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Evaluation of Salt Tolerance in Potato (*Solanum* spp.)

by

Tala Khrais

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Master of Science

Department of Plant Science
McGill University, Macdonald Campus

1996

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To Naela and Moh'D

with Love

Evaluation of Salt Tolerance in Potato

(*Solanum* spp.)

Tala Khrais

M.Sc.

Tissue Culture

Plant Science

Abstract

This research was carried out to identify salt tolerant potato genotypes *in vitro* among 131 tetraploid potato cultivars (*Solanum tuberosum*), 9 diploid simple hybrid clones (4 clones of *S. chacoense* x *S. tuberosum*, 4 clones of *S. phureja/S. stenotomum* x *S. tuberosum*, and 1 clone of *S. tuberosum x S. tuberosum*), 1 primitive cultivated diploid *S. phureja/S. stenotomum* accession, 12 tetraploid complex hybrids, and 13 diploid *S. chacoense* accessions. Four levels of NaCl (0, 40 ,80, and 120 mM) were used. The cultivars, and the simple and complex hybrids were tested for salt tolerance at the vegetative stage in the nodal cutting bioassay. The thirteen *S. chacoense* accessions were tested for salt tolerance at the germination and early seedling growth stage, in a seedling bioassay. Eleven of these *S. chacoense* accessions were further tested at the vegetative stage, in the nodal cutting bioassay. There was a progressive decline in the morphological parameters measured, with increased salt levels, in the nodal cutting bioassay. The parameters were used collectively in ranking the different genotypes, averaged over three NaCl levels (40, 80, and 120 mM). Twenty potato cultivars, two clones of the simple hybrid *S. chacoense x S. tuberosum*, and one complex hybrid were all considered salt tolerant at the vegetative stage. Ranking of seven *S. chacoense* accessions was similar between early seedling growth and later vegetative stage. Two of these accessions were promising as sources of salt tolerance.

Evaluation de la Tolérance à la Salinité

de la Pomme de Terre (*Solanum* spp.)

Tala Khrais

Maîtrise

Cultures des Tissus

Phytologie

Résumé

Cette recherche *in vitro* a été menée pour identifier des genotypes de pommmes de terre tolérants à la salinité. Quatre niveaux de NaCl (0, 40, 80, and 120 mM) ont été utilisés. Le groupe de genotypes testé comprenait 131 cultivars tétraploids (*Solanum tuberosum*), 9 clones diploids des hybrids simples de *S. tuberosum*, et un diploid cultivé primitif (*S. phureja/S. stenotomum*), 12 tétraploids hybrids complexes, et 13 diploids écotypes originaires d' Argentine (*S. chacoense*). Les cultivars, et les hybrids simples et complexes, ont été testés pour leur tolérance à la salinité au stade végétatif en utilisant le dosage biologique du segment nodal. Les treize écotypes de *S. chacoense* ont été testés pour leur tolérance à la salinité pendant la germination et au début de la croissance des plantules en utilisant un dosage biologique pour plantules. Onze écotypes de *S. chacoense* ont été aussi testés au stade végétatif. Les résultats ont démontré une décroissance progressive des paramètres morphologiques suivant une augmentation dans les niveaux de sel lors du dosage biologique du segment nodal. Ces paramètres ont été utilisés collectivement dans la classification des differents genotypes en calculant la moyenne sur les trois niveaux de NaCl (40, 80, and 120 mM). Vingt cultivars (*Solanum tuberosum*), deux clones de *S. chacoense* x *S. tuberosum*, et un hybrid complex ont été considérés tolérants à la salinité au stade végétatif. La classification a été similaire au stade plantule et au stade végétatif de sept écotypes de *S. chacoense*. Deux écotypes ont été classifiés tolérants à la salinité.

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Abbreviation list:

The following list gives the abbreviations that were used in the thesis:

Full word	Abbreviation
Coefficient of variance	C.V.
Cluster analysis	CA
Cubic clustering criterion	CCC
Concentration	CNC; Conc.
Cultivar(s)	Cv.(s.)
Degree of freedom	DF
Pseudo F statistic	F
Genotype	G
Final germination percentage	G%
Hour	H
Multiple comparison method	MCM
Month	Mo.
Nodal cutting bioassay	NCB
Number of clusters	NCL
Root dry weight	RDW
Root fresh weight	RFW
Root length	RL
Seedling bioassay	SB
Shoot dry weight	SDW
Shoot fresh weight	SFW
Shoot length	SL
Symbol	SYM
Pseudo t^2 statistic	t^2
Total dry weight	TDW
Total fresh weight	TFW
True potato seeds	TPS
Week	Wk

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Chapter 1 - General Introduction

Potato (*Solanum tuberosum*) belongs to the *Solanacea* family. The genus *Solanum* contains more than 1,000 species of which almost 230 are tuberiferous. *S. tuberosum*, a tetraploid, is the only worldwide distributed species (Hawkes, 1992). The total gene pool available in the genus *Solanum* for research and improvement of agronomical characteristics consists of: the wild species, the primitive edible cultivars and their hybrids, and the advanced varieties of present and past cultivation (Foldø, 1987).

Potato is a globally important food crop. The Food and Agriculture Organization ranked potato fourth in annual food production (283×10^6 T), following maize (*Zea mays*), wheat (*Triticum aestivum*), and rice (*Oryza sativa*). In Canada, 28,000 ha were planted with potatoes, at an average yield of 27,600 kg ha^{-1}, and a total production of 3519×10^3 T (FAO, 1994).

Salt-affected soils are a global problem. No continent is free from this. The total agricultural land available on earth is 14 billion ha. Six billion ha are located in arid or semi-arid areas, of which one sixth is salt-affected. Irrigated land occupies 0.23 billion ha, and one third of this is salt-affected (Ashraf, 1994). In Canada, the saline soils are primarily located on the seacoasts and in the prairie regions of Alberta and Saskatchewan (Chapman, 1975).

Breeding for salt tolerance in any crop must cover the nationally used cultivars , as much of the world collection as possible, and the close relatives of the desired crop. Salt tolerant crops would increase food production in soils undergoing reclamation, or under conditions where saline water is the only means of irrigation, such as where drainage water, brackish underground water, or even diluted sea water must be used (Shannon and Qualset, 1984). [Single quotation marks are used for cultivar names throughout the thesis, except where the abbreviation "cv." or the word "cultivar" immediately proceeds the name (American Society for Horticultural Science, 1991)].

The international importance of the potato crop, and the vast range of its wild relatives encourage research in the production of salt tolerant potatoes. Field trials have conventionally been used for screening for salt tolerance in potato. Such trials were affected by climatic variation, and by discontinuous distribution of salts in the soils (Morpurgo and Rodriguez, 1987). Nowadays, *in vitro* trials, using nodal cutting and seedling bioassays, hold promise in the screening for salt tolerant potato genotypes (Zhang et al., 1993).

The objectives of this research were to evaluate *in vitro* salt tolerance of: 1) 131 tetraploid potato (*Solanum tuberosum*) cvs., at the vegetative stage. 2) 12 diploid simple hybrids of *S. tuberosum*, crossed with either *S. chacoense*, or *S. phureja/S. stenotomum*, 2 primitive cultivated

diploids *S. phureja/S. stenotomum* accessions, and 13 of their tetraploid complex progenies, at the vegetative stage. 3) 13 diploid *S. chacoense* accessions, at the early seedling growth stage, of which 11 were also tested at the vegetative stage.

Chapter 2 - Literature Review

2.1. Potato *Solanum* spp.

In Hawkes' (1994) classification, the genus *Solanum* subgenus Potatoe, section Petota is divided into two subsections; Estolonfera that does not form tubers, and Potatoe that is tuber-bearing. The latter are divided into a total of 19 series. The most important is the series Tuberosa. It contains the largest number of potato species.

Tuber-bearing *Solanum* spp. have two alternate methods of reproduction; sexual and vegetative. *Solanum* spp. adapt to changing environments through sexual reproduction. After the successful establishment of the genotypes, clonal propagation takes over (Hawkes and Hjerting, 1969).

S. stenotumum JUZ. et BUK., and *S. phureja* belong to the series Tuberosa. Both species are diploid (2n=24, EBN=2). *S. stenotumum* is the most primitive species of the cultivated potato. The area of its distribution extends from central Peru to central Bolivia. It is cultivated at high altitudes (2.5-3.5 km above sea level). There, *S. stenotomum* tuberizes in 5-6 mo (Hawkes, 1992). This species went through artificial selection in the warmer Andean valleys, and eventually became adapted to frost-free areas. The tubers lost their dormancy requirement, and tuberization occurred in 3-4 mo under short day conditions. These eastern valley potatoes were given the name *S. phureja* (Hawkes, 1994). *S.*

phureja is in the ancestry of many cultivars (cvs.) such as Conestoga, Tobique, and Yukon Gold (Plaisted and Hoopes, 1989).

The wild potato *S. chacoense* Bitt. is a tuber-bearing species belongs to the series, *Commersoniana* Buk. The species name *chacoense* was derived from the word Chaco, its native province in Argentina. The distribution of this wild species covers many countries in S. America, including Argentina, Paraguay, Uruguay, eastern Brazil, and central Bolivia (Hawkes and Hjerting, 1969).

S. chacoense is considered a vigorous and adaptable wild species. A great diversity exists in its morphology, physiology, and genetics. It lives in both natural and artificial habitats. It occurs in both the sun and the shade. Though it is more common on plains and foothills, it spreads in vast altitudinal ranges, from sea level to over 2.3 km (Hawkes and Hjerting, 1969).

The diploid species *S. chacoense* (2n=24, EBN=2) has been used by plant breeders for many desirable agronomic characters. It has a wide range of disease, pest, and drought tolerance. The tubers have high protein contents, and the highest dry matter content of all the wild species (Hawkes and Hjerting, 1969). *S. chacoense* is in the ancestry of many cvs. including Atlantic, Conestoga, Denali, Islander, Lenape, Russette, Sunrise, Trent, and Yankee Chipper (Plaisted and Hoopes, 1989). Intraspecific variation among 40 *S. chacoense*

accessions was shown by Hosaka and Hanneman (1988) based on chloroplast DNA (ctDNA) specific restriction fragment patterns resulting from three restriction enzyme digestions; *Bam*HI, *Hind*III, and *Pvu*II. Three (ctDNA) types were observed among the accessions.

2.2 Salinity

Generally, salinity is defined as the occurrence of various salts in soil or water in concentrations that may interfere with plant growth. The term includes (Na^{+}), calcium (Ca^{+2}), magnesium (Mg^{+2}), and potassium (K^{+}) chlorides, sulphates, and bicarbonates (Lewis, 1984). Many studies have focused on plant responses to NaCl only. Understanding the basic plant response to the simple component of the salinity equation is the logical place to start. Later, applied solutions representing natural saline soil conditions could be further investigated (Jones, 1992).

Salinity is expressed in various ways: Electrical conductivity (EC) as decisiemens per meter (dSm^{-1}), weight on a per volume basis as milligrams per litre (mgl^{-1}), parts per million (ppm), or ionic (charged particle) concentration of a particular salt in a solution in millimoles (mM). No exact relationship exists between these measurement methods. A soil extract, that has an EC of 1 dSm^{-1}, has a molar concentration of about 11 mM NaCl, and contains 640 mgl^{-1} total dissolved

salts (Lewis, 1984).

Saline soils are soils having an EC of saturation extracts greater than 4 dSm^{-1} (44 mM NaCl). They contain less than 15% exchangeable Na^{+} (Troeh et al., 1980). For simplicity the unit (mM) is used throughout the thesis.

2.2.1 Physiology of salt tolerance

Plants are divided into two physiological groups according to growth responses to salinity: halophytes (salt lovers), and glycophytes (non-halophytes). These are not discrete groups, since there is a wide range of plant species responses. Halophytes are plants that survive to complete their life cycle at high salinity levels of at least 300 mM. (Flowers et al., 1977).

Salinity has detrimental effects on plant cells. The first harmful effect is osmotic stress (Jones, 1992). Osmotic stress results in dehydration and loss of turgor (Serrano and Gaxiola, 1994). As a result, osmotic adjustments will occur in plant cells. This is accomplished via ion accumulation in the vacuoles, and/or synthesis of osmolytes, and accumulation in the cytoplasm. Osmolytes include: polyols (e.g., glycerol), sugars (e.g., sucrose), and amino acids (e.g., proline) (Serrano and Gaxiola, 1994). The second harmful effect of salinity involves cellular ion toxicity. It results from an increased concentration of intracellular ions. This

develops during water loss and the uptake of Na^+ and Cl^-. Therefore, toxicity of intracellular enzymes will occur. These enzymes only operate in a narrow range of ionic conditions (< 50 mM Na^+ and Cl^-). Essential cation (K^+ and Ca^{+2}) uptake will also be inhibited (Serrano and Gaxiola, 1994). Salt exclusion and salt inclusion are two distinct mechanisms preventing ion toxicity, at the cellular level (Ashraf, 1994). Halophytes are mainly salt includers and salt inclusion in the cells of halophytes is followed either by salt compartmentation in vacuoles, or in special salt glands on leaf surfaces (Ashraf, 1994; Shannon et al., 1994). Most tolerant glycophytes are salt excluders, and the cells of the glycophytes remove Na^+ and/or Cl^- salts either by specific pumps or passively by membrane impermeability. Osmotic adjustment depends on the synthesis of different osmolytes (Shannon et al., 1994; Tal, 1984).

