narrow cylindrical

Growth habit of spout: intermediate

Length of lateral branches: short

Predominant colour: purple

Pubescence: pubescent

Lenticels pigmentation of sprout: absent

STEM

Stem colour: purple with some green

Stem section: angular

Stem wings: dentate

Stem anthocyanic coloration: very strong

Average main stem: 3.35

PLANT

Foliage structure: intermediate type

Growth habit: semi-upright

Height: medium

LEAF

Openness: close

Presence of secondary leaflet: weak

Abaxial leaf pubescence: glabrescent

Adaxial leaf pubescence: strongly pubescent

Green colour: dark

Anthocyanic coloration on midrib of upper side: strongly pigmented

Terminal and lateral leaflets frequency of coalescence: medium

Size of terminal leaflet: medium

Size of lateral leaflets: small size

Second pair of lateral leaflets (width in relation length): close

Leaflet: waviness of margin: absent or very weak

Leaflet: depth of veins: medium

Leaflet: glossiness of upperside: dull

Leaf dissection: weakly dissected

FLOWER

Calyx colour:

Predominant colour of corolla:

Secondary colour of corolla:



ACCESSION: UCT-27MU

NAME: Murta

TUBER

Shape: round (rounded)

Unusual shape: absent

Predominant skin colour: red

Secondary skin colour: absent

Distribution of secondary skin colour: absent

Tuber skin type: smooth

Predominant flesh colour: yellow-cream

Secondary flesh colour: absent

Distribution of secondary flesh colour: absent

Note of eyes per tuber: intermediate

Average number of eyes: 5.9

Depth of tuber eyes: deep

Distribution of tuber eyes: evenly distributed

Presence of eyebrows: markedly

SPROUT

Shape: ovate

Growth habit of spout: close

Length of lateral branches: medium

Predominant colour: violet

Pubescence: glabrous

Lenticels pigmentation of sprout: absent

STEM

Stem colour: green

Stem section: angular

Stem wings: straight

Stem anthocyanic coloration: absent or very weak

Average main stem: 4.0

PLANT

Foliage structure: intermediate type

Growth habit: semi-upright

Height: medium

LEAF

Openness: intermediate

Presence of secondary leaflet: medium

Abaxial leaf pubescence: glabrescent

Adaxial leaf pubescence: glabrescent

Green colour: light

Anthocyanic coloration on midrib of upper side: slightly pigmented

Terminal and lateral leaflets frequency of coalescence: absent or very low

Size of terminal leaflet: big size

Size of lateral leaflets: medium size

Second pair of lateral leaflets (width in relation length): close

Leaflet: waviness of margin: absent or very weak

Leaflet: depth of veins: medium

Leaflet: glossiness of upperside: dull

Leaf dissection: weakly dissected

FLOWER

Calyx colour:

Predominant colour of corolla:

Secondary colour of corolla:



ACCESSION: UCT-28MiR

NAME: Michuñe Rojo

TUBER

Shape: absent

Unusual shape: fusiform

Predominant skin colour: red

Secondary skin colour: absent

Distribution of secondary skin colour: absent

Tuber skin type: smooth

Predominant flesh colour: white

Secondary flesh colour: absent

Distribution of secondary flesh colour: absent

Note of eyes per tuber: intermediate

Average number of eyes: 8.7

Depth of tuber eyes: shallow

Distribution of tuber eyes: evenly distributed

Presence of eyebrows: marked

SPROUT

Shape: wide cylindrical

Growth habit of spout: intermediate

Length of lateral branches: medium

Predominant colour: violet

Pubescence: glabrescent

Lenticels pigmentation of sprout: absent

STEM

Stem colour: green with some purple

Stem section: angular

Stem wings: medium

Stem anthocyanic coloration: medium

Average main stem: 2.0

PLANT

Foliage structure: branching type

Growth habit: upright

Height: medium

LEAF

Openness: close

Presence of secondary leaflet: weak

Abaxial leaf pubescence: strongly pubescent

Adaxial leaf pubescence: strongly pubescent

Green colour: dark

Anthocyanic coloration on midrib of upper side: slightly pigmented

Terminal and lateral leaflets frequency of coalescence: absent or very low

Size of terminal leaflet: big size

Size of lateral leaflets: medium

Second pair of lateral leaflets (width in relation length): close

Leaflet: waviness of margin: weak

Leaflet: depth of veins: medium

Leaflet: glossiness of upperside: glossy

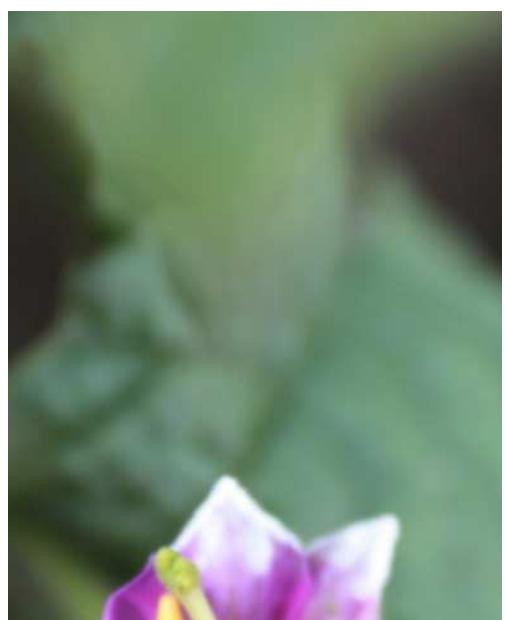
Leaf dissection: weakly dissected

FLOWER

Calyx colour:

Predominant colour of corolla:

Secondary colour of corolla:



ACCESSION: UCT-29Mol

NAME: Molejona

TUBER

Shape: rounded

Unusual shape: absent

Predominant skin colour: red-purple

Secondary skin colour: absent

Distribution of secondary skin colour: absent

Tuber skin type: smooth

Predominant flesh colour: cream

Secondary flesh colour: absent

Distribution of secondary flesh colour: absent

Note of eyes per tuber: intermediate

Average number of eyes: 5.5

Depth of tuber eyes: intermediate

Distribution of tuber eyes: evenly distributed

Presence of eyebrows: marked

SPROUT

Shape: conical

Growth habit of spout: intermediate

Length of lateral branches: medium

Predominant colour: purple

Pubescence: glabrous

Lenticels pigmentation of sprout: present

STEM

Stem colour: green

Stem section: angular

Stem wings: straight

Stem anthocyanic coloration: weak

Average main stem: 4.0

PLANT

Foliage structure: intermediate type

Growth habit: semi-upright

Height: short

LEAF

Openness: close

Presence of secondary leaflet: weak

Abaxial leaf pubescence: pubescent

Adaxial leaf pubescence: pubescent

Green colour: light

Anthocyanic coloration on midrib of upper side: absent or very weak

Terminal and lateral leaflets frequency of coalescence: low

Size of terminal leaflet: medium size

Size of lateral leaflets: small size

Second pair of lateral leaflets (width in relation length): medium

Leaflet: waviness of margin: absent or very weak

Leaflet: depth of veins: medium

Leaflet: glossiness of upperside: glossy

Leaf dissection: weakly dissected

FLOWER

Calyx colour:

Predominant colour of corolla:

Secondary colour of corolla:

MOLEJONA



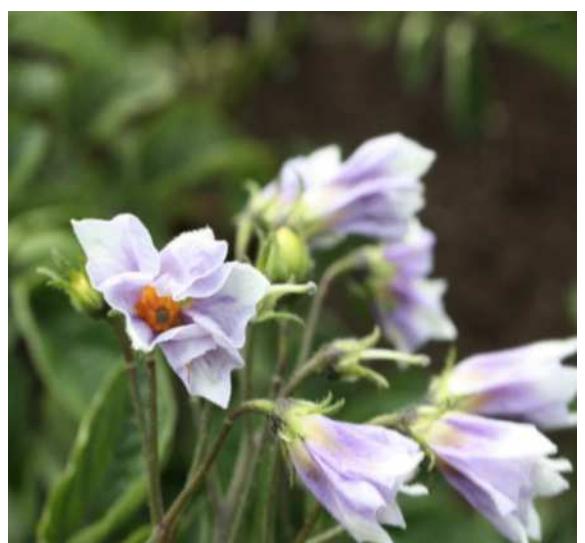
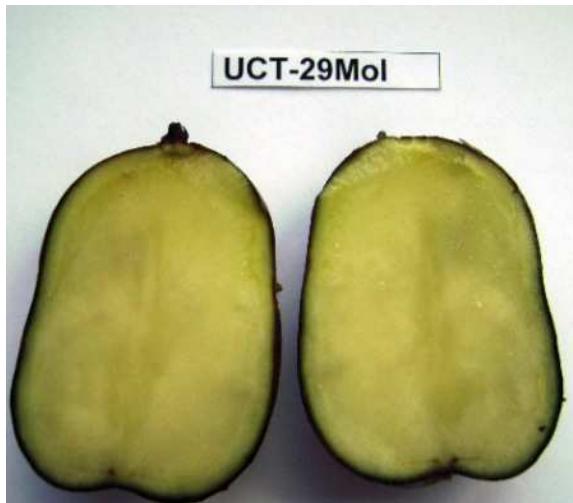
UCT-29Mol



UCT-29Mol



UCT-29Mol



ACCESSION: UCT-3CI

NAME: Clavela

TUBER

Shape: ovate

Unusual shape: absent

Predominant skin colour: yellow

Secondary skin colour: pink

Distribution of secondary skin colour: scattered

Tuber skin type: smooth

Predominant flesh colour: cream

Secondary flesh colour: absent

Distribution of secondary flesh colour: absent

Note of eyes per tuber: few

Average number of eyes: 5.5

Depth of tuber eyes: shallow

Distribution of tuber eyes: evenly distributed

Presence of eyebrows: inconspicuous

SPROUT

Shape: wide cylindrical

Growth habit of spout: open

Length of lateral branches: short

Predominant colour: fuchsia

Pubescence: pubescent

Lenticels pigmentation of sprout: absent

STEM

Stem colour: green

Stem section: angular

Stem wings: straight

Stem anthocyanic coloration: absent or very weak

Average main stem: 4.0

PLANT

Foliage structure: intermediate type

Growth habit: semi-upright

Height: short

LEAF

Openness: intermediate

Presence of secondary leaflet: weak

Abaxial leaf pubescence: pubescent

Adaxial leaf pubescence: pubescent

Green colour: dark

Anthocyanic coloration on midrib of upper side: absent or weak

Terminal and lateral leaflets frequency of coalescence: high

Size of terminal leaflet: big size

Size of lateral leaflets: medium

Second pair of lateral leaflets (width in relation length): close

Leaflet: waviness of margin: weak

Leaflet: depth of veins: medium

Leaflet: glossiness of upperside: glossy

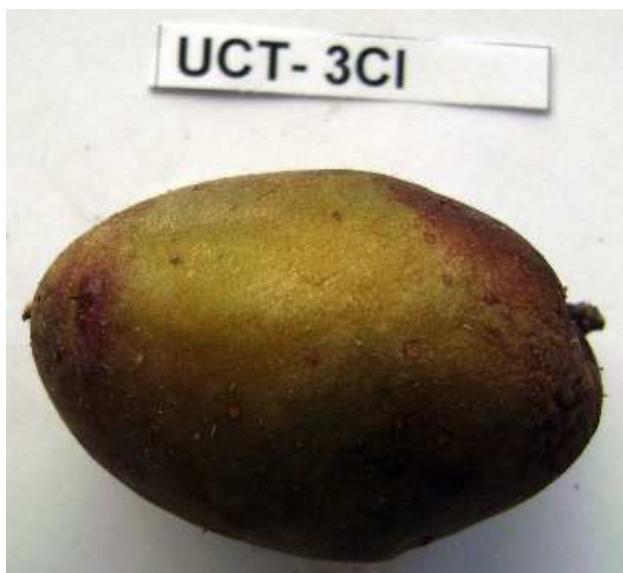
Leaf dissection: weakly dissected

FLOWER

Calyx colour: green with some purple

Predominant colour of corolla: deep blue

Secondary colour of corolla: white



ACCESSION: UCT-1Ma

NAME: Michuñe azul

TUBER

Shape: absent

Unusual shape: constrained fusiform

Predominant skin colour: dark purple-black

Secondary skin colour: absent

Distribution of secondary skin colour: absent

Tuber skin type: smooth

Predominant flesh colour: white

Secondary flesh colour: violet

Distribution of secondary flesh colour: vascular ring and medulla

Note of eyes per tuber: intermediate

Average number of eyes: 10.5

Depth of tuber eyes: medium

Distribution of tuber eyes: evenly distributed

Presence of eyebrows: inconspicuous

SPROUT

Shape: conical

Growth habit of spout: close

Length of lateral branches: medium

Predominant colour: violet

Pubescence: pubescent

Lenticels pigmentation of sprout: present

STEM

Stem colour: green with some purple

Stem section: angular

Stem wings: straight

Stem anthocyanic coloration: weak

Average main stem: 3.9

PLANT

Foliage structure: intermediate type

Growth habit: semi-upright

Height: high

LEAF

Openness: intermediate

Presence of secondary leaflet: weak

Abaxial leaf pubescence: strongly pubescent

Adaxial leaf pubescence: strongly pubescent

Green colour: dark

Anthocyanic coloration on midrib of upper side: strongly pigmented

Terminal and lateral leaflets frequency of coalescence: absent or very weak

Size of terminal leaflet: big size

Size of lateral leaflets: big size

Second pair of lateral leaflets (width in relation length): close

Leaflet: waviness of margin: weak

Leaflet: depth of veins: medium

Leaflet: glossiness of upperside: dull

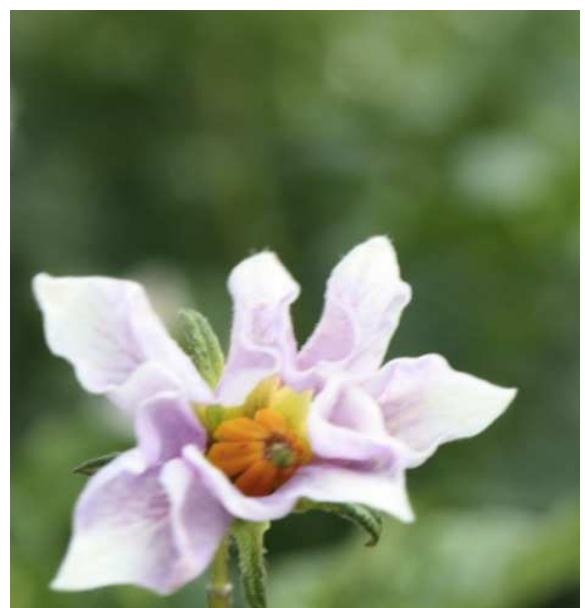
Leaf dissection: weakly dissected

FLOWER

Calyx colour: red

Predominant colour of corolla: light purple

Secondary colour of corolla: absent



ACCESSION: UCT-16At

NAME: Azul tabla

TUBER

Shape: long-oblong

Unusual shape: absent

Predominant skin colour: dark purple-black

Secondary skin colour: absent

Distribution of secondary skin colour: absent

Tuber skin type: smooth

Predominant flesh colour: cream

Secondary flesh colour: violet

Distribution of secondary flesh colour: vascular ring and medulla

Note of eyes per tuber: intermediate

Average number of eyes: 11.7

Depth of tuber eyes: intermediate

Distribution of tuber eyes: evenly distributed

Presence of eyebrows: markedly

SPROUT

Shape: ovate

Growth habit of spout: intermediate

Length of lateral branches: medium

Predominant colour: fuchsia

Pubescence: strongly pubescent

Lenticels pigmentation of sprout: absent

STEM

Stem colour: green with some purple

Stem section: angular

Stem wings: undulate (wavy)