Munns et al. (1995) hypothesized a two-phase growth response to salt stress. The first phase was an osmotic response. A considerable growth reduction would develop during this phase. Nevertheless, the osmotic pressure of the salt outside the roots would affect the tolerant and sensitive cvs. equivalently. The second phase is salt-specific response; an added decrease in growth would be caused by salt building up to toxic levels within the plants. This decline would cause an additional decrease in growth of the salt-sensitive cvs. within a species. These cvs. could be either

the least able to exclude the salt from the transpiration stream, or the least capable of compartmentalizing the salt in vacuoles. The duration of the first phase might rely on several factors. The first phase is supposed to be longer for good salt excluders (e.g., barley, *Hordeum vulgare*) than poor salt excluders (e.g. lupin, *Lupinus* spp.). It might depend on the growth rate of the species, a fast growing annual crop is expected to show earlier genotypic differences than a slow growing perennial plant. It might also rely on the temperature during the growing season. Therefore, temperate cereals (e.g., wheat, *Triticum aestivum*) have a longer first phase than rice. The increase in temperature and the salt level would also increase the salt uptake within a certain genotype. This 2 phase model was tested using 15 wheat and barley genotypes. All genotypes exhibited an identical growth decline for 4 wk. After the first phase, the salt sensitive genotypes had greater growth reduction.

2.2.2 Morphology and anatomy of salt tolerance

In the halophytes, distinct morphological and anatomical features include: succulence, thickened leaf cuticle, and the presence of salt glands. Salt glands are structural devices developed on the epidermis of leaves and stems, which secrete salt out of the halophytic plants (Ram and Nabors, 1985).

Salt-affected glycophytes are often darker green, stunted, with shorter and fewer internodes. They may develop a rosette growth habit. Some plants become more succulent, resulting in a higher percent water content (Shannon et al., 1994).

Reduced shoot growth in glycophytes is a common response to salinity. It is the result of reduction in the number of leaves formed on the main axis, and an inhibition of lateral bud initiation and growth. The shoot/root ratio (S/R) may decrease with the increase in salinity. This results in a more efficient utilization of water and nutrients (e.g., wheat). On the other hand, the shoot growth of several species was less inhibited by salinity than the root growth. This resulted in an increase in the shoot/root ratio (S/R) (e.g., *Sorghum bicolor)* (Shannon et al., 1994).

2.2.3 Breeding for salt tolerance

The genetic basis behind the phenotypic variability in salt tolerance is essential information in any breeding program for salt tolerance. This includes the number of genes involved, the genes' mode of action, and the pattern of inheritance. Salt tolerance is speculated to be a quantitative trait, regulated by few major genes. In tomato, an ancestral relative of potato, a single major gene controlled salt tolerance (Ashraf, 1994). None of this

valuable information is available for *Solanum* spp.

The improvement of salt tolerance in a few important crops was exploited mainly through selection. In cross-pollinated species, or artificially crossed self-pollinated species, the genetic variability of the genotypes in salt tolerance could be tested. Screening could be done on a large population, under very high selection pressure (< 1% survival rate). For example, 10,000 seeds of 'Akbar', a salt sensitive cv. of corn (*Zea mays*) were screened, at 180 mM NaCl. One line was identified as salt tolerant. Only few successful attempts were reported in either the interspecific or intergeneric hybridization (Ashraf, 1994). In *Solanum* spp., there have been no efforts in selection of salt tolerant breeding lines, nor any attempts at interspecific or inter generic hybridization.

2.3 Salt tolerance research

Salt tolerance research *in vivo* has included field, greenhouse, and outdoor pot experiments. Field salinity studies have met with several common problems. Soil salinity varies with time, location, and soil depth (Shannon, 1984). Plant reactions to salinity are complicated by large genotype and environment interactions (Ekanayake and Dodds, 1993). Several factors might affect plant responses to salinity including humidity and temperature. These factors affect

transpiration. Some crops grown under field conditions are more salt-sensitive than when grown in the greenhouse (Shannon et al., 1994). The greenhouse is a semi-controlled growth environment compared with the field. It can remove various limiting factors of field experiments and can be cheaper (Zeroni, 1988).

In vitro techniques have been used by plant breeders in developing salt tolerant species. These techniques include somatic hybridization, embryo rescue, and pollen or anther culture (Chandler et al., 1988). Organ culture techniques, such as the nodal cuttings used in this thesis, may offer potential for quick evaluation of germplasm for salt tolerance (Naik and Waidholm, 1993). The aseptic culture of whole plant organs or segments will maintain the plant characteristics (Donnelly and Vidaver, 1988).

2.3.1 *In vivo* screening of potato

In vivo studies of salt tolerance in potato started more than 40 years ago. Bernstein et al. (1951) studied the effect of salinity on the potato cv. White Rose in a field experiment. A mixture of NaCl:$CaCl_2$ (1:1) was applied. The molar concentrations were equal to 0, 34, 68, and 103 mM. A relative decrease in the tuber yield was reported. The yield was calculated as the mean fresh weight of tubers per unit area averaged over 3 harvest days.

Field tests were conducted in 2 consecutive years by Barnes and Peele (1958) on 'Sebago'. The molar concentrations were about 10-30 mM. No differences were recorded in the yield per unit area, averaged over the 2 years, perhaps due to the narrow range of NaCl concentrations utilized.

Seven potato cvs., including: Cardinal, Chieftain Multa, Norland, Patrones, Red Bed, and Red LaSoda were tested for salt tolerance in an outdoor pot experiment (Ahmad and Abdullah, 1979). Plants were treated with saline water of 0.2-1.0% mixed salts (NaCl, $MgSO_4$, $CaCl_2$, and $NaHCO_3$). Salt tolerance was determined on the mean fresh weight of tubers. This was calculated as the fresh weight of tubers/plant divided by the number of tubers/plant. Relative reduction in this term was considered the relative reduction in yield. Relative yield of tubers of 'Cardinal', 'Patrones', and 'Multa' increased with the increase in salt levels up to 0.8%. The concentration of 1% mixed salts was inhibitory to yield. At this concentration, 'Patrones', 'Norland', and 'Red Lasoda' were classified as tolerant to salinity (their relative yields decreased by 20-50%), and the others were sensitive (their relative yields decreased by 50-85%).

The cv. Kufri Chandramukhi was evaluated for its salt tolerance in a field experiment (Paliwal and Yadav, 1980). A mixture of NaCl, $CaCl_2$, and $NaHCO_3$ salts was used, at total molar concentrations of 4, 20, 40, and 80 mM. No differences were found between the yield per unit area at 20 and 40 mM.

At 80 mM, the yield was decreased by 45%.

The salt tolerance of cv. Spunta was studied in a field experiment (Bouaziz, 1980). Four levels of NaCl were used of about 4, 25, 39, and 53 mM. The average fresh weight of tubers/plant decreased with the increase in salt levels down to 80% of the control at 53 mM. As a result, it was considered a potentially tolerant cv.. However, the salinity levels utilized were relatively low.

Potato cvs. Russet Burbank, Red Pontiac, Norchip, and Norgold Russet were evaluated for their salt tolerance in a greenhouse pot experiment (Bilski et al., 1988a). Solutions of NaCl and Na_2SO_4 were applied at 0, 40, 80, and 120 mM, and 0, 20, and 40 mM, respectively. The effect of salt was only evaluated on vegetative growth. The relative reduction in the haulm dry weight and in the number of plants surviving were used in a comparison of cvs.. There was a relative decrease in these two parameters for all the cvs. exposed to salinity. The cvs. ranked differently at the different salt levels. 'Norchip', 'Red Pontiac', and 'Norgold Russet' ranked first in relative haulm dry weight at 40, 80, and 120 mM NaCl, respectively. Averaged over all treatments, 'Red Pontiac' ranked first in relative haulm dry weight, and 'Norgold Russet' ranked first in number of plants that survived. For both parameters (survival and haulm dry weight), 'Russet Burbank' was the least tolerant. On a molar basis, SO_4^{-2} was more toxic than Cl^-, but a close correlation occurred.

The salt tolerance of 11 accessions of 6 wild *Solanum* species was evaluated (Bilski et al., 1988b). The effect of NaCl and Na_2SO_4 was evaluated on seedling growth, under similar treatment conditions. The relative reduction in haulm dry weight and in the number of plants surviving were used in the comparison of genotypes. S. *chacoense* ranked first in foliage dry weight and plant survival. *S. gourlayi*, *S. microdontum*, *S. sparsipilum*, and *S. bulbocastanum* were intermediate. *S. papita* was the least tolerant. A close correlation existed between the haulm dry weight and survival. This indicated that both were good indicators of salt tolerance. Heat and drought tolerant *S. chacoense* and *S. papita* ranked first and least in tolerance to salinity, indicating the absence of linkage between these factors.

Outdoor pot experiments were conducted to evaluate the salt tolerance of four European cvs. including: Alpha, Blanka, Cara, Desirée, and the local Israeli cvs. Idit, and Ori (Levy et al., 1988). Three concentrations of NaCl:$CaCl_2$ of 4:1 ratio (W:W). The three solutions contained 1.2, 2.0 ,and 3.0 $gl^{-1}$of NaCl, and 0.3, 0.5, and 0.7 gl^{-1} of $CaCl_2$. The molar concentration of the solutions were 20.5, 34.2, and 51.3 mM NaCl and 2.7, 4.5, and 6.3 mM $CaCl_2$. The average tuber fresh and dry weights per plant were reduced for all the cvs.. The relative reductions in yields were due to reduced tuber dry weights. This parameter was used in measuring tolerance. 'Alpha' ranked first in relative yield at the intermediate and high salt levels.

The cvs. Cilvia, Erntestolz, Grata, and Hansa were examined for their salt tolerance, in two pot experiments conducted in the greenhouse for 2 consecutive years (Bruns and Caesar, 1990). Three concentrations of mixed salt solutions of NaCl and Na_2SO_4 were applied at concentrations equal to 44, 88, and 131 mM, at 3 stages of crop development. Tolerance altered with ontogeny. When salinity was applied 1 wk after emergence, it delayed shoot development, especially at 131 mM, but was later compensated b higher growth rates. The second application was at the onset of tuber formation. This was the most sensitive stage. All four cvs. showed earlier senescence and large yield reductions when salt was applied at this stage, at the higher salt levels (88 and 131 mM). Treatments applied during tuber development, at the third stage, had only marginal effects on shoot development but shortened the vegetative period. Salt tolerance was evaluated based on the mean tuber fresh weight/plant. A salt concentration of 44 mM did not affect yield regardless of application stage. It even increased the yield of 'Grata' and 'Cilvia'. In the first year, favourable weather conditions resulted in clear differences with respect to salt levels and application times. In the second year, high temperatures resulted in lower yields, and smaller differences between the controls and plants treated with salt. 'Cilvia' and 'Ernestolz' yielded better than 'Grata' and 'Hansa' at high salt levels and under unfavourable weather conditions.

Levy (1992) studied the response of different potato genotypes in three field experiments. Three salinity levels were used: usual irrigation quality (11-15 mM NaCl), intermediate salt levels (42-47 mM NaCl), and high levels (67-76 mM NaCl). Different numbers of cvs. were tested in three different experiments. 'Alpha', 'Cara', and 'Desirée' were tested in the first experiment (A). 'Atica' was added in the second experiment (B). Ten additional cvs. and clones were evaluated (Aracy, Baronesa, Draga, Nicola, Serrana Inta, DTO-28, DTO-33, Lt-2, Lt-4, Lt-7) in the third experiment (C). Only three cvs. (Desirée, Cara, and Alpha) were tested repeatedly in the three experiments. These experiments were mainly established to explore different management strategies. In (A), the application of saline water was done after plant establishment. Planting was done early in the season. In (B), the treatments were applied directly after tubers were planted. In (C), 1 wk was allowed for initial sprouting and emergence. Planting was done late in the season. No treatment was designated for the tuberization stage, compared with the treatments applied by Bruns and Caesar (1990). The yield was calculated as the fresh weight of tubers per unit area. The yield was greater for these three cvs. in the control plot of C than the control plots of A and B. This was due to late planting and later crop development under conditions of higher temperatures and evaporation. The yield reduction at the high salinity level was greater for these

cvs. at B, followed by C and A. 'Alpha' ranked first in salt tolerance, averaged over the three experiments, with a 40 % relative reduction in yield.

The cv. Desirée was evaluated for salt tolerance, in a field experiment (Heuer and Nadler, 1995; Nadler and Heuer, 1995). The treatments included a control, and 2 salinity treatments of a concentration of 16.5, 33, and 66 mM NaCl, respectively. All treatments were applied after plant emergence. The effect of salinity was evaluated on vegetative growth. Plant height (cm), leaf area (m^2 /plant), and the haulm fresh weight (gm/plant) were affected by salinity. Total tuber weight (t/ha) was not affected by any treatment, due to low tuber dry weight production in the control treatment, and the actual effect of the treatments occurred after the period of tuber initiation (Nadler and Heuer, 1995).

The effect of salinity on potato crop yield *in vivo* altered with ontogeny (Bruns and Caesar, 1990, and Levy, 1992). These authors reported that moderately saline water (about 40-45 mM NaCl) could be used for irrigation, without severe damage to the potato crop. However, irrigation with saline water was best delayed until the plants were established in the field. Also, it was best to use fresh water during the onset of tuberization.

Few studies evaluated the salt tolerance of a single cv.. Different authors rarely evaluated the same cvs., except for the two studies by Levy et al., (1988) and Levy, (1992) where

the cvs. Alpha, Cara, and Desirée were repeatedly tested. As mentioned, Alpha ranked first in both studies. Different studies used various salt mixtures but, NaCl was consistently used as the main component. Salt tolerance was assessed at the vegetative stage and/or at tuber harvest. The ranking of cvs. for salt tolerance was primarily based on the relative reduction in yield. Yield was mainly assessed as mean fresh weight of tubers per unit area, in the field trials, or mean fresh weight per plant in the greenhouse or outdoor pot experiments.

2.3.2 *In vitro* screening of potato

Two potato cvs. (Hansa and Fruhbote), and three wild species (*S. phureja, S. sparsipilum,* and *S. chacoense)* were tested for salt tolerance (Arslan et al., 1987). Single node cuttings from *in vitro* plantlets were used. A mixture of NaCl and MgCl was used in a Murashige and Skoog (1962) basal medium. The concentrations were 0, 40, 80, and 160 mM. Salt tolerance was evaluated after 6 wk. A relative reduction in vegetative growth parameters was reported including shoot length and fresh weight. 'Fruhbote' had the greatest shoot length and shoot fresh weight at all salt levels. *S. phureja* and *S. sparsipilum* ranked second at 80 and 120 mM NaCl, respectively. Root length was not affected by salinity level for most of the genotypes. However, *S. sparsipilum* and

'Fruhbote' showed increased root lengths of 134.4% and 102.9%, at 40 and 160 mM, respectively. Ranking of the cvs. was variable across the NaCl levels. No correlation between the results of the different growth parameters was conducted. Nevertheless, averaged over the salt levels and relative to the control, 'Fruhbote' was more salt tolerant than 'Hansa'. *S. sparsipilum* was more tolerant than *S. phureja*, and *S.chacoense* was the least tolerant.

The response of eight potato clones and cvs. (Desirée, Br 69.84, DIO-33, P-3, F-3, Cex, Lt-2, and Lt-5) was investigated by Morpurgo and Rodriguez (1987). Stem cuttings (5 nodes/cutting) were used. Two NaCl levels were used (0 and 103 mM NaCl) in an Murashige and Skoog (1962) basal medium. After 3 wk, results were taken in absolute values. No statistical significance was documented. Shoot fresh weight increased in 'Desirée' and Br 69.84, decreased in DIO-33 and D-3, and no growth occurred in the other clones at 103 mM NaCl. Root fresh weight, dry weight, and length were reduced in all genotypes, but 'Desirée' and BR 69.84 showed the least damage from NaCl.

This study was followed by a test of ten cvs. and clones (Serrana, Rosita, Yungay, Mariva, P-3, Lt-5, DTO-33, G-1, Lt-6, and Lt-2) (Murpurgo, 1991). Two NaCl levels were used (0 and 154 mM). All *in vitro* parameters were reduced including: shoot length (cm), shoot and root fresh weights (g/flask). Root fresh weight was the most affected trait, confirming the

earlier results of Morpurgo and Rodriguez (1987). A highly significant correlation ($r=0.88^{**}$) was found between root fresh weight *in vitro* and tuber fresh weight(g/plant) in field-grown plants exposed to 40-80 mM NaCl. Other parameters were not significantly correlated.

Elhag (1991) used single node cuttings, to compare the salt tolerance of 86 potato genotypes *in vitro* with *in vivo* pot experiments. Eleven cvs. were tested (Alpha, Culpa, Desirée, Diamant, Draga, Erntestolz, Hansa, Kennebec, Marfona, Roxy, and Spunta). Five clones, and 70 wild and primitive cultivated species were also evaluated. The levels of NaCl used were 0, 40, 80, and 120 mM both *in vitro* and in the pot trials. Shoot length and shoot dry weight were suitable for characterizing salt tolerance among the potato genotypes. The genotypes were ranked *in vitro* based on the sum of the ranking of the relative growth parameters at 40, 80, and 120 mM. The correlation between both SL and SFW *in vitro* and *in vivo*, and the tuber fresh weight/plant in pot trials, was positive and highly significant. The six most salt tolerant genotypes, were the wild species: *S. chacoense*, *S. sparsipilum*, *S. vernei*, *S. spegazzinii*, *S. tarijense*, and *S. gourlayi*. Three accessions of *S. chacoense* were tested. One accession was superior and the other two accessions had relative low tolerance. This underlined the potential differences in salt tolerance among accessions of a species. Within the cvs., Desirée was the top ranked in both SL and

SFW.

A comparative study of *in vivo and in vitro* responses to salinity was conducted by Naik and Widholm (1993). Six cvs. were tested including: Kennebec, Norchip, Red Pontiac, Russet Burbank, Russet Norkotah, and Superior. In the greenhouse, the cvs. were challenged with 6 levels of NaCl (0, 5, 100, 150, 200, and 250 mM), after 70% of sprout emergence. The same NaCl levels were used *in vitro* to evaluate the responses of 1 cm long apical root segments, 1 cm single node or apical cuttings, and cell suspension cultures. In the cell suspension cultures, cells of 0.5 gm fresh weight were tested for salt tolerance. The callus cultures were initiated from leaf rachis explants of greenhouse-grown plants. After 25 d, the authors evaluated vegetative growth, but not yield parameters *in vivo*, analyzed as a percent of the control. Results were also taken after 8, 15, and 12 d, from the root, stem, and cell suspension culture methods, respectively. The cvs. Red Pontiac and Norchip were apparently the most salt tolerant, based on a close correlation between root length in the root culture bioassay, and plant fresh weight *in vivo*, averaged over all the salt levels excluding the control. Therefore, Naik and Widholm's 1993 study was the first to recommend root segment cultures for assessing salt tolerance of potato genotypes *in vitro*. The authors only measured rooting parameters (% rooting, and root number per cutting). They did not evaluate other vegetative growth parameters for

correlation with haulm fresh weight measured *in vivo*. The relative decrease in the mean cell fresh weight per flask, averaged over the salt levels, for the cvs. was used in determining salt tolerance in the cell suspension culture. Poor correlation occurred between growth parameters in the stem, and cell suspension cultures with *in vivo* results. Naik and Widholm (1993) disparaged the use of stem cultures on this basis. Their interpretations are debatable since correlations were not attempted with yield *in vivo*.

Seven cvs. including Atlantic, Kennebec, Russet Burbank, Norland, Shepody, Spunta, and Superior, two hybrids of *S. tuberosum* X *S. chacoense*, and four hybrids of *S. gourlayi*/or *microdontum* were evaluated for their salt tolerance using the nodal cutting bioassay (Zhang et al., 1993). Nodal cuttings 1 cm long with 1 axillary bud, obtained from *in vitro* plantlets, which were propagated on Murashige and Skoog (1962) solid medium, supplemented with (mgl^{-1}): myo inositol (100), thiamine.HCl (0.4), and Ca pantothenate (2.0). Cultures were incubated under 16/8 h D/N, 40 μE photon flux density, with temperatures of 23$\pm$ 2°C D/N. For salinity screening, three levels of NaCl were used (0, 80, and 120 mM NaCl). One accession of each of the wild species *S. chacoense*, *S. gourlayi*, and S. *microdontum* were also tested for their salt tolerance using the seedling bioassay. The seeds were surface sterilized, rinsed in sterile double distil water, treated with filter sterilized GA_3 for 24 hr to break dormancy, again

rinsed in sterile water, and cultured 1 per test tube, in the above mentioned media. The seeds spent one wk in the dark followed by 3 wk in the light, under the same temperature as light regimes in the nodal cutting bioassay. For the cvs., shoot and root length and root dry weight, but not shoot dry weight, were significantly depressed by the salt treatments. 'Spunta' and 'Russet Burbank' were the least affected, and 'Norland' was the most affected by salinity, in shoot length, root length, and root dry weight. For the hybrids, all parameters were depressed by salinity. Hybrids derived from *S. chacoense* were relatively more salt tolerant than hybrids derived from *S. gourlayi* or *microdontum* in shoot and root length, and shoot and root dry weight. In the seedling bioassay, final germination percentage, and shoot and root fresh weight but not shoot dry weight, were depressed by salinity. In these parameters, *S. chacoense* ranked first, and *S. gourlayi* was the least tolerant, at 80 and 120 mM NaCl.

Chapter 3 - Evaluation of Salt Tolerance of 131 Potato *S. tuberosum* Cvs. at the Vegetative Stage *in Vitro*

3.1 Introduction

The use of *in vitro* techniques for screening *Solanum* spp. for salt tolerance was first reported by Arslan et al., 1987). Some studies were centred solely on *in vitro* screening (Arslan et al. 1987; Morpurgo and Rodriguez 1987; Zhang et al. 1993). Others have examined the correlation between *in vitro* and *in vivo* salt tolerance, to validate their *in vitro* bioassays (Elhag,1991; Murpurgo, 1991; Naik and Widholm 1993).

The source material was always *in vitro* plantlets, and the common technique was the use of single node cuttings that contained one axillary bud (Arslan et al. 1987, Elhag 1991, Zhang et al., 1993). Naik and Widholm (1993) also used apical cuttings, and found those were less influenced by NaCl than the single node cuttings. Stem cuttings (5 nodes/cutting) were used by Morpurgo and Rodriguez (1987), and Murpurgo (1991), but had no obvious advantage over the single node cuttings, and were less economical of plant material.

NaCl was mainly used in the *in vitro* experiments, except that of Arslan et al. (1987), in which a mixture of NaCl and MgCl was used. Different ranges of salt levels were used in assessing salt tolerance. Morpurgo and Rodriguez (1987) and Murpurgo (1991) were the only ones to use a high level of NaCl

versus the control (103 or 153 mM). It was recommended by the authors that a range of lower NaCl levels be used to quantify the response to salt stress (Murpurgo, 1991). Ranges of four, six, and three NaCl levels were used, respectively, by Arslan et al. (1987), Naik and Widholm (1993), and Zhang et al. (1993). Elhag (1991) tested 8 levels of NaCl (0, 20, 40, 60, 80, 120, 140, and 160 mM). A distinct progressive reduction in shoot length (SL) and shoot fresh weight (SFW) was reported at 3 NaCl levels 40, 80, and 120 mM, compared to the control. The level 120 mM was the greatest at which a relative reduction in these criteria was quantified. Further testing by Elhaj, was done at these three levels.

Different morphological parameters were recommended in the different reports. Root fresh weight (RFW) was positively correlated with *in vivo* tuber yield (Murpurgo, 1991), while SL and SFW were significantly correlated with each other, in both *in vivo* and *in vitro* experiments, and with *in vivo* tuber yield (Elhag, 1991).

Many cvs. of potato (*Solanum tuberosum*) have been developed and grown on a commercial scale. These cvs. have the advantage of acceptable trade qualities in comparison with wild species or interspecific hybrids. A salt tolerant cv. could be directly cultivated in salt-affected soils, or if genetic infertility is not a barrier, become an immediately suitable candidate for parentage in a breeding program for salt tolerance. Most modern potato cvs. have been developed

in either Europe or North America. Only a few European cvs. were tested for salt tolerance *in vitro* by Arslan et al. (1987), Morpurgo and Rodriguez (1987), and Murpurgo (1991), and few North American cvs. were evaluated by Naik and Widholm (1993). Elhag (1991) evaluated only one North American cv. and ten European cvs.. Zhang et al. (1993) tested one European cv. and seven North American cvs.. None of the above authors tested a wide range of cvs. of both origins. The reason for their choice of cvs. was local popularity or was not apparent. Few cvs. have been tested more than once *in vitro* by different authors. Three North American cvs. (Kennebec, Russet Burbank, and Superior) were tested by both Naik and Widholm (1993) and Zhang et al. (1993). Both groups concluded that 'Superior' and 'Kennebec' were salt sensitive, but only Zhang et al. (1993) concluded that 'Russet Burbank' was salt tolerant. 'Spunta' was evaluated by both Elhag (1991) and Zhang et al. (1993), the authors reached contrary opinions regarding this cv.. 'Hansa' was reported to be salt sensitive by both Arslan et al. (1987) and Elhag (1991). Salt tolerant rankings are relative and may vary with the source clone. A significant number of European and North American cvs. had not been assessed when this study was under taken. The evaluation of a broader list of cvs. *in vitro* is expected to result in a diverse collection of salt tolerant cvs, of different agronomical qualities suitable for use in various salt-affected soils, in different regions of the world.

The objective of this section of the research was to rank a total of 131 potato *S. tuberosum* cvs. in salt tolerance, of which 34 were European and 97 were North American. Nodal cuttings appeared suitable for *in vitro* salinity screening but the precise levels of NaCl for evaluation of such a wide range of genotypes was uncertain. A nodal cutting bioassay (NCB) was used, as reported by Zhang et al. (1993), over 4 NaCl levels: 0, 40, 80, and 120 mM. Ranking was based on root and shoot growth parameters.

3.2 Materials and methods

3.2.1 Plant materials and propagation

In vitro plantlets of 131 cvs. (Appendix 1 A; 1 B) were supplied by the Potato Propagation Centre, Fredericton, N.B. The cvs. were maintained *in vitro* by nodal cutting propagation in micropropagation medium (Appendix 5).

3.2.2 Experimental design

Seven consecutive trials of NCB (Appendix 4) were conducted in a three factorial experiment. The three factors were NaCl levels, the cvs., and the time of the trial. Different sets of cvs. were tested in each trial. Each cv.

was tested twice for salt tolerance.