Stem anthocyanic coloration: purple with some green

Average main stem: 4.00

PLANT

Foliage structure: intermediate type

Growth habit: semi-upright

Height: medium

LEAF

Openness: close

Presence of secondary leaflet: weak

Abaxial leaf pubescence: pubescent

Adaxial leaf pubescence: glabrescent

Green colour: light

Anthocyanic coloration on midrib of upper side: strongly pigmented

Terminal and lateral leaflets frequency of coalescence: absent or very weak

Size of terminal leaflet: big size

Size of lateral leaflets: medium

Second pair of lateral leaflets (width in relation length): close

Leaflet: waviness of margin: medium

Leaflet: depth of veins: shallow

Leaflet: glossiness of upperside: glossy

Leaf dissection: almost dissected

FLOWER

Calyx colour: green with some purple

Predominant colour of corolla: yellow

Secondary colour of corolla: absent

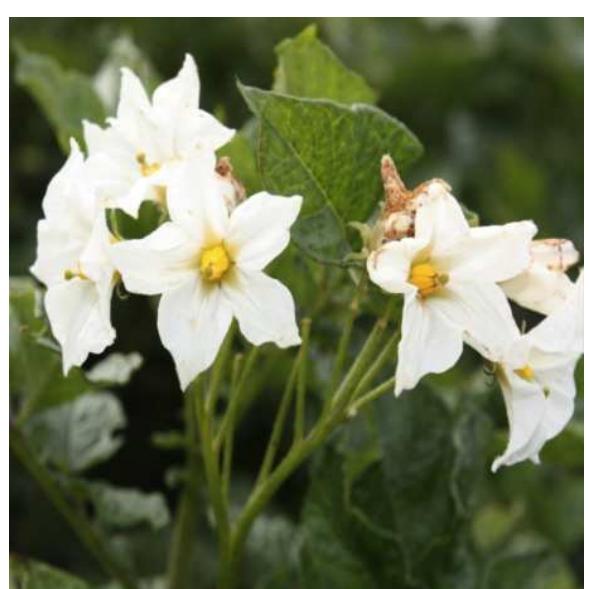
**UCT-16At**



**UCT-16At**



**UCT-16At**



ACCESSION: UCT-30Ño

NAME: Ñocha

TUBER

Shape: elongate

Unusual shape: absent

Predominant skin colour: reddish

Secondary skin colour: yellow

Distribution of secondary skin colour: scattered

Tuber skin type: smooth

Predominant flesh colour: white

Secondary flesh colour: red

Distribution of secondary flesh colour: scattered area

Note of eyes per tuber: intermediate

Average number of eyes: 11.5

Depth of tuber eyes: medium

Distribution of tuber eyes: evenly distributed

Presence of eyebrows: markedly

SPROUT

Shape: conical

Growth habit of spout: intermediate

Length of lateral branches: short

Predominant colour: pink

Pubescence: glabrescent

Lenticels pigmentation of sprout: absent

STEM

Stem colour: green

Stem section: angular

Stem wings: straight

Stem anthocyanic coloration: weak

Average main stem: 3.5

PLANT

Foliage structure: intermediate type

Growth habit: semi-upright

Height: medium

LEAF

Openness: intermediate

Presence of secondary leaflet: weak

Abaxial leaf pubescence: strongly pubescent

Adaxial leaf pubescence: strongly pubescent

Green colour: light

Anthocyanic coloration on midrib of upper side: slightly pigmented

Terminal and lateral leaflets frequency of coalescence: absent or very low

Size of terminal leaflet: big size

Size of lateral leaflets: medium

Second pair of lateral leaflets (width in relation length): close

Leaflet: waviness of margin: medium

Leaflet: depth of veins: shallow

Leaflet: glossiness of upperside: dull

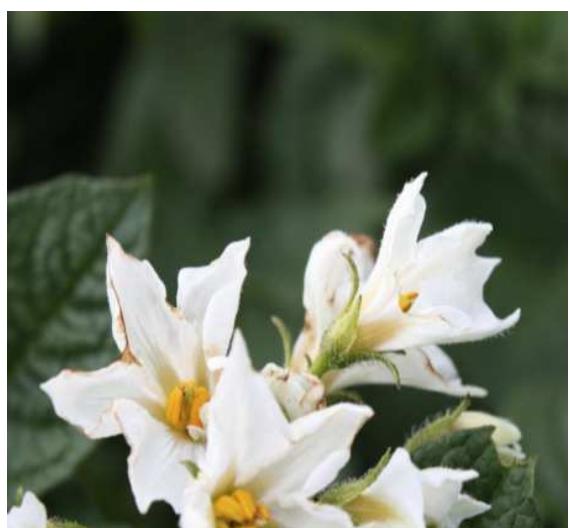
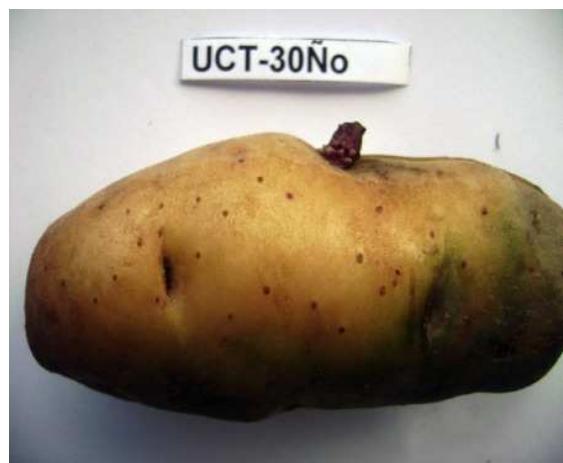
Leaf dissection: weakly dissected

FLOWER

Calyx colour:

Predominant colour of corolla:

Secondary colour of corolla:



ACCESSION: UCT-19Aq

NAME:Azul de quento

TUBER

Shape: round

Unusual shape: absent

Predominant skin colour: brownish

Secondary skin colour: absent

Distribution of secondary skin colour: absent

Tuber skin type: smooth

Predominant flesh colour: cream

Secondary flesh colour: absent

Distribution of secondary flesh colour: absent

Note of eyes per tuber: intermediate

Average number of eyes: 8.4

Depth of tuber eyes: deep

Distribution of tuber eyes: evenly distributed

Presence of eyebrows: markedly

SPROUT

Shape: conical

Growth habit of spout: intermediate

Length of lateral branches: short

Predominant colour: fuchsia

Pubescence: pubescent

Lenticels pigmentation of sprout: present

STEM

Stem colour: purple with some green

Stem section: angular

Stem wings: undulate (wavy)

Stem anthocyanic coloration: absent or very weak

Average main stem: 3.7

PLANT

Foliage structure: intermediate type

Growth habit: semi-upright

Height: low

LEAF

Openness: close

Presence of secondary leaflet: weak

Abaxial leaf pubescence: pubescent

Adaxial leaf pubescence: pubescent

Green colour: light

Anthocyanic coloration on midrib of upper side: absent or very weak

Terminal and lateral leaflets frequency of coalescence: absent or very weak

Size of terminal leaflet: big size

Size of lateral leaflets: medium

Second pair of lateral leaflets (width in relation length): close

Leaflet: waviness of margin: medium

Leaflet: depth of veins: medium

Leaflet: glossiness of upperside: high

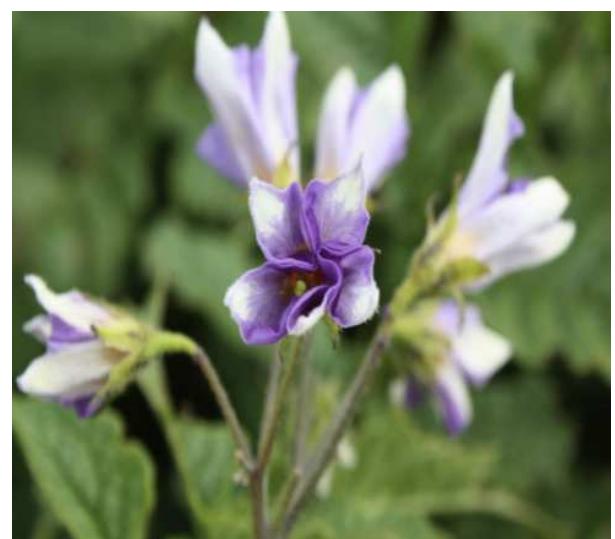
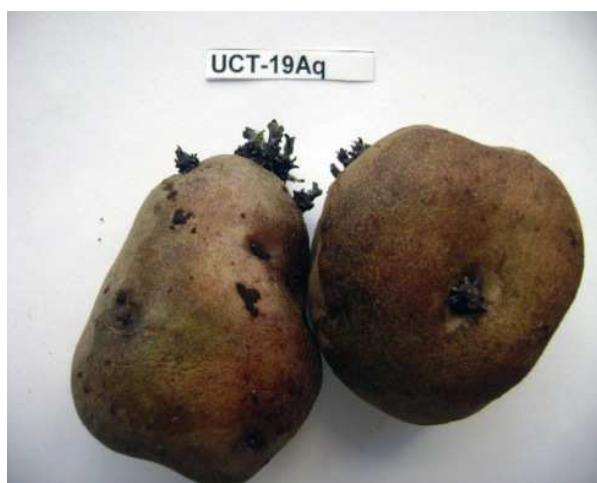
Leaf dissection: moderately dissected