3.2.3 Data analysis

The means of five plantlets for each cv. for each trial, were used in the analysis. The data were analyzed using the General Linear Model (GLM). The means of the main effects were separated by Scheffe's Test (p=0.05). Correlation coefficients between the 4 NaCl levels (0, 40, 80, and 120 mM) were calculated for each growth parameter. Cluster analysis (CA) was used to group the different genotypes. The CA method used was Ward's minimum-variance method (SAS, 1989). The data matrix was standardized to remove the arbitrary effects due to the different scales of measurement of the variables. The CA results were shown graphically by using a dendrogram (cluster tree) which displayed the paired potato genotypes in clusters. The increase in the cubic clustering criterion (CCC) and the pseudo F statistic and the decrease in the t^2 statistics were used as indicators of the number of clusters formed and where the tree was cut to form a classification.

3.3 Results and discussion

Shoot length (SL), shoot fresh weight (SFW), and shoot dry weight (SDW) were influenced by the effects of salt concentration (CNC), time of each of the seven consecutive trials (TIME), genotype (G), and the interaction terms (G*CNC,

CNC*TIME, and G*TIME) (Appendix 6 A-C). There was a progressive decrease in the shoot growth parameters with increased NaCl levels (Fig 3.2). The reductions due to the increased NaCl levels, for SL, SFW, and SDW, were 36%, 27%, and 28% at 40 mM, 61%, 60%, and 53% at 80 mM, and 68%, 63%, and 59% at 120 mM, averaged over 131 cvs., and 7 trials. SL had the greatest reduction at the 3 NaCl levels (40, 80, and 120 mM) (Figure 3.1 A-C).

Root length (RL), root fresh weight (RFW), and root dry weight (RDW), were influenced by the main effects, and G*CNC interaction except for RL and time. CNC*TIME had a significant effect only on RFW (Appendix 6 D-F). There was a progressive decrease in the root growth parameters with increased NaCl levels. The relative reductions for 131 cvs., averaged over 7 trials, for RL, RFW, and RDW, respectively, were 21%, 37%, and 38% at 40 mM, 44%, 70%, and 67% at 80 mM, and 73%, 82%, and 83% at 120 mM (Figure 3.1 D-F). RL had the least reduction at the 3 NaCl levels (40, 80, and 120 mM).

Growth in 0 mM control media was correlated with the results at 40 mM for all the traits, except for RL, and RDW and was not correlated with growth at higher NaCl levels (Tables 3.1; 3.2). So, salt tolerance at 80 and 120 mM had no apparent correlation with vigour at 0 mM. A positive correlation was found between growth at 40 and 80, 40 and 120, and 80 and 120 mM (Tables 3.1; 3.2). SL, SFW, SDW, and RFW results averaged over the total 131 cvs. and the 3 NaCl levels

(40, 80, and 120 mM) were positively correlated for the 7 trials (Tables 3.3; 3.4; 3.5; and 3.6).

The CA of the 6 morphological parameters (SL, SFW, SDW, RL, RFW, and RDW), averaged over 7 trials and across 3 NaCl levels (40, 80, and 120 mM NaCl), resulted in 2 distinct clusters. The tolerant cluster contained 20 cvs. at an R^2 of 74% (Appendix 9). Three were of European origin (Bintje, Erntelstolz, and Junior), while the rest were North American (Acadia Russet, Amisk, Atlantic, Belleisle, Chipeta, Coastal Chip, Eide Russet, Green mountain, Norqueen, Onaway, Rhinered, Russet Norkotah, Saginaw Gold, Spartan Pearl, Sierra, Tobique, and Trent. The Differences in shoot growth with increasing levels of NaCl among two of the salt tolerant cvs. (Topique and Norqueen) and a salt sensetive cv. ofelia are shown in Fig. 3.2..

Some of the cvs. in the present study have also been investigated by others *in vitro*. The five European cvs. (Diamant, Draga, Erntestolz, Marfona, and Spunta), and the North American cv. Kennebec were tested in the present study and by Elhag (1991), under the same NaCl levels. In agreement with Elhag (1991) 'Erntestolz' was the only salt tolerant one in this group (Appendix 1-B; 9).

The North American cvs. Kennebec, Norchip, Red Pontiac, Russet Burbank, Russet Norkotah, and Superior were tested in this study and by Naik and Widholm (1993). Only 'Russet Norkotah' occurred in the salt tolerant cluster, in this study

(Appendix 1-B; 9). The rest were salt sensitive, including 'Norchip', and 'Red Pontiac' which were found by Naik and Widholm (1993) to be salt tolerant. The discrepancies between the results of these two studies might be explained by differences in the NaCl levels, and in the growth parameters used for ranking in these studies. The cvs. Atlantic, Kennebec, Russet Burbank, Spunta, and Superior were tested in both the present study and by Zhang et al. (1993). These cvs. were ranked in the second cluster in this study (Appendix 1-B; 9). This does not contradict the relative salt tolerance of 'Spunta' and 'Russet Burbank' reported by Zhang et al. (1993), considering that their study was based on a much smaller set of cvs..

'Erntestolz' was found to be salt tolerant *in vivo* by Bruns and Caesar (1990), and 'Russet Burbank' was described as salt sensitive *in vivo* by Bilski et al. (1988a), and this agreed with the results of the present study. Still, some discrepancies were also present, between previous *in vivo* results and the present study. 'Red LaSoda' and 'Red Pontiac' were both salt sensitive in the present study, but were reported to be relatively salt tolerant by Bilski et al. (1988a), and Ahmad and Abdullah (1979). Again, a small number of cvs. were tested in their studies. Their top-ranked genotypes were not necessarily very salt tolerant in absolute terms. Cardinal and Chieftain were salt sensetive in the present study (Appendix 1-B; 9) and in the findings of Ahmad

and Abdullah (1979).

The importance of the reported adaptability, drought tolerance, and the immediate parentage were investigated for the cvs. ranked in this study. 'Bintje' was the only European cv. that was reported to be widely adaptable (Appendix 1-B; 9), which occurred in the salt tolerant cluster. 'Atlantic', and 'Coastal Chip' were two salt tolerant North American cvs. described as widely adapted, with no indication as to which abiotic stress was involved. These two cvs. shared the same parents ('Wauseon' x 'B5141-6'). 'Wauseon' was in the parentage of an additional three cvs. screened in the present study: Campbell 11-13, Cupids, and Sunrise, but these were salt sensitive. 'B5141-6' appeared in the parentage of the tolerant cv. Trent, and three sensitive cvs.: Denali, Russet, and Snowden. So, 'B5141-6' may have contributed some salt tolerance. 'Chipeta', which was in the tolerant cluster, was reported to be particularly adapted to drought. Six cvs. reported by New Brunswick Department of Agriculture (1993) to be widely adapted to abiotic stress were salt sensitive including: Caribe, Chieftain, Irish Cobbler, Katahdin, Kennebec, and Red Pontiac. The drought sensitive cvs. BelRus, Castile, Hilite Russet, and Norchip were all salt sensitive in this study. 'Norqueen' and 'Onaway' were the only two cvs. which were noted for drought tolerance that were in the salt tolerant cluster (Appendix 1-B; 9). From this study, it was apparent that drought tolerance might not necessarily be

related to salt tolerance. 'Katahdin' and three of its progeny were also in the sensitive group including: 'Red Lasoda', 'Red Pontiac', and 'Sebago', but its progeny 'Onaway' was salt tolerant. 'Russet Burbank', 'Blue Mac', 'Keswick', and 'Norchip', and their progenies 'Coastal Russet', 'AC Domino', 'Fundy', and 'Islander', respectively, were all salt sensitive. Salt sensitivity might be dominant, as has been reported for tomato (*Lycopersicon esculentum*), an ancestral relative of potato (Ashraf, 1994). 'Keswick' and 'LaChipper' were among the salt sensitive cvs., yet they were both derived from the salt tolerant 'Green Mountain'. The North American cvs. Acadia Russet, Amisk, Belleisle, Eide Russet, Green Mountain, Rhinered, Saginaw Gold, Sierra, Spartan Pearl, Tobique, and Trent were all in the salt tolerant cluster (Appendix 1-B; 9). These cvs. had not been tested previously *in vitro*, and information on their tolerance for abiotic stresses was not available. 'Norgold Russet' was in the parentage of two of these salt tolerant cvs., Acadia Russet and Eide Russet, while Targhee was in the parentage of Sierra and Amisk. Neither 'Norgold Russet', nor 'Targhee' were evaluated in the present study. However, 'Norgold Russet' ranked first in the study of Bilski et al. (1988a).

3.4 Conclusion

In the nodal cutting bioassay, the shoot and root parameters were both adversely affected by increased NaCl levels. Vigour in the control medium (0 mM) was only correlated with vegetative growth at the 40 mM NaCl level. The vegetative growth among the 3 NaCl levels (40, 80, and 120 mM) was positively correlated.

Potato cvs. might be a useful genetic source for the improvement of salt tolerance in potato. CA proved to be a practical statistical procedure for grouping these cvs., which were separated into two main groups. Twenty cvs. ranked above the others in the more salinity tolerant cluster. The salt and drought tolerant North American cvs. Onaway, and Norqueen, and the salt tolerant European cv. Erntestolz, might be particularly promising cvs. for direct use in salt-affected areas of the world, or have uses in a breeding program for salt tolerance.

Several cvs. were salt sensitive in this study but considered relatively salt tolerant by others including: Norchip, Red Pontiac, Red LaSoda, Russet Burbank, and Spunta. The disparity in ranking for salt tolerance for these cvs. is explained by the relatively smaller number of cvs. screened in the other studies, or the different criteria, and/or different salt levels used in the comparisons.

Twelve cvs. (1 European and 11 North American) of the 20,

identified to be salt tolerant here, had not been previously screened for salt, nor drought tolerance, nor reported for wide adaptability to abiotic stresses. These were Acadia Russet, Amisk, Belleisle, Eide Russet, Green Mountain, Junior, Rhinered, Saginaw Gold, Sierra, Spartan Pearl, Tobique, and Trent.

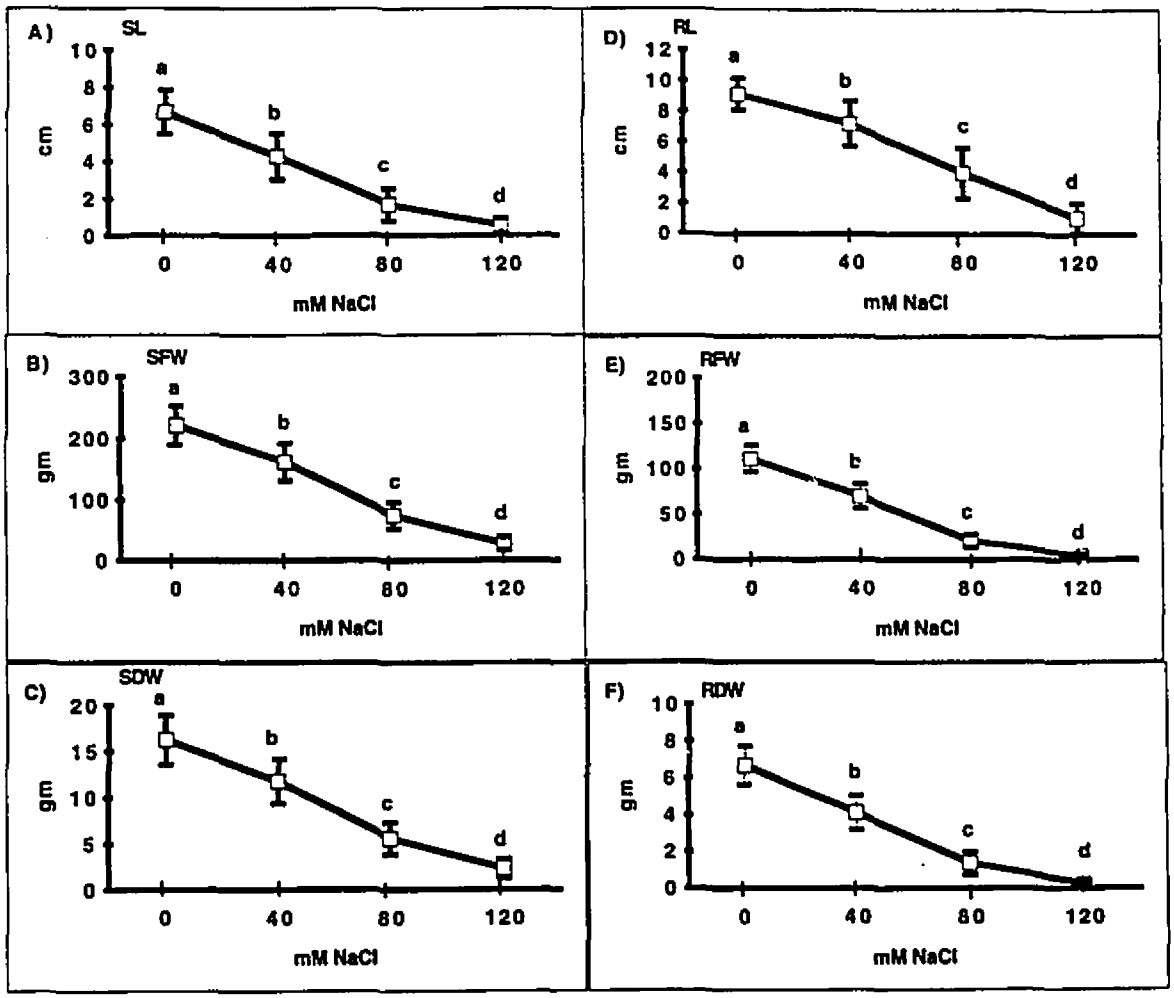


Figure 3.1 The effect of NaCl salinity on shoot and root growth parameters of 131 potato cvs.. Means of the shoot and root growth parameters followed by the same letter do not differ by the Scheffe's test at the 0.05 level. Bars show S.E. [SL = shoot length; SFW = shoot fresh weight; SDW = shoot dry weight; RL = root length; RFW = root fresh weight; RDW = root dry weight].