FLOWER

Calyx colour: purple with some green

Predominant colour of corolla: white

Secondary colour of corolla: white



ACCESSION: UCT-2Lv

NAME: Lengua vaca

TUBER

Shape: elliptic, ovate-elongate

Unusual shape: absent

Predominant skin colour: yellow

Secondary skin colour: absent

Distribution of secondary skin colour: absent

Tuber skin type: smooth

Predominant flesh colour: yellow-cream

Secondary flesh colour: absent

Distribution of secondary flesh colour: absent

Note of eyes per tuber: intermediate

Average number of eyes: 6.5

Depth of tuber eyes: shallow

Distribution of tuber eyes: evenly distributed

Presence of eyebrows: marked

SPROUT

Shape: ovate

Growth habit of spout: open

Length of lateral branches: medium

Predominant colour: violet-pink

Pubescence: glabrous

Lenticels pigmentation of sprout: absent

STEM

Stem colour: green with some purple

Stem section: angular

Stem wings: straight

Stem anthocyanic coloration: absent or very weak

Average main stem: 3.8

PLANT

Foliage structure: intermediate type

Growth habit: medium

Height: medium-low

LEAF

Openness: intermediate

Presence of secondary leaflet: medium

Abaxial leaf pubescence: pubescent

Adaxial leaf pubescence: pubescent

Green colour: light

Anthocyanic coloration on midrib of upper side: absent or very weak

Terminal and lateral leaflets frequency of coalescence: absent or very low

Size of terminal leaflet: big size

Size of lateral leaflets: big size

Second pair of lateral leaflets (width in relation length): close

Leaflet: waviness of margin: absent or very weak

Leaflet: depth of veins: shallow

Leaflet: glossiness of upperside: glossy

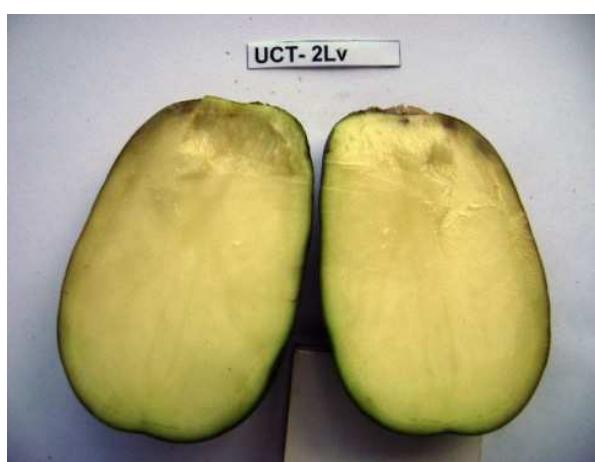
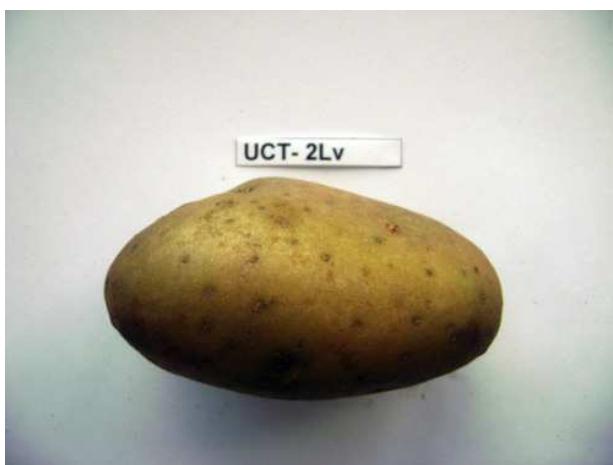
Leaf dissection: weakly dissected