Figure 3.2 Morphological differences among the salt tolerant 'Topique' and 'Norqueen', and the salt sensitive 'Ofelia' in the nodal cutting bioassay, under NaCl levels of (0, 40, 80, and 120 mM NaCl), after 4 weeks.

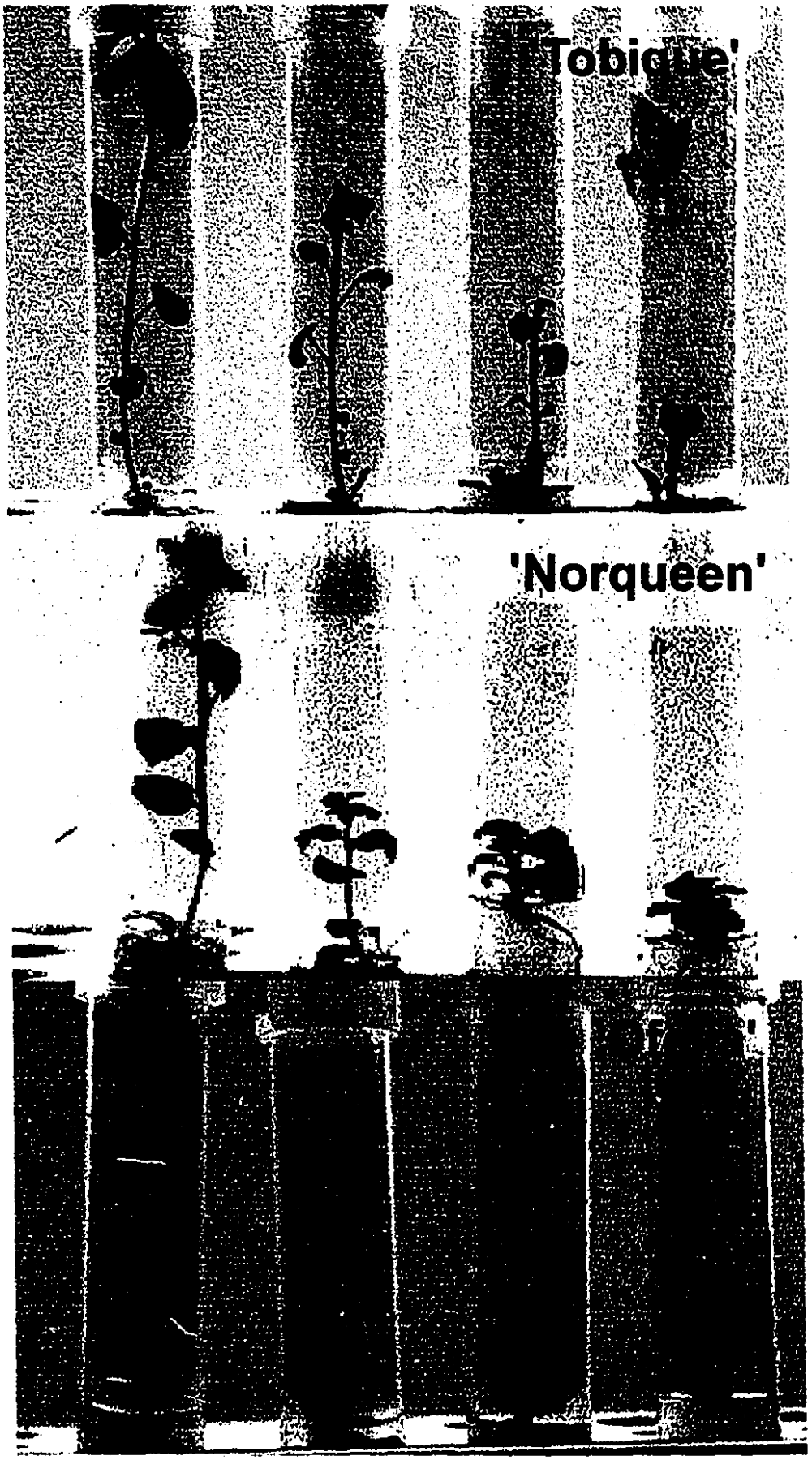

'Tobique'
'Norqueen'
0
40
80
120
mM NaCl

Table 3.1 Simple correlation coefficients among 4 NaCl levels for the shoot growth parameters, averaged over 131 potato cvs. and 7 trials.

	mM	0	40	80	120
SL	0	_	0.71 **	0.49 NS	0.32 NS
	40		_	0.60 *	0.64 *
	80			_	0.65 **
	120				_
SFW	0	_	0.78 **	0.50 NS	0.40 NS
	40		_	0.66 *	0.59 *
	80			_	0.70 **
	120				_
SDW	0	_	0.69 **	0.53 NS	0.20 NS
	40		_	0.67 **	0.6 *
	80			_	0.85 **
	120				_

NS Not significant *Significant at p = 0.05
**Significant at p = 0.01. [SL = shoot length; SFW = shoot fresh weight; SDW = shoot dry weight].

Table 3.2 Simple correlation coefficients among 4 NaCl levels for the root growth parameters, averaged over 131 potato cvs. and 7 trials

	mM	0	40	80	120
RL	0	_	0.47 NS	0.20 NS	0.07 NS
	40		_	0.44 NS	0.19 NS
	80			_	0.43 NS
	120				_
RFW	0	_	0.65 *	0.40 NS	0.05 NS
	40		_	0.70 *	0.60 *
	80			_	0.63 *
	120				_
RDW	0	_	0.07 NS	0.11 NS	0.01 NS
	40		_	0.13 NS	0.12 NS
	80			_	0.12 NS
	120				_

NS Not significant *Significant at p = 0.05
**Significant at p = 0.01 [RL = root length, RFW = root fresh weight, RDW = root dry weight]

Table 3.3 Simple correlation coefficients for shoot length, among 7 trials, averaged over 3 NaCl levels (40, 80, and 120 mM) and 131 potato cvs.

	Time1	Time2	Time3	Time4	Time5	Time6	Time7
Time1							
Time2	0.99**						
Time3	0.98*	0.97*					
Time4	0.97*	0.96**	0.99**				
Time5	0.99**	0.98**	0.99**	0.99*			
Time6	0.92**	0.91*	0.96*	0.98*	0.95*		
Time7	0.99*	0.99*	0.99*	0.98*	0.99**	0.94**	

NS Not significant *Significant at p = 0.05
**Significant at p = 0.01. Time(1-7) refer to the effect of the seven consecutive trials conducted in two years.

Table 3.4 Simple correlation coefficients for shoot fresh weight, among 7 trials, averaged over 3 NaCl levels (40, 80, and 120 mM) and 131 potato cvs..

	Time1	Time2	Time3	Time4	Time5	Time6	Time7
Time1							
Time2	0.98*						
Time3	0.95**	0.90*					
Time4	0.96*	0.93***	0.98**				
Time5	0.99*	0.97**	0.96*	0.95*			
Time6	0.97**	0.97**	0.95**	0.98*	0.96*		
Time7	0.99**	0.98**	0.96*	0.96*	0.99*	0.98*	

NS Not significant *Significant at p =0.05
**Significant at p = 0.01. Time(1-7) refer to the effect of the seven consecutive trials conducted in two years.

Table 3.5 Simple correlation coefficients for shoot dry weight, among 7 trials, averaged over 3 NaCl levels (40, 80, and 120 mM), and 131 potato cvs.

	Time1	Time2	Time3	Time4	Time5	Time6	Time7
Time1							
Time2	0.99**						
Time3	0.96**	0.92*					
Time4	0.95**	0.91**	0.99*				
Time5	0.97**	0.94*	0.99*	0.99**			
Time6	0.96**	0.94**	0.98*	0.98**	0.99**		
Time7	0.99**	0.98**	0.98*	0.98*	0.99**	0.98**	

NS Not significant *Significant at p =0.05
Time(1-7) refer to the effect of the seven consecutive trials conducted in two years.

Table 3.6 Simple correlation coefficients for root fresh weight, among 7 trials, averaged over 3 NaCl levels (40, 80, and 120 mM), and 131 potato cvs.

	Time1	Time2	Time3	Time4	Time5	Time6	Time7
Time1							
Time2	0.99***						
Time3	0.96***	0.92***					
Time4	0.95**	0.91***	0.99***				
Time5	0.97***	0.94***	0.99***	0.99***			
Time6	0.96***	0.94***	0.98***	0.98***	0.99***		
Time7	0.99***	0.98***	0.98***	0.98***	0.99***	0.98***	

NS Not significant *Significant at p =0.05
**Significant at p = 0.01. Time (1-7) refer to the effect of the seven consecutive trials conducted in two years.

CHAPTER 4 - Evaluation of the Salt Tolerance of *S. chacoense* Accessions, the Diploid Simple Hybrids of *S. tuberosum*, *S. chacoense*, and *S. phureja* and/or *S. stenotomum*, and their Tetraploid Complex Progenies, at the Vegetative Growth Stage.

4.1 Introduction

The salt tolerance of the wild species *S. chacoense* has been confirmed in several studies (See Chapter 5 section 5.1 p 70). In contrast, the primitive cultivated diploids (S. phureja, and *S. stenotonmum*) were reported to be sensitive, among 70 species tested (Elhag, 1991). The differences in salt tolerance among the different accessions of *S. chacoense*, collected from different sites in South America, have not been fully investigated. There have been no reports comparing the salt tolerance of *S. chacoense*, *S. phureja*, and *S. stenotonmum*, with that of interspecific hybrids of these species with *S. tuberosum*. The initial objectives of this section of the study were to compare the salt tolerance of: 1) 11 diploid *S. chacoense* accessions (Appendix 2) The diploid interspecific simple hybrids of *S. chacoense* x *S. tuberosum* (PA1-5) or *S. phureja/S. stenotonmum* x *S. tuberosum* (PB1-6); 3) An intraspecific diploid simple hybrid (*S. tuberosum* x *S. tuberosum*)(PC), 4) Two accessions of primitive cultivated diploids (*S. phureja/S. stenotonmum*)(PD1-2) (Appendix 3A); and 5) 13 complex tetraploid progenies resulting from crosses

(Appendix 3B) For simplicity, the complex tetraploid progenies will be referred to as the progenies in this chapter.

4.2 Materials and methods

4.2.1 Plant materials and propagation

The tubers of the total of 12 clones of *S. chacoense* x *S. tuberosum*, *S. phureja*, and/or *S. stenotonmum* x *S. tuberosum*, and *S. tuberosum* x *S. tuberosum* and 2 accessions of *S. phureja* and/or *S. stenotonmum*, and seeds of their 13 progenies were obtained from Dr. Henry De Jong, Agriculture and Agrifood Canada Research Station, Fredericton, NB (Appendix 2 A, B). True potato seeds (TPS) of 11 *Solanum chacoense* Bitt. accessions were obtained from Dr. J. B. Bamberg of the United States Department of Agriculture, Inter-Regional Potato Introduction Station, Sturgeon Bay, Wisconsin (Appendix 3).

The tubers were grown in the greenhouse at ambient temperatures and under natural light, for 1 mo. Stem cuttings were surface sterilized by washing in running tap water for 30 min, immersed in a 10% bleach solution (a commercial preparation containing 5.25% sodium hypochlorite) for 20 min with occasional agitation, and rinsed 3 times with sterile distilled water. Shoot apices (5-10mm) and nodal segment explants were aseptically removed, and transferred into

micropropagation medium (Appendix 5). The shoot apices were supported on filter paper bridges in liquid medium, while the nodal segments were cultured on solid medium. The surviving plantlets, from both sources, went through 2 consecutive 4 wk cycles of subculture on micropropagation media. Each subculture was followed by a NCB trial. The explants of four clones (PA3, PB5, PB6, and PD) did not survive in culture.

Samples of 20 and 10 seeds were taken of the progenies and *S. chacoense* accessions, respectively. The seeds were surface sterilized, and GA_3-treated as in the seedling bioassay (SB) (See Chapter 5 section 5.2.2 p 69), and germinated in the seedling bioassay media (Appendix 5). Seeds of one progeny did not germinate (HB10). The plantlets of the progenies and *S. chacoense* accessions went through 2 consecutive 4 wk cycles of subculture on micropropagation media. Each subculture was followed by a NCB trial.

4.2.2 Experimental design

Two trials of the NCB were conducted. Each trial was a 3-factorial experiment. The 4 levels of NaCl (0, 40, 80, and 120 mM), the 32 genotypes of *Solanum* spp., and the time of each of the 2 trials were the 3 factors. Two identical trials were conducted in two consecutive months (December- January 1994).

4.2.3 Data analysis

See Chapter 3, section 3.2.3, p 28.

4.3 Results and discussion

The shoot growth parameters (SL, SFW, and SDW) were influenced by the effect of CNC, TIME, G and G*CNC, G*TIME, and CNC*TIME (Appendix 7 A-C). There was a progressive decrease in the shoot growth parameters with increased NaCl levels. The reductions in SL, SFW, and SDW were, respectively, (26%, 27%, and 25%) at 40 mM, (54%, 55%, and 30%) at 80 mM, (69%, 63%, and 75%) at 120 mM, averaged over 32 genotypes, and 2 trials (Figure 4.1 A-C, Figure 4.2).