FLOWER

Calyx colour: green with some purple

Predominant colour of corolla: deep blue

Secondary colour of corolla: white



ACCESSION: UCT-31Ob

NAME: Ojitos blanco

TUBER

Shape: absent

Unusual shape: fusiform

Predominant skin colour: dark purple-black

Secondary skin colour: white-cream

Distribution of secondary skin colour: splashed

Tuber skin type: smooth

Predominant flesh colour: white

Secondary flesh colour: violet

Distribution of secondary flesh colour: vascular ring and medulla

Note of eyes per tuber: intermediate

Average number of eyes: 11.5

Depth of tuber eyes: very deep

Distribution of tuber eyes: evenly distributed

Presence of eyebrows: marked

SPROUT

Shape: wide cylindrical

Growth habit of spout: intermediate

Length of lateral branches: short

Predominant colour: purple

Pubescence: pubescent

Lenticels pigmentation of sprout: absent

STEM

Stem colour: green with some purple knots

Stem section: angular

Stem wings: straight

Stem anthocyanic coloration: weak

Average main stem: 3.1

PLANT

Foliage structure: intermediate type

Growth habit: semi-upright

Height: medium

LEAF

Openness: close

Presence of secondary leaflet: medium

Abaxial leaf pubescence: pubescent

Adaxial leaf pubescence: pubescent

Green colour: dark

Anthocyanic coloration on midrib of upper side: slightly pigmented

Terminal and lateral leaflets frequency of coalescence: low frequency

Size of terminal leaflet: big size

Size of lateral leaflets: medium

Second pair of lateral leaflets (width in relation length): close

Leaflet: waviness of margin: absent or very weak

Leaflet: depth of veins: shallow

Leaflet: glossiness of upperside: dull

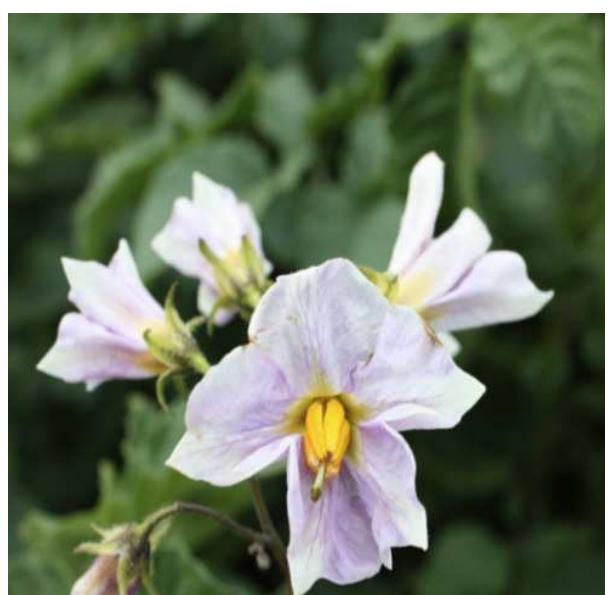
Leaf dissection: weakly dissected

FLOWER

Calyx colour: green with some purple

Predominant colour of corolla: light purple

Secondary colour of corolla: white



ACCESSION: UCT-32Ci

NAME:Cielito

TUBER

Shape: round

Unusual shape: absent

Predominant skin colour: brownish

Secondary skin colour: red

Distribution of secondary skin colour: scattered

Tuber skin type: smooth

Predominant flesh colour: white

Secondary flesh colour: absent

Distribution of secondary flesh colour: absent

Note of eyes per tuber: intermediate

Average number of eyes: 5.7

Depth of tuber eyes: medium

Distribution of tuber eyes: evenly distributed

Presence of eyebrows: marked

SPROUT

Shape: conical

Growth habit of spout: intermediate

Length of lateral branches: long

Predominant colour: violet

Pubescence: strongly pubescent

Lenticels pigmentation of sprout: present

STEM

Stem colour: green

Stem section: angular

Stem wings: straight

Stem anthocyanic coloration: absent or very weak

Average main stem: 4.3

PLANT

Foliage structure: leaf type

Growth habit: spreading

Height: medium

LEAF

Openness: intermediate

Presence of secondary leaflet: weak

Abaxial leaf pubescence: glabrescent

Adaxial leaf pubescence: pubescent

Green colour: light

Anthocyanic coloration on midrib of upper side: absent or very weak

Terminal and lateral leaflets frequency of coalescence: high

Size of terminal leaflet: medium

Size of lateral leaflets: medium

Second pair of lateral leaflets (width in relation length): close

Leaflet: waviness of margin: weak

Leaflet: depth of veins: shallow

Leaflet: glossiness of upperside: medium

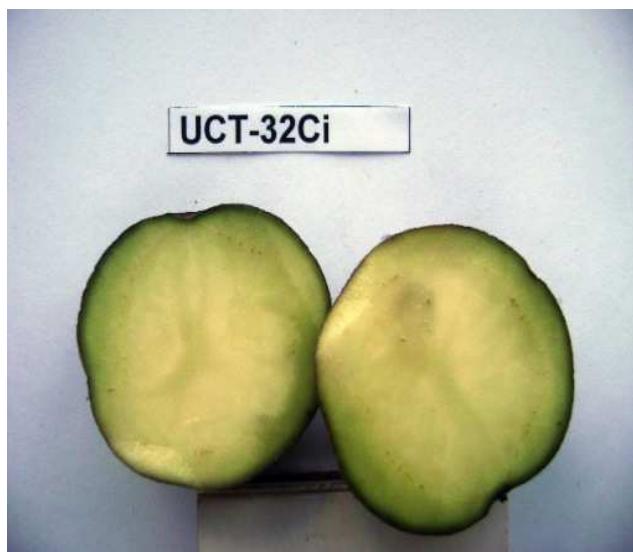
Leaf dissection: weakly dissected

FLOWER

Calyx colour:

Predominant colour of corolla:

Secondary colour of corolla:



ACCESSION: UCT-20Ro

NAME: Rosada

TUBER

Shape: round

Unusual shape: absent

Predominant skin colour: yellow

Secondary skin colour: absent

Distribution of secondary skin colour: absent

Tuber skin type: smooth

Predominant flesh colour: cream

Secondary flesh colour: absent

Distribution of secondary flesh colour: absent

Note of eyes per tuber: intermediate

Average number of eyes: 6.0

Depth of tuber eyes: medium

Distribution of tuber eyes: evenly distributed

Presence of eyebrows: inconspicuous

SPROUT

Shape: ovate

Growth habit of spout: open

Length of lateral branches: short

Predominant colour: pink

Pubescence: pubescent

Lenticels pigmentation of sprout: absent

STEM

Stem colour: green with some purple knots

Stem section: angular

Stem wings: straight

Stem anthocyanic coloration: absent or very weak

Average main stem: 3.5

PLANT

Foliage structure: intermediate type

Growth habit: semi-upright

Height: medium

LEAF

Openness: intermediate

Presence of secondary leaflet: weak

Abaxial leaf pubescence: pubescent

Adaxial leaf pubescence: pubescent

Green colour: dark

Anthocyanic coloration on midrib of upper side: slightly pigmented

Terminal and lateral leaflets frequency of coalescence: absent or very low

Size of terminal leaflet: medium

Size of lateral leaflets: medium

Second pair of lateral leaflets (width in relation length): close

Leaflet: waviness of margin: absent or very weak

Leaflet: depth of veins: shallow

Leaflet: glossiness of upperside: medium

Leaf dissection: almost dissected

FLOWER

Calyx colour: green with some purple

Predominant colour of corolla: light purple

Secondary colour of corolla: white



ACCESSION: UCT-33Cab

NAME:Cabrita

TUBER

Shape: oval

Unusual shape: absent

Predominant skin colour: purple

Secondary skin colour: yellow

Distribution of secondary skin colour: scattered

Tuber skin type: smooth

Predominant flesh colour: white

Secondary flesh colour: violet

Distribution of secondary flesh colour: vascular ring and medulla

Note of eyes per tuber: intermediate

Average number of eyes: 8.6

Depth of tuber eyes: intermediate

Distribution of tuber eyes: evenly distributed

Presence of eyebrows: superficial

SPROUT

Shape: wide cylindrical

Growth habit of spout: open

Length of lateral branches: medium

Predominant colour: violet

Pubescence: glabrescent

Lenticels pigmentation of sprout: absent

STEM

Stem colour: green

Stem section: angular

Stem wings: straight

Stem anthocyanic coloration: absent or very weak

Average main stem: 4.3

PLANT

Foliage structure: intermediate type

Growth habit: semi-upright

Height: medium

LEAF

Openness: intermediate

Presence of secondary leaflet: medium

Abaxial leaf pubescence: strongly pubescent

Adaxial leaf pubescence: glabrous

Green colour: light

Anthocyanic coloration on midrib of upper side: slightly pigmented

Terminal and lateral leaflets frequency of coalescence: low frequency

Size of terminal leaflet: medium

Size of lateral leaflets: small size

Second pair of lateral leaflets (width in relation length): close

Leaflet: waviness of margin: moderately undulating

Leaflet: depth of veins: medium

Leaflet: glossiness of upperside: dull

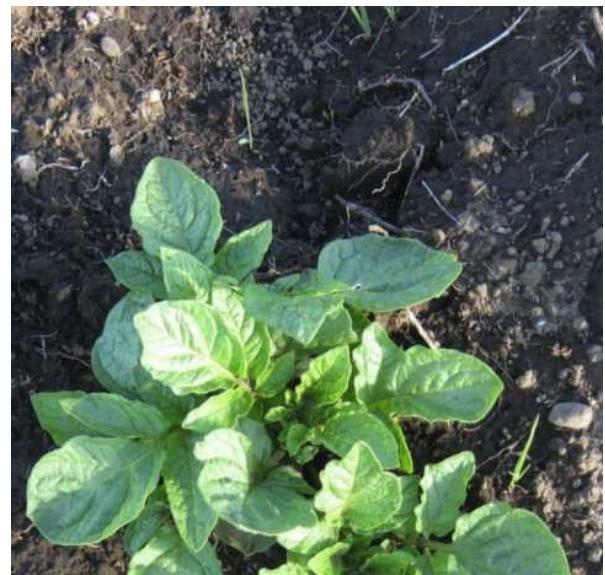
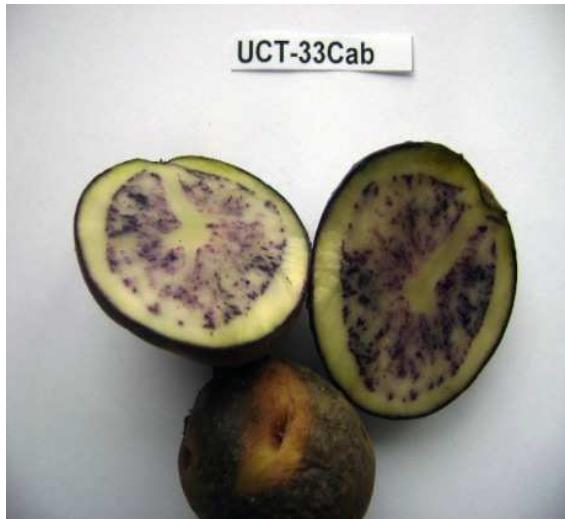
Leaf dissection: weakly dissected

FLOWER

Calyx colour:

Predominant colour of corolla:

Secondary colour of corolla:



ACCESSION: UCT-15MgRo

NAME: Meca gato roja

TUBER

Shape: elongate

Unusual shape: absent

Predominant skin colour: red

Secondary skin colour: absent

Distribution of secondary skin colour: absent

Tuber skin type: smooth

Predominant flesh colour: white

Secondary flesh colour: red

Distribution of secondary flesh colour: scattered areas

Note of eyes per tuber: intermediate

Average number of eyes: 9.1

Depth of tuber eyes: shallow

Distribution of tuber eyes: evenly distributed

Presence of eyebrows: inconspicuous

SPROUT

Shape: wide cylindrical

Growth habit of spout: intermediate

Length of lateral branches: medium

Predominant colour: pink

Pubescence: glabrous

Lenticels pigmentation of sprout: absent

STEM

Stem colour: green with some purple knots

Stem section: angular

Stem wings: undulating (wavy)

Stem anthocyanic coloration: medium

Average main stem: 2.8

PLANT

Foliage structure: branched type

Growth habit: semi-upright

Height: medium

LEAF

Openness: intermediate

Presence of secondary leaflet: weak

Abaxial leaf pubescence: strongly pubescent

Adaxial leaf pubescence: strongly pubescent

Green colour: dark

Anthocyanic coloration on midrib of upper side: strongly pigmented

Terminal and lateral leaflets frequency of coalescence: low

Size of terminal leaflet: medium

Size of lateral leaflets: medium

Second pair of lateral leaflets (width in relation length): close

Leaflet: waviness of margin: weak

Leaflet: depth of veins: medium

Leaflet: glossiness of upperside: glossy

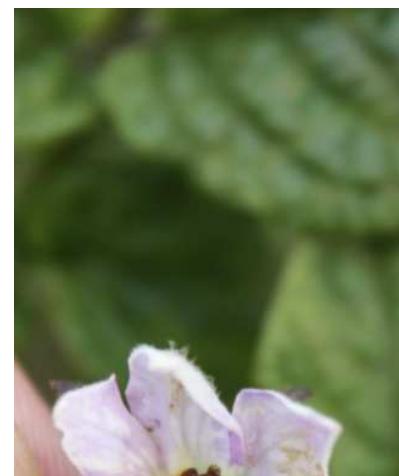
Leaf dissection: weakly dissected

FLOWER

Calyx colour: green with some purple

Predominant colour of corolla: deep red

Secondary colour of corolla: white



ACCESSION: UCT-21Ac

NAME: Azul cristalina

TUBER

Shape: rounded

Unusual shape: absent

Predominant skin colour: yellow-cream

Secondary skin colour: absent

Distribution of secondary skin colour: absent

Tuber skin type: smooth

Predominant flesh colour: cream

Secondary flesh colour: absent

Distribution of secondary flesh colour: absent

Note of eyes per tuber: intermediate

Average number of eyes: 7.3

Depth of tuber eyes: deep

Distribution of tuber eyes: evenly distributed

Presence of eyebrows: markedly

SPROUT

Shape: conical

Growth habit of spout: intermediate

Length of lateral branches: long

Predominant colour: fuchsia

Pubescence: strongly pubescent

Lenticels pigmentation of sprout: present

STEM

Stem colour: green with some purple knots

Stem section: angular

Stem wings: straight

Stem anthocyanic coloration: medium

Average main stem: 4.9

PLANT

Foliage structure: branched type

Growth habit: semi-upright

Height: medium

LEAF

Openness: intermediate

Presence of secondary leaflet: medium

Abaxial leaf pubescence: pubescent

Adaxial leaf pubescence: pubescent

Green colour: light

Anthocyanic coloration on midrib of upper side: absent or very weak

Terminal and lateral leaflets frequency of coalescence: absent or very low

Size of terminal leaflet: medium

Size of lateral leaflets: medium

Second pair of lateral leaflets (width in relation length): close

Leaflet: waviness of margin: weak

Leaflet: depth of veins: shallow

Leaflet: glossiness of upperside: medium

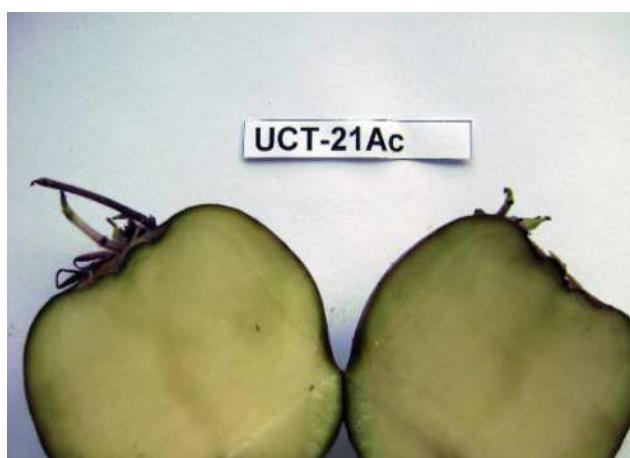
Leaf dissection: weakly dissected

FLOWER

Calyx colour: purple with some green

Predominant colour of corolla: deep purple

Secondary colour of corolla: white



ACCESSION: UCT-34Cor

NAME: Cordillera

TUBER

Shape: round

Unusual shape: absent

Predominant skin colour: purple-red

Secondary skin colour: absent

Distribution of secondary skin colour: absent

Tuber skin type: partially crosslinked

Predominant flesh colour: cream

Secondary flesh colour: absent

Distribution of secondary flesh colour: absent

Note of eyes per tuber: few

Average number of eyes: 4.6

Depth of tuber eyes: intermediate

Distribution of tuber eyes: evenly distributed

Presence of eyebrows: marked

SPROUT

Shape: wide cylindrical

Growth habit of spout: intermediate

Length of lateral branches: short

Predominant colour: fuchsia

Pubescence: pubescent

Lenticels pigmentation of sprout: absent

STEM

Stem colour: green

Stem section: angular

Stem wings: dentate

Stem anthocyanic coloration: absent or very weak

Average main stem: 3.5

PLANT

Foliage structure: intermediate type

Growth habit: semi-upright

Height: medium

LEAF

Openness: intermediate

Presence of secondary leaflet: medium

Abaxial leaf pubescence: pubescent

Adaxial leaf pubescence: pubescent

Green colour: dark

Anthocyanic coloration on midrib of upper side: strongly pigmented

Terminal and lateral leaflets frequency of coalescence: absent or very low

Size of terminal leaflet: big size

Size of lateral leaflets: medium size

Second pair of lateral leaflets (width in relation length): close

Leaflet: waviness of margin: weak

Leaflet: depth of veins: shallow

Leaflet: glossiness of upperside: dull

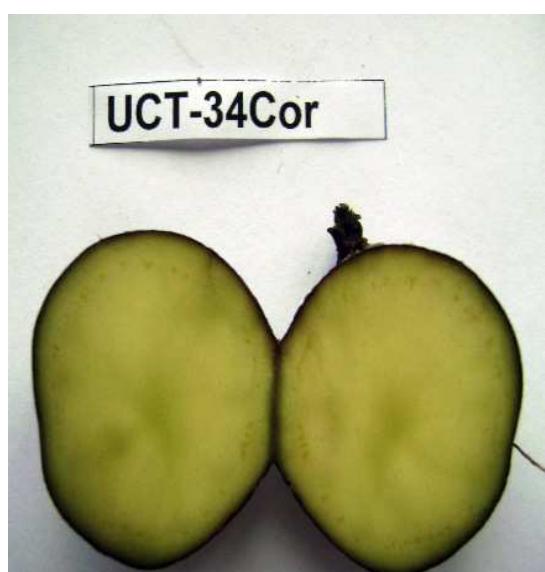
Leaf dissection: almost dissected

FLOWER

Calyx colour:

Predominant colour of corolla:

Secondary colour of corolla:



ACCESSION: UCT-35AzC

NAME: Azul caucheque

TUBER

Shape: ovate

Unusual shape: absent

Predominant skin colour: purple-dark

Secondary skin colour: absent

Distribution of secondary skin colour: absent

Tuber skin type: smooth

Predominant flesh colour: white

Secondary flesh colour: violet

Distribution of secondary flesh colour: vascular ring and medulla

Note of eyes per tuber: intermediate

Average number of eyes: 7.7

Depth of tuber eyes: deep

Distribution of tuber eyes: evenly distributed

Presence of eyebrows: markedly

SPROUT

Shape: obovate

Growth habit of spout: open

Length of lateral branches: long

Predominant colour: fuchsia

Pubescence: glabrous

Lenticels pigmentation of sprout: present

STEM

Stem colour: purple with some green

Stem section: angular

Stem wings: straight

Stem anthocyanic coloration: very strong

Average main stem: 3.3

PLANT

Foliage structure: branched type

Growth habit: upright

Height: tall

LEAF

Openness: close

Presence of secondary leaflet: medium

Abaxial leaf pubescence: strongly pubescent

Adaxial leaf pubescence: strongly pubescent

Green colour: dark

Anthocyanic coloration on midrib of upper side: strongly pigmented

Terminal and lateral leaflets frequency of coalescence: absent or very low

Size of terminal leaflet: medium

Size of lateral leaflets: medium

Second pair of lateral leaflets (width in relation length): close

Leaflet: waviness of margin: absent or very weak

Leaflet: depth of veins: shallow

Leaflet: glossiness of upperside: glossy

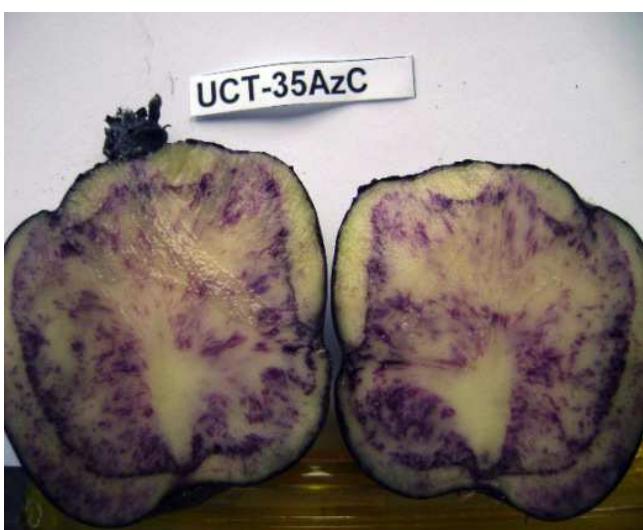
Leaf dissection: weakly dissected

FLOWER

Calyx colour:

Predominant colour of corolla:

Secondary colour of corolla:



ACCESSION: UCT-8Gb

NAME: Guadacho blanco

TUBER

Shape: elongate

Unusual shape: absent

Predominant skin colour: yellow

Secondary skin colour: absent

Distribution of secondary skin colour: absent

Tuber skin type: smooth

Predominant flesh colour: yellow-cream

Secondary flesh colour: absent

Distribution of secondary flesh colour: absent

Note of eyes per tuber: intermediate

Average number of eyes: 7.4

Depth of tuber eyes: shallow

Distribution of tuber eyes: evenly distributed

Presence of eyebrows: inconspicuous

SPROUT

Shape: wide cylindrical

Growth habit of spout: close

Length of lateral branches: medium

Predominant colour: violet

Pubescence: pubescent

Lenticels pigmentation of sprout: absent

STEM

Stem colour: green with purple knots

Stem section: angular

Stem wings: straight

Stem anthocyanic coloration: absent or very weak

Average main stem: 3.6

PLANT

Foliage structure: intermediate type

Growth habit: semi-upright

Height: medium

LEAF

Openness: open

Presence of secondary leaflet: strong

Abaxial leaf pubescence: strongly pubescent

Adaxial leaf pubescence: strongly pubescent

Green colour: dark

Anthocyanic coloration on midrib of upper side: strongly pigmented

Terminal and lateral leaflets frequency of coalescence: absent or very low

Size of terminal leaflet: medium

Size of lateral leaflets: medium

Second pair of lateral leaflets (width in relation length): medium

Leaflet: waviness of margin: absent or very weak

Leaflet: depth of veins: shallow

Leaflet: glossiness of upperside: glossy

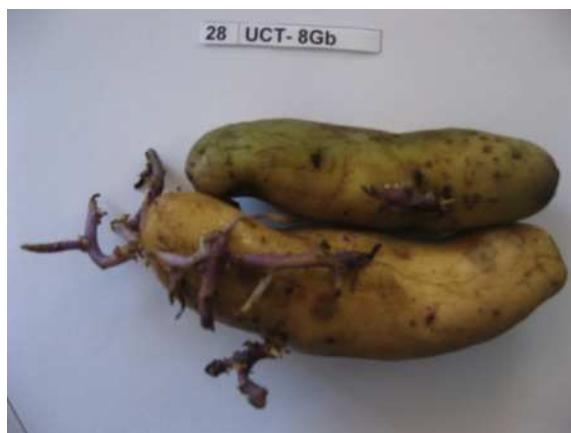
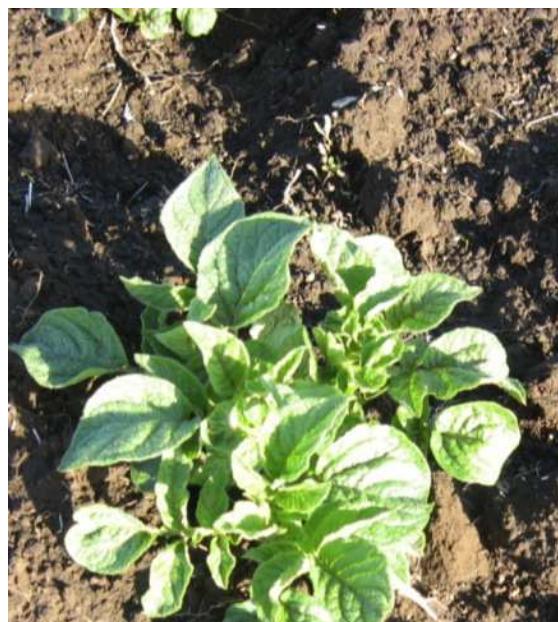
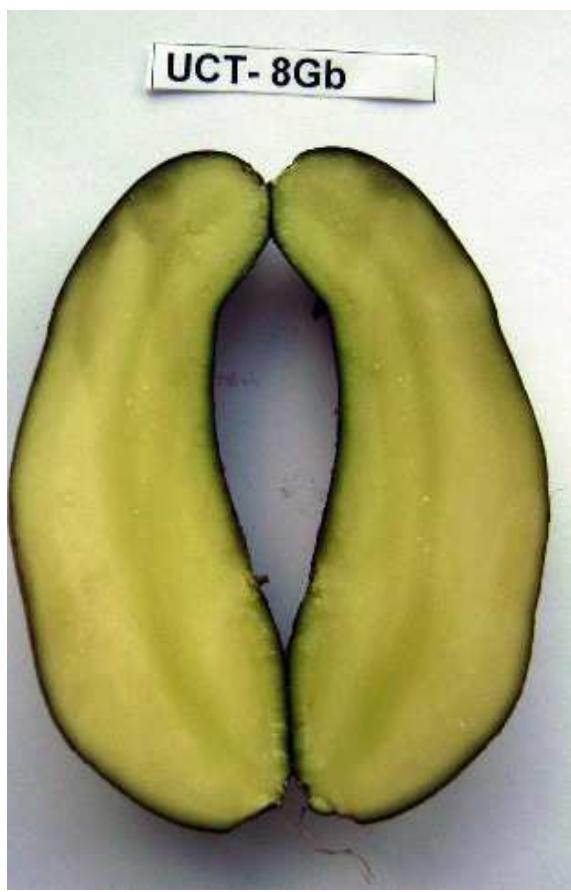
Leaf dissection: weakly dissected