RL and RDW were influenced by the main effects of CNC, TIME, and G, and G*CNC. RFW was affected by CNC, TIME, and G and G*CNC, G*TIME, and CNC*TIME (Appendix 7 D-F). There was also a progressive reduction in the root growth parameters with increased NaCl levels. The relative reduction for 32 genotypes, averaged over the 2 trials, for RL, RFW, and RDW, were, respectively, (16%, 39%, and 46%) at 40 mM, (40%, 70%, and 66%) at 80 mM, (62%, 73%, and 59%) at 120 mM (Figure 4.1 D-F).

Average shoot growth and average RFW were significantly higher in the first than the second trial (Table 4.1). The

effect of subculturing on growth was only significant for the control and at the lowest NaCl level of 40 mM (Table 4.2).

A positive correlation was found in growth among NaCl levels of 80, 40, and 120 mM, except for RL (Table 4.3; and 4.4). The growth at the control level (0 mM) was correlated with growth at 40 mM NaCl, except for RFW. Growth at 80 and 120 mM NaCl had no significant correlation with vigour at the control level of 0 mM NaCl in the media.

Among the *S. chacoense* accessions, CH7 ranked first in SL, RFW, and RDW, and CH10 ranked first in SFW and SDW (Table 4.5; 4.6; 4.7; 4.8; 4.9; 4.10). Among the simple hybrids, *S. chacoense* x *S. tuberosum* clone PA2 ranked first in SL, SFW, and RFW, and PA4 ranked first in SDW. The clones of *S. chacoense* X *S. tuberosum*, PB3, and PD1, did not differ in RL or RDW results (Table 4.5; 4.6; 4.7; 4.8; 4.9; and 4.10).

Among the progenies, HB8 ranked first in all parameters, except for RDW (Table 4.5; 4.6; 4.7; 4.8; 4.9; 4.10). Among the total simple hybrids and their progenies, the simple hybrid *S. chacoense* x *S. tuberosum* clone PA2 ranked first for SL, SFW, and SDW, and the progeny HB8 ranked first for RL, and RFW (Table 4.5; 4.6; 4.7; 4.8; 4.9; 4.10).

The shoot growth parameters showed that all the clones of the main simple hybrid in all the crosses *S. chacoense* X *S. tuberosum* were superior to their progenies in salt tolerance, except for the clone PA1 (Table 4.5; 4.6; 4.7). Its progenies (HB8 and HB9) were more salt tolerant. The different

reciprocal simple hybrids in each of the crosses were less tolerant than their progenies, except for the primitive cultivated diploid *S. phureja* and/or *S. stenotomum* (clone PD1) which was better than its progeny in salt tolerance (HB1), and the hybrid *S. tuberosum* x *S. tuberosum* (PC) was only more salt tolerant than its progeny (HB7) only in SL (Table 4.5; 4.6; 4.7). These trends were not obvious in the root growth parameters. Using different morphological traits, the 32 genotypes were ranked differently. CH7, CH10, PA4, and HB8 ranked first in SL and RDW; SFW; SDW; RL and RFW, respectively (Table 4.5; 4.6; 4.7; 4.8; 4.9; 4.10).

The CA of the 6 morphological parameters (SL, SFW, SDW, RL, RFW, and RDW), averaged over 2 trials, and across 3 NaCl levels (40, 80, and 120 mM NaCl) resulted in 2 distinct clusters for *S. chacoense* accessions, at an R^2 of 78% (Appendix 10). CH3, CH4, CH7, and CH10 were in the most salt tolerant cluster. The second cluster contained the rest of the accessions.

The dendrogram from the CA for the simple hybrids identified three clusters at an R^2 of 76% (Appendix 11). The two clones of *S. chacoense* x *S. tuberosum* (PA2 and PA4) were in the most salt tolerant cluster. The second cluster contained the other two clones of *S. chacoense* x *S. tuberosum* (PA5 and PA1), one primitive cultivated diploid *S. phureja*/*S. stenotomum* (PD1), and one clone of *S. phureja*/*S. stenotomum* x *S. tuberosum* (PB3). The last cluster contained the other

clones of *S. phureja/S. stenotomum* x *S. tuberosum* (PB1, PB2, and PB4), and *S. tuberosum x S. tuberosum* (PC). Since the clones of the hybrid *S. chacoense* X *S. tuberosum* were concentrated in the first two clusters, this simple hybrid was considered more salt tolerant than the other simple hybrids. The primitive edible cv. *S. phureja* and/or *S. stenotomum* was considered second in salt tolerance, grouping with the least tolerant clones of the simple hybrid *S.chacoense* X *S. tuberosum*. The simple hybrid *S. phureja/S. stenotomum* X *S. tuberosum* was ranked third, where one clone was grouped in the second cluster and the other two clones were grouped in the last cluster. The hybrid *S. tuberosum* X *S. tuberosum* was considered the least salt tolerant, appearing in the last cluster. One primitive edible cv. *S. phureja* and/or *S. stenotomum* was apparently more salt tolerant than the simple hybrid *S. phureja/S. stenotomum* X *S. tuberosum*. The salt tolerance was probably diluted due to the crossing with *S. tuberosum*. This was also indicated by the fact that the hybrid *S. tuberosum* X *S. tuberosum* was the least tolerant.

For the progenies, there were two distinguishable clusters at R^2 of 73 % (Appendix 12). The hybrid HB8 was the only member in the most salt tolerant cluster. The second cluster consisted of the rest of the progenies. HB8 was considered the most outstanding progeny in salt tolerance, though its parents (PA1 x PB2) were in the second, and third clusters, respectively (Appendix 11).

CA for the simple hybrids and the progenies showed that two clones of the simple hybrid *S. chacoense* X *S. tuberosum* were more salinity tolerant than their progenies (Appendix 13) Two clones of the simple hybrid *S. phureja* and/or *S. stenotomum* x *S. tuberosum*, and the simple hybrid *S. tuberosum* X *S. tuberosum* (PB1, PB2, and PC) were surpassed by their progenies. The hybrid HB8 was more salt tolerant than its parents. The rest of the simple hybrids, and the primitive cultivated diploids, were grouped with their progenies.

For the total 32 genotypes, at an R^2 of 72%, there were 2 clusters (Appendix 14). The first cluster contained five *S. chacoense* accessions (CH3, CH4, CH7, CH10, and CH12), and the clones of the hybrid *S. chacoense* X *S. tuberosum* (PA2 and PA4), and one progeny HB8. The second cluster contained the rest of the genotypes. Five of the *S. chacoense* accessions were in the first cluster, but only two clones of the hybrid *S.chacoense* X *S. tuberosum* and one hybrid progeny appeared in the same cluster. It might therefore be suggested that the wild species *S. chacoense* had greater tolerance than the hybrid *S.chacoense* X *S. tuberosum*.

4.4 Conclusion

In this NCB, the shoot and root parameters were adversely affected by the increase in NaCl levels. Growth vigour in the control medium (0 mM) was only correlated with vegetative growth at the 40 mM NaCl level. The vegetative growth among the 3 NaCl levels (40, 80, and 120 mM) was positively correlated. CA of the six morphological parameters, was a very useful and practical way of ranking the different combinations of the 32 genotypes into distinct groups. Both the multiple comparison method (MCM) and CA agreed on the top-ranked genotypes among *S. chacoense* accessions, the hybrids, and the progenies. CA was even more useful than MCM in ranking the total 32 genotypes. It was difficult to decide on which genotype to choose as the most salt tolerant when using the different morphological traits separately. CH7, CH10, PA4, and HB8 ranked first in SL and RDW; SFW; SDW; RL and RFW respectively. CA was able to group all these four genotypes into one salt tolerant cluster. Also, CA results suggested that few accessions of the wild species *S. chacoense* exceeded the hybrids *S. chacoense* X *S. tuberosum* in salt tolerance. The primitive cultivated species *S. phureja* and/or *S. stenotomum* seemed more salt tolerant than the clones of the hybrid *S. phureja/S. stenotomum* X *S. tuberosum*. It is possible that crossing *S. tuberosum* with these genotypes has

the effect of reducing salt tolerance. This result was further illustrated by the fact that *S. tuberosum* x *S. tuberosum* was grouped in the last cluster within the hybrids. While two clones of *S. chacoense* x *S. tuberosum* surpassed their progenies in salt tolerance, two clones of the hybrid *S. phureja/ S. stenotomum* x *S. tuberosum* and the hybrid *S. tuberosum* x *S. tuberosum* were exceeded by their progenies. This was indicated in both the multiple comparison and the CA results.

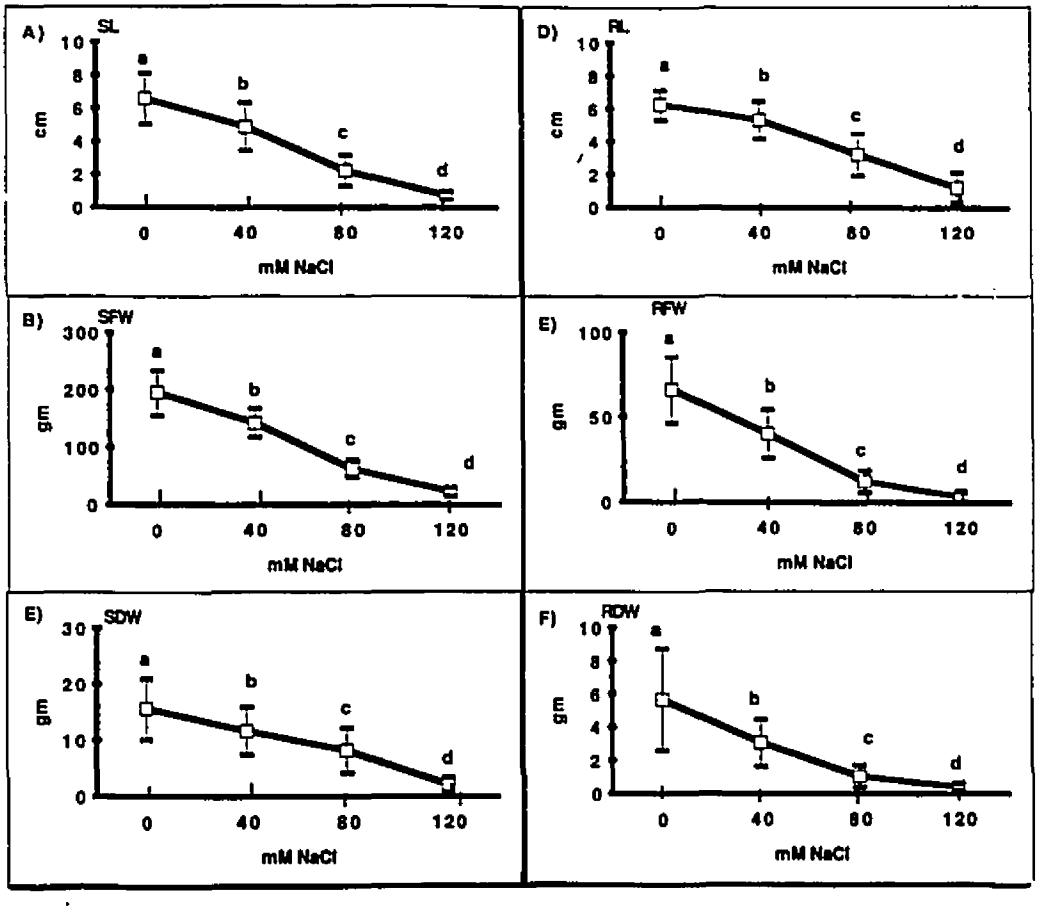


Figure 4.1 The effect of NaCl salinity on shoot and root growth parameters of 32 *Solanum* spp. genotypes in the nodal cutting bioassay *in vitro*. Means of the shoot and root growth parameters followed by the same letter do not differ by the Scheffe's test at the 0.05 level. Bars show S.E. [SL = shoot length; SFW = shoot fresh weight; SDW = shoot dry weight; RL = root length, RFW = root fresh weight, RDW = root dry weight].

Figure 4.2 Morphological differences among the salt tolerant genotypes (a) simple hybrid parent (*S. chacoense* x *S. tuberosum*) clone (PA2), (b) the progeny (HB8) and the salt sensitive genotypes (c) the primitive cultivated diploids *S. phureja* and/or *S. stenotomum* (PD1) and (d) *S. chacoense* accession (CH13) under NaCl levels of (0, 40, 80, and 120 mM), after 4 wks, in the nodal cutting bioassay.

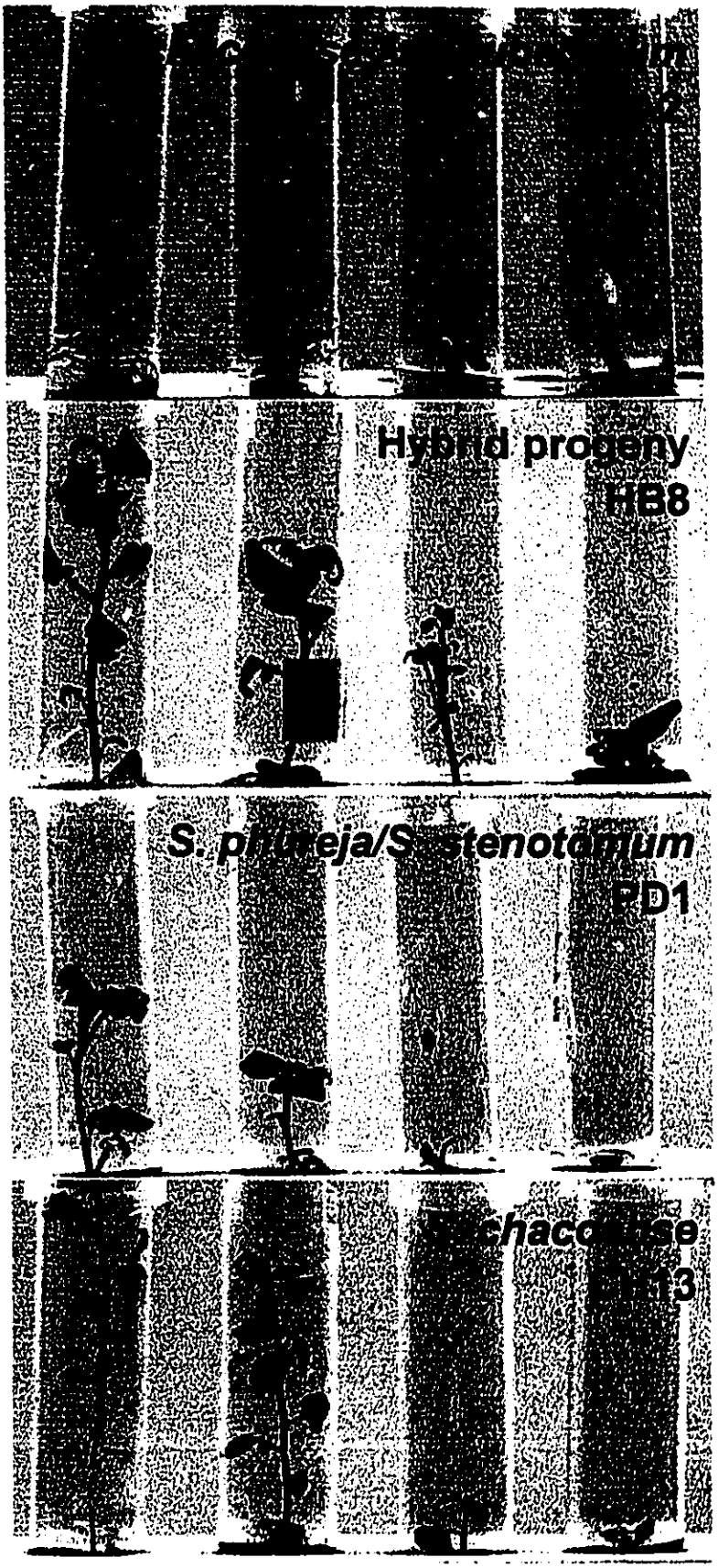

Hybrid progeny
HB8
S. phureja/S. stenotomum
PD1
Tuberosum
13
0
40
80
120
mM NaCl

Table 4.1 The effect of the two consecutive trials (Time) of the nodal cutting bioassay on the mean shoot length (cm), shoot fresh weight(gm), shoot dry weight (gm), and root fresh weight(gm).

Time	SL	SFW	SDW	RFW
1	3.82±0.31 a	114.85±15.5 a	10.07±2.5 a	34.21±5.2 a
2	3.38±0.30 b	97.22±12.4 b	8.78±3.2 b	26.90±6.7 b

Means followed by the same letter within a column are not significantly different (Scheffe's Test, p≤0.05%). [SL = shoot length; SFW = shoot fresh weight; SDW = shoot dry weight; RFW = root fresh weight].

Table 4.2 The effect of the two consecutive trials of the nodal cutting bioassay on the mean shoot length, shoot fresh weight, shoot dry weight, and root fresh weight, under 4 NaCl levels (0, 40, 80, and 120 mM).

mM NaCl	SL	SFW	SDW	RFW
0	7.06±0.6 a	212.97±20 a	14.18±3.0 a	73.83±5.0 a
	6.14±0.5 b	177.51±5 b	7.14±2.0 b	58.36±7.0 b
40	5.26±0.5 a	158.74±3 a	12.96±1.0 a	45.72±4.0 a
	4.53±0.7 b	126.47±10 b	10.49±2.0 b	35.08±2.0 b
80	2.36±0.2 a	67.53±5 a	8.34±1.0 a	14.60±2.0 a
	2.09±0.4 a	58.14±7 a	8.11±1.0 a	10.11±1.5 a
120	0.60±0.1 a	20.15±2 a	1.84±0.2 a	2.70±0.3 a
	0.79±0.3 a	26.75±4 a	2.32±0.4 a	4.03±0.2 a

Means followed by the same letter within a column are not significantly different (LSD Test, p≤0.05%). [SL = shoot length; SFW = shoot fresh weight; SDW = shoot dry weight; RFW = root fresh weight].

Table 4.3 Simple correlation coefficients among 4 NaCl levels (0, 40, 80, and 120 mM) for the shoot growth parameters tested for 32 *Solanum* spp. genotypes.

	mM	0	40	80	120
SL	0	-	0.80**	0.55NS	0.17NS
	40		-	0.85**	0.61*
	80			-	0.76*
	120				-
SFW	0	-	0.80**	0.52NS	0.25NS
	40		-	0.79**	0.68*
	80			-	0.74*
	120				-
SDW	0	-	0.73**	0.59NS	0.22NS
	40		-	0.64*	0.69*
	80			-	0.60*
	120				-

NS Not significant *Significant at p = 0.05
**Significant at p = 0.01. [SL = shoot length; SFW = shoot fresh weight; SDW = shoot dry weight].

Table 4.4 Simple correlation coefficients among 4 NaCl levels (0, 40, 80, and 120 mM) for the root growth parameters tested for 32 *Solanum* spp. genotypes.

	mM NaCl	0	40	80	120
RL	0	-	0.62**	0.32NS	0.10NS
	40		-	0.60*	0.39NS
	80			-	0.74**
	120				-
RFW	0	-	0.40NS	0.40NS	0.16NS
	40		-	0.66*	0.61*
	80			-	0.84**
	120				-
RDW	0	-	0.79**	0.46NS	0.24NS
	40		-	0.68*	0.60*
	80			-	0.80**
	120				-

NS Not significant *Significant at p = 0.05
**Significant at p = 0.01. [RL = root length, RFW = root fresh weight, RDW = root dry weight].

Table 4.5 Mean shoot length (SL) of 32 *Solanum* spp. genotypes including: *S. chacoense* accessions (CH1-CH7), the simple diploid hybrids clones (PA1-PA5; PB1-PB4; PC; PD), and their complex hybrid progenies (HB1-HB13), averaged across 3 NaCl levels, and 2 trials, in the nodal cutting bioassay.

Genotype	SL(cm) ± S.E	
S. chacoense		
CH1	4.55 ± 1.20	bcdefg
CH2	4.01 ± 1.03	bcdefgh
CH3	4.59 ± 1.08	bcdegf
CH4	4.90 ± 1.17	bcdef
CH5	4.28 ± 2.01	bcdefgh
CH7	10.10 ± 2.82	a
CH9	4.61 ± 1.11	bcdefg
CH10	6.10 ± 1.55	bc
CH11	4.55 ± 1.37	bcdefg
CH12	4.40 ± 1.20	bcdefgh
CH13	3.54 ± 1.50	cdefghi
Hybrid		
PA1	3.06 ± 0.50	defghi
PA2	6.38 ± 1.50	b
PA4	5.33 ± 1.00	bcde
PA5	3.91 ± 1.18	bcdefgh
PB1	0.87 ± 0.10	j
PB2	0.79 ± 0.10	j
PB3	2.16 ± 0.93	ghij
PB4	1.81 ± 0.76	hij
PC	1.84 ± 0.25	hij
PD1	2.62 ± 0.50	fghij
Progeny		
HB1	2.30 ± 0.84	fghij
HB2	2.75 ± 0.80	efghij
HB3	2.39 ± 0.50	fghij
HB4	3.25 ± 1.17	defghij
HB5	2.11 ± 0.85	ghij
HB6	2.66 ± 0.92	fghij
HB7	1.39 ± 0.25	j
HB8	5.63 ± 1.62	bcd
HB9	3.61 ± 1.12	cdefghi
HB11	2.88 ± 0.86	efghij
HB12	3.13 ± 1.34	defghij
HB13	2.43 ± 0.75	fghij

Means followed by the same letter are not significantly different at the p = 0.05 level, using Scheffe's test.

Table 4.6 Mean shoot fresh weight (SFW) of 32 *Solanum* spp. genotypes including: *S. chacoense* accessions (CH1-CH7), the simple diploid hybrids clones (PA1-PA5; PB1-PB4; PC; PD), and their complex hybrid progenies (HB1-HB13), averaged across 3 NaCl levels, and 2 trials, in the nodal cutting bioassay.

Genotype	SFW(gm) ± S.E	
S. chacoense		
CH1	103.60 ± 23.2	cdefg
CH2	83.32 ± 24.0	efg
CH3	168.96 ± 49.0	bcdef
CH4	213.70 ± 57.2	abc
CH5	82.46 ± 20.0	efg
CH7	202.90 ± 46.2	abcd
CH9	111.88 ± 28.6	bcdefg
CH10	287.10 ± 79.5	a
CH11	104.79 ± 31.8	cdefg
CH12	193.22 ± 25.0	abcde
CH13	60.65 ± 17.5	fg
Hybrid		
PA1	88.66 ± 36.9	efg
PA2	282.93 ± 75.0	a
PA4	222.45 ± 79.0	ab
PA5	106.99 ± 35.1	cdefg
PB1	13.10 ± 2.5	g
PB2	5.35 ± 1.5	g
PB3	31.62 ± 11.3	g
PB4	47.66 ± 15.0	g
PC	26.05 ± 5.0	g
PD1	95.55 ± 25.0	defg
Progeny		
HB1	71.77 ± 24.8	fg
HB2	86.81 ± 20.0	efg
HB3	52.56 ± 15.0	g
HB4	63.04 ± 22.4	fg
HB5	38.24 ± 6.0	g
HB6	61.31 ± 22.7	fg
HB7	31.11 ± 7.50	g
HB8	189.90 ± 25.0	abcde
HB9	93.77 ± 28.6	defg
HB11	88.74 ± 32.7	efg
HB12	104.91 ± 43.1	cdefg
HB13	84.09 ± 22.5	efg

Means followed by the same letter are not significantly different at the p = 0.05 level, using Scheffe's test.

Table 4.7 Mean shoot dry weight (SDW) of 32 *Solanum* spp. genotypes including: *S. chacoense* accessions (CH1-CH7), the simple diploid hybrids clones (PA1-PA5; PB1-PB4; PC; PD), and their complex hybrid progenies (HB1-HB13), averaged across 3 NaCl levels, and 2 trials, in the nodal cutting bioassay.

Genotype	SDW(gm) ± S.E	
S. chacoense		
CH1	10.08 ± 2.21	defgh
CH2	6.87 ± 1.6	fghi
CH3	13.31 ± 3.4	cdefg
CH4	15.84 ± 3.6	bcde
CH5	6.12 ± 2.8	fghi
CH7	17.69 ± 3.8	bcd
CH9	8.38 ± 2.3	efghi
CH10	21.48 ± 5.1	b
CH11	8.13 ± 2.1	efghi
CH12	13.93 ± 7.5	bcdef
CH13	5.49 ± 2.6	ghi
Hybrid		
PA1	7.15 ± 2.8	fghi
PA2	19.40 ± 9.9	bc
PA4	30.00 ± 9.9	a
PA5	8.05 ± 3.4	efghi
PB1	1.60 ± 0.9	i
PB2	1.04 ± 0.7	i
PB3	3.34 ± 1.3	hi
PB4	4.12 ± 1.3	hi
PC	2.80 ± 1.7	hi
PD1	9.00 ± 0.5	efghi
Progeny		
HB1	5.82 ± 2.1	ghi
HB2	7.54 ± 3.7	fghi
HB3	4.17 ± 2.3	hi
HB4	5.50 ± 2.1	ghi
HB5	3.60 ± 1.8	hi
HB6	5.80 ± 2.2	ghi
HB7	2.90 ± 1.5	hi
HB8	18.40 ± 6.0	bc
HB9	8.45 ± 3.2	efghi
HB11	7.73 ± 3.6	fghi
HB12	10.33 ± 3.6	defgh
HB13	5.10 ± 3.2	hi

Means followed by the same letter are not significantly different at p = 0.05 level, using Scheffe's test.

Table 4.8 Mean root length (RL) of 32 *Solanum* spp. genotypes, including: *S. chacoense* accessions (CH1-CH7), the simple diploid hybrids clones (PA1-PA5; PB1-PB4; PC; PD), and their complex hybrid progenies (HB1-HB13), averaged across 3 NaCl levels, and 2 trials, in the nodal cutting bioassay.

Genotype	RL(cm) ± S.E.	
S. chacoense		
CH1	4.02 ± 1.20	abcde
CH2	4.41 ± 1.03	abcde
CH3	7.25 ± 0.81	ab
CH4	5.40 ± 0.52	abcd
CH5	4.08 ± 1.48	abcde
CH7	6.28 ± 0.90	abc
CH9	3.65 ± 0.76	bcde
CH10	4.87 ± 0.21	abcde
CH11	2.91 ± 0.90	cde
CH12	2.92 ± 1.20	cde
CH13	3.78 ± 1.37	abcde
Hybrid		
PA1	5.04 ± 1.92	abcde
PA2	4.26 ± 1.33	abcde
PA4	4.78 ± 1.84	abcde
PA5	1.79 ± 0.37	de
PB1	1.40 ± 0.77	e
PB2	1.49 ± 0.97	e
PB3	4.03 ± 1.43	abcde
PB4	3.17 ± 1.87	cde
PC	1.24 ± 1.14	e
PD1	4.17 ± 2.23	abcde
Progeny		
HB1	3.98 ± 1.78	abcde
HB2	3.20 ± 1.49	cde
HB3	2.31 ± 1.04	de
HB4	4.76 ± 1.27	abcde
HB5	3.24 ± 1.46	cde
HB6	3.41 ± 1.45	bcde
HB7	2.78 ± 1.41	cde
HB8	7.59 ± 0.65	a
HB9	6.25 ± 1.24	abc
HB11	4.84 ± 1.58	abcde
HB12	5.08 ± 1.57	abcde
HB13	3.80 ± 1.44	abcde

Means followed by the same letter are not significantly different at p = 0.05 level, using Scheffe's test.