FLOWER

Calyx colour: green with some purple

Predominant colour of corolla: deep red

Secondary colour of corolla: white



ACCESSION: UCT-9MgM

NAME: Meca gato morada

TUBER

Shape: absent

Unusual shape: fusiform

Predominant skin colour: purple

Secondary skin colour: absent

Distribution of secondary skin colour: absent

Tuber skin type: smooth

Predominant flesh colour: cream

Secondary flesh colour: violet

Distribution of secondary flesh colour: vascular ring and medulla

Note of eyes per tuber: intermediate

Average number of eyes: 9.8

Depth of tuber eyes: very deep

Distribution of tuber eyes: evenly distributed

Presence of eyebrows: marked

SPROUT

Shape: narrow cylindrical

Growth habit of spout: intermediate

Length of lateral branches: short

Predominant colour: purple

Pubescence: pubescent

Lenticels pigmentation of sprout: absent

STEM

Stem colour: green with purple knots

Stem section: angular

Stem wings: straight

Stem anthocyanic coloration: absent or very weak

Average main stem: 3.75

PLANT

Foliage structure: intermediate type

Growth habit: semi-upright

Height: medium

LEAF

Openness: intermediate

Presence of secondary leaflet: weak

Abaxial leaf pubescence: strongly pubescent

Adaxial leaf pubescence: strongly pubescent

Green colour: dark

Anthocyanic coloration on midrib of upper side: slightly pigmented

Terminal and lateral leaflets frequency of coalescence: absent or very low

Size of terminal leaflet: big

Size of lateral leaflets: medium

Second pair of lateral leaflets (width in relation length): medium

Leaflet: waviness of margin: absent or very weak

Leaflet: depth of veins: shallow

Leaflet: glossiness of upperside: medium

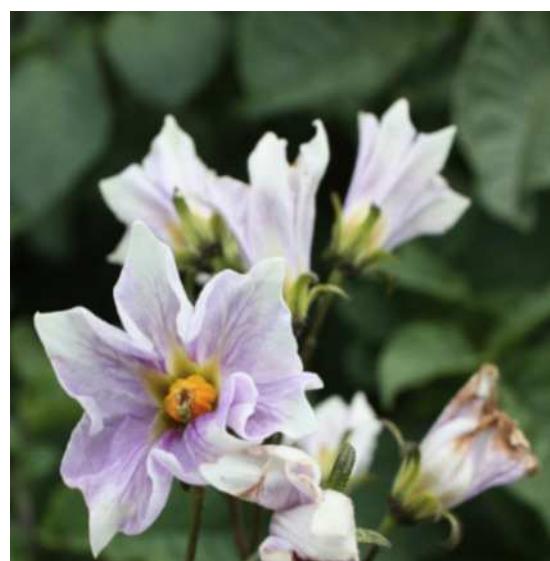
Leaf dissection: weakly dissected

FLOWER

Calyx colour: green with some purple

Predominant colour of corolla: deep blue

Secondary colour of corolla: white



ACCESSION: UCT-10MgL

NAME: Meca gato larga

TUBER

Shape: elongated

Unusual shape: absent

Predominant skin colour: dark purple-black

Secondary skin colour: absent

Distribution of secondary skin colour: absent

Tuber skin type: smooth

Predominant flesh colour: white

Secondary flesh colour: purple

Distribution of secondary flesh colour: All flesh except medulla

Note of eyes per tuber: intermediate

Average number of eyes: 12.8

Depth of tuber eyes: very deep

Distribution of tuber eyes: evenly distributed

Presence of eyebrows: marked

SPROUT

Shape: wide cylindrical

Growth habit of spout: intermediate

Length of lateral branches: medium

Predominant colour: fuchsia

Pubescence: pubescent

Lenticels pigmentation of sprout: absent

STEM

Stem colour: green with purple knots

Stem section: angular

Stem wings: straight

Stem anthocyanic coloration: absent or very weak

Average main stem: 4.0

PLANT

Foliage structure: intermediate type

Growth habit: weak

Height: medium

LEAF

Openness: intermediate

Presence of secondary leaflet: weak

Abaxial leaf pubescence: strongly pubescent

Adaxial leaf pubescence: strongly pubescent

Green colour: dark

Anthocyanic coloration on midrib of upper side: slightly pigmented

Terminal and lateral leaflets frequency of coalescence: absent or very low

Size of terminal leaflet: big size

Size of lateral leaflets: medium

Second pair of lateral leaflets (width in relation length): close

Leaflet: waviness of margin: absent or very weak

Leaflet: depth of veins: shallow

Leaflet: glossiness of upperside: medium

Leaf dissection: weakly dissected

FLOWER

Calyx colour: green with some purple

Predominant colour of corolla: deep red

Secondary colour of corolla: white



ACCESSION: Cultivar Desirée

TUBER

Shape: long-oblong

Unusual shape: no

Predominant skin colour: pink

Secondary skin colour: absent

Distribution of secondary skin colour: absent

Tuber skin type: smooth

Predominant flesh colour: yellow-cream

Secondary flesh colour: absent

Distribution of secondary flesh colour: absent

Note of eyes per tuber:

Average number of eyes:

Depth of tuber eyes: shallow

Distribution of tuber eyes: predominantly apical

Presence of eyebrows: inconspicuous

SPROUT

Shape: conical

Growth habit of spout: close

Length of lateral branches: short

Predominant colour: pink

Pubescence: strongly pubescent

Lenticels pigmentation of sprout: absent

STEM

Stem colour: green

Stem section: angular

Stem wings: undulating (wavy)

Stem anthocyanic coloration: weak

Average main stem: 2.7

PLANT

Foliation structure: intermediate type

Growth habit: semi-upright

Height: low

LEAF

Openness: intermediate

Presence of secondary leaflet: medium

Abaxial leaf pubescence: pubescent

Adaxial leaf pubescence: pubescent

Green colour: medium (grayish green)

Anthocyanic coloration on midrib of upper side: weak

Terminal and lateral leaflets frequency of coalescence: absent or very low

Size of terminal leaflet: big size

Size of lateral leaflets: medium

Second pair of lateral leaflets (width in relation length): medium

Leaflet: waviness of margin: absent or very weak

Leaflet: depth of veins: shallow

Leaflet: glossiness of upperside: medium

Leaf dissection: weakly dissected

FLOWER

Calyx colour: deep purple

Predominant colour of corolla: light pink

Secondary colour of corolla: white

PHENOLOGY

Dormancy: long (4-5 months)

Maturity: medium late (150 days)

DISEASES

Response to late blight: moderately susceptible

Potato virus Y: resistant

Leaf roll virus: susceptible



ACCESSION: Cultivar Karu

TUBER

Shape: long-obovate

Unusual shape: no

Predominant skin colour: red

Secondary skin colour: absent

Distribution of secondary skin colour: absent

Tuber skin type: smooth

Predominant flesh colour: yellow

Secondary flesh colour: absent

Distribution of secondary flesh colour: absent

Note of eyes per tuber: intermediate

Average number of eyes: 7.0

Depth of tuber eyes: shallow

Distribution of tuber eyes: predominately apical

Presence of eyebrows: inconspicuous

SPROUT

Shape: ovate

Growth habit of spout: open

Length of lateral branches: short

Predominant colour: purple

Pubescence: glabrescent

Lenticels pigmentation of sprout: absent

STEM

Stem colour: green

Stem section: angular

Stem wings: straight

Stem anthocyanic coloration: medium

Average main stem: 3.2

PLANT

Foliation structure: intermediate type

Growth habit: semi-upright

Height: low

LEAF

Openness: intermediate

Presence of secondary leaflet: strong

Abaxial leaf pubescence: pubescent

Adaxial leaf pubescence: pubescent

Green colour: medium (deep green)

Anthocyanic coloration on midrib of upper side: medium

Terminal and lateral leaflets frequency of coalescence: absent or very low

Size of terminal leaflet: big size

Size of lateral leaflets: medium size

Second pair of lateral leaflets (width in relation length): close

Leaflet: waviness of margin: absent or very weak

Leaflet: depth of veins: shallow

Leaflet: glossiness of upperside: medium

Leaf dissection: weakly dissected

FLOWER

Calyx colour:

Predominant colour of corolla: violet

Secondary colour of corolla:

PHENOLOGY

Dormancy: long (5 months)

Maturity: (135-145 days)

DISEASES

Response to late blight: moderately susceptible

Potato virus Y: resistant

Leaf roll virus: moderately resistant