Table 4.9 Mean root fresh weight (RFW) of 32 *Solanum* spp. genotypes including: *S. chacoense* accessions (CH1-CH7), the simple diploid hybrids clones (PA1-PA5; PB1-PB4; PC; PD), and their complex hybrid progenies (HB1-HB13), averaged across 3 NaCl levels, and 2 trials, in the nodal cutting bioassay.

Genotype	RFW(gm) ± S.E.	
S. chacoense		
CH1	12.48 ± 3.81	ef
CH2	25.88 ± 11.43	bcdef
CH3	48.85 ± 15.60	abcde
CH4	52.48 ± 14.96	abcd
CH5	15.61 ± 7.73	def
CH7	61.44 ± 19.89	ab
CH9	24.44 ± 8.10	bcdef
CH10	37.91 ± 12.72	abcdef
CH11	23.07 ± 8.95	bcdef
CH12	50.86 ± 26.02	abcde
CH13	12.04 ± 5.92	ef
Hybrid		
PA1	39.38 ± 18.45	abcdef
PA2	55.18 ± 26.29	abc
PA4	50.82 ± 28.40	abcde
PA5	12.12 ± 4.76	ef
PB1	5.70 ± 3.50	f
PB2	2.50 ± 1.76	f
PB3	28.83 ± 14.51	bcdef
PB4	24.78 ± 16.41	bcdef
PC	8.95 ± 8.28	f
PD1	31.18 ± 17.13	abcdef
Progeny		
HB8	70.17 ± 21.95	a
HB12	40.97 ± 22.94	abcdef
HB2	39.55 ± 25.54	abcdef
HB6	39.23 ± 23.53	abcdef
HB11	36.31 ± 17.15	abcdef
HB9	33.67 ± 17.01	abcdef
HB13	33.58 ± 24.86	abcdef
HB1	28.64 ± 15.88	bcdef
HB4	21.00 ± 8.88	cdef
HB5	14.55 ± 9.04	def
HB7	13.77 ± 9.21	def
HB3	12.39 ± 7.34	ef

Means followed by the same letter are not significantly different at p = 0.05 level, using Scheffe's test.

Table 4.10 Mean root dry weight (RDW) of 32 *Solanum* spp. genotypes including: *S. chacoense* accessions (CH1-CH7), the simple diploid hybrids clones (PA1-PA5; PB1-PB4; PC; PD), and their complex hybrid progenies (HB1-HB13), averaged across 3 NaCl levels, and 2 trials, in the nodal cutting bioassay.

Genotype	RDW(gm) ± S.E.	
S. chacoense		
CH1	1.25 ± 0.38	b
CH2	1.77 ± 0.75	ab
CH3	2.78 ± 1.11	ab
CH4	4.21 ± 1.36	ab
CH5	0.96 ± 0.52	b
CH7	10.23 ± 5.92	a
CH9	1.48 ± 0.44	b
CH10	3.79 ± 0.90	ab
CH11	1.27 ± 0.49	b
CH12	2.67 ± 1.56	ab
CH13	1.32 ± 0.61	b
Hybrid		
PA1	2.16 ± 1.05	ab
PA2	3.43 ± 1.46	ab
PA4	3.19 ± 1.69	ab
PA5	1.73 ± 0.43	ab
PB1	1.04 ± 0.67	b
PB2	0.13 ± 0.07	b
PB3	1.76 ± 0.83	ab
PB4	1.47 ± 0.88	b
PC	0.61 ± 0.56	b
PD1	2.22 ± 1.24	ab
Progeny		
HB1	1.70 ± 1.00	ab
HB2	4.19 ± 2.91	ab
HB3	2.89 ± 1.00	ab
HB4	1.39 ± 0.56	b
HB5	1.22 ± 0.80	b
HB6	1.81 ± 1.09	ab
HB7	4.87 ± 2.00	ab
HB8	7.71 ± 1.15	ab
HB9	2.31 ± 1.20	ab
HB11	2.26 ± 1.33	ab
HB12	2.57 ± 1.38	ab
HB13	1.75 ± 1.19	ab

Means followed by the same letter are not significantly different at p = .05 level, using Scheffe's test.

Chapter 5 - A Comparison of the Salt Tolerance of *S. chacoense* Accessions at the Germination and Early Seedling Growth, vs. the Vegetative Growth Stages.

5.1 Introduction

Arslan et al. (1987) were first to screen *S. chacoense* for salinity tolerance *in vitro*. They did not report the number of accessions they screened, but could not recommend this species for salt tolerance. The potential of *S. chacoense* for superior salt tolerance *in vivo*, compared with other wild species, was first reported by Bilski et al. (1988 b), who only screened one accession number of *S. chacoense*. *S. chacoense* ranked first, accessions of *S. gourlayi*, *S. microdontum*, *S. bulbocastanum*, *and S. sparisipilum* were intermediate. *S. papita* was the least tolerant. Bilski et al. (1988,b) averaged their results over the salt levels of 40, 80, and 120 mM. Survival rate, and haulm fresh weight were used in the comparison.

Of the 86 *Solanum* spp. tested by Elhag (1991), 3 accessions of *S. chacoense* were screened . Only 1 of these was among the top 6 genotypes in salt tolerance at the levels of 40, 80, and 120 mM NaCl. The parameters used for evaluating salt tolerance at the vegetative growth stage were SL and SFW for both *in vitro*- and *in vivo*-grown plants. These growth parameters were positively correlated with each

other and with yield (g/plant) *in vivo*. Hybrids derived from *S. chacoense* were more salt tolerant than hybrids derived from *S. gourlayi* or *microdontum* (Zhang et al., 1993). The parameters used in assessing salt tolerance during vegetative growth were SL, SDW, RL, RDW, at NaCl levels of 80 and 120 mM. *S. chacoense* ranked first in salt tolerance during germination and early seedling growth, when compared with the two wild species *S. gourlayi* and *S. microdontum*, at the above levels of NaCl. The criteria for ranking were the final germination percentage, SL, RL, and RDW.

When this study was initiated few *S. chacoense* accessions had been evaluated for salinity tolerance, and there were no previous reports comparing the salt tolerance of *S. chacoense* accessions or any other wild *Solanum* sp. during germination and seedling growth versus the later vegetative growth. For these reasons, the objectives of this section of the study were: 1) to rank the salt tolerance of thirteen *S. chacoense* accessions, investigating differences among the accessions during germination and early seedling growth 2) to compare the salt tolerance of the eleven *S. chacoense* accessions ranked at the vegetative stage with their salt tolerance at the early seedling stage. To achieve this goal, a seedling bioassay (SB) was used, described by Zhang et al., (1993), and a comparison was drawn between the ranking of the accessions in the NCB, with the SB.

5.2 Materials and methods

5.2.1 Plant materials

True potato seeds (TPS) of thirteen *Solanum chacoense* Bitt. accessions were obtained from Dr. J. B. Bamberg of the United States Department of Agriculture Research Service, Inter-Regional Potato Introduction Station, Sturgeon Bay, Wisconsin (Appendix 3).

5.2.2 Seedling bioassay

The TPS were placed into microporous specimen capsules (#13215,SPI Supplies, Division of Structure Prope, Inc., West Chester, PA, U.S.A.). Ten seeds were placed into each capsule. The capsules were immersed in 10 % (w/w) commercial bleach for 20 min, rinsed 3 times with sterile distilled water, soaked in 2 gl^{-1} filter-sterilized gibberellic acid (GA_3) solution for 24 hr to eliminate dormancy, if present, and again rinsed 3 times with sterilized distilled water. The capsules were opened and 20 seeds were transferred, 2 per test tube, onto 15 ml of the seedling bioassay medium (SBM) (Appendix 5). The cultures were kept in the dark for 1 wk, followed by 3 wks in the light, under appropriate culture conditions (Appendix 4, B).

5.2.3 Experimental design

Four levels of NaCl were tested 0, 40, 80, and 120 mM. Twenty seeds were used for each NaCl level. Two trials of the SB were conducted in two consecutive months (July-August). Each trial was a three-factor experiment, including the NaCl levels, the accessions, and time.

5.2.4 Data analysis

Final percent germination (G%) was defined as the ratio of the seedlings which germinated over the total seed sample treated at each NaCl level, for each accession, and for each trial. An arcsin transformation was performed on the data. The seedling growth parameters shoot and root length (SL and RL), total fresh weight (TFW), and total dry weight (TDW) (48 hr at 60 °C) were collected. The means for each genotype, and for each trial, were used in the analysis. Data was analyzed using the same statistical procedures as in Chapter 3 section 3.2. p 28. CA was done on the means of 4 morphological parameters (SL, RL, TFW, and TDW) measured for each genotype across 3 NaCl levels (40 ,80, and 120 mM) averaged over the 2 trials.

5.3 Results and discussion

G% was only influenced by two main effects, NaCl concentration (CNC), and genotype (G) (Appendix 8). A progressive reduction in G% was observed with the increase in CNC (Figure 5.1). SL, RL, and TFW were influenced by the main factors (G and CNC). TDW was affected by the three main factors (G, CNC, and TIME), and the interaction term G*TIME (Appendix 8). SL decreased with the increase in NaCl levels at both 80 and 120 mM. RL and TDW were adversely affected at only 120 mM. There were no differences in TFW between the control (0 mM), and 40 mM, or between 40 and 80 mM (Figure 5.1). The increase in NaCl levels affected the various growth parameters quite differently. While the increase in medium NaCl levels decreased SL at 80 mM and 120 mM, RL was only inhibited at 120 mM. The reduction in water uptake by seedlings on medium with increased NaCl levels might have been compensated by an increase in the TDW. This might be due to the contribution of either Na^+ or Cl^- to the dry matter. This might explain the absence of reduction in TFW at 40 mM, compared with the control, and at 80 mM compared with 40 mM. This was indicated by the absence of differences in TDW among cultures growing on three NaCl levels (0, 40, and 80 mM). At 120 mM, both TFW and TDW were reduced. Similar results were found by Yeo and Flowers (1985) who studied the response of salt-sensitive rice (*Oryza sativa* L.) to NaCl and Na^+/Ca^{+2} combinations. The differences in SFW were more pronounced

than SDW. This was explained by the obvious differences in the water content. The organic dry weight changes were partly masked by the contribution of either Na^{+1} or Cl^{-1} ions to the TDW. The inconsistency of the effect of increased NaCl levels on seedling growth of forty rice cvs. was reported by Reddy and Vaidyanath (1992). The characters measured were the G%, SL, SFW, SDW, RL, RFW, and RDW. They found that the different parameters were affected differently.

Averaged over the three NaCl levels (40, 80, and 120 mM NaCl), CH7 and CH13 ranked first and second respectively in G%. The accessions CH11, and CH9 did not germinate, and were eliminated from further analysis. Significant differences among the accessions were only detected in SL. The accessions CH7, CH10, CH12, and CH13 ranked above the other accessions (Figure 5.2 A, B).

The CA of the 11 *S. chacoense* accessions that germinated under salinity stress gave two clusters (Appendix 15). CH7, CH10, CH12 and CH13 were present in the first cluster. The second cluster consisted of CH1, CH3, CH4, CH5, CH2, CH6, CH8. CH7, CH10, CH12, and CH13 repeatedly, occurred as top ranking accessions in the MCM results of G% and SL and the CA of the four morphological parameters.

The NCB detected significant differences among accessions for more morphological traits than did the SB.
Significant differences were detected only in G% and SL for the seedling bioassay, while there were significant

differences in all the morphological parameters in the NCB.

In the NCB, CH7 ranked first in SL, RFW, and RDW. CH10 ranked first in SFW and SDW. In the SB, MCM of SL G% ranked CH7, and CH10 the first. Also, these two accessions were in the more salt tolerant cluster in both bioassays. Therefore, CH7 and CH10 were consistently top ranking in salt tolerance at the germination and early seedling growth, and at the vegetative stage, based on MCM and CA results. CH10 was tested previously by Bilski et al. (1988 b), who ranked it first in salt tolerance for both the germination tests, and at *in vivo* vegetative growth in the greenhouse.

In the current study, CH7 proved to be as salt tolerant as CH10. CH1, CH2, and CH5 were all sensitive at both stages of growth. CH9 and CH11 had no germination in SB, and were not salt tolerant in the NCB. However, CH13 and CH12 were only salt tolerant in the SB, and CH3 and CH4 were only salt tolerant in the NCB. These results were drawn based on both the MCM and the CA.

5.4 Conclusion

The increase in NaCl levels affected the various measured parameters quite differently. A progressive decrease was observe in G%. SL only decreased at 80 mM and 120 mM. RL was only inhibited at 120 mM. There was no reduction in TFW at 40 mM, compared with the control (0 mM), and at 80 mM compared with 40 mM. This was also indicated by the absence of differences in TDW among cultures growing on three NaCl levels (0, 40, and 80 mM). This study affirmed the previous reports on the salt tolerance of this wild species, but differences were detectable in salt tolerance among *S. chacoense* accessions. During germination and early seedling growth, CH7, CH10, CH12, and CH13 were outstanding accessions.

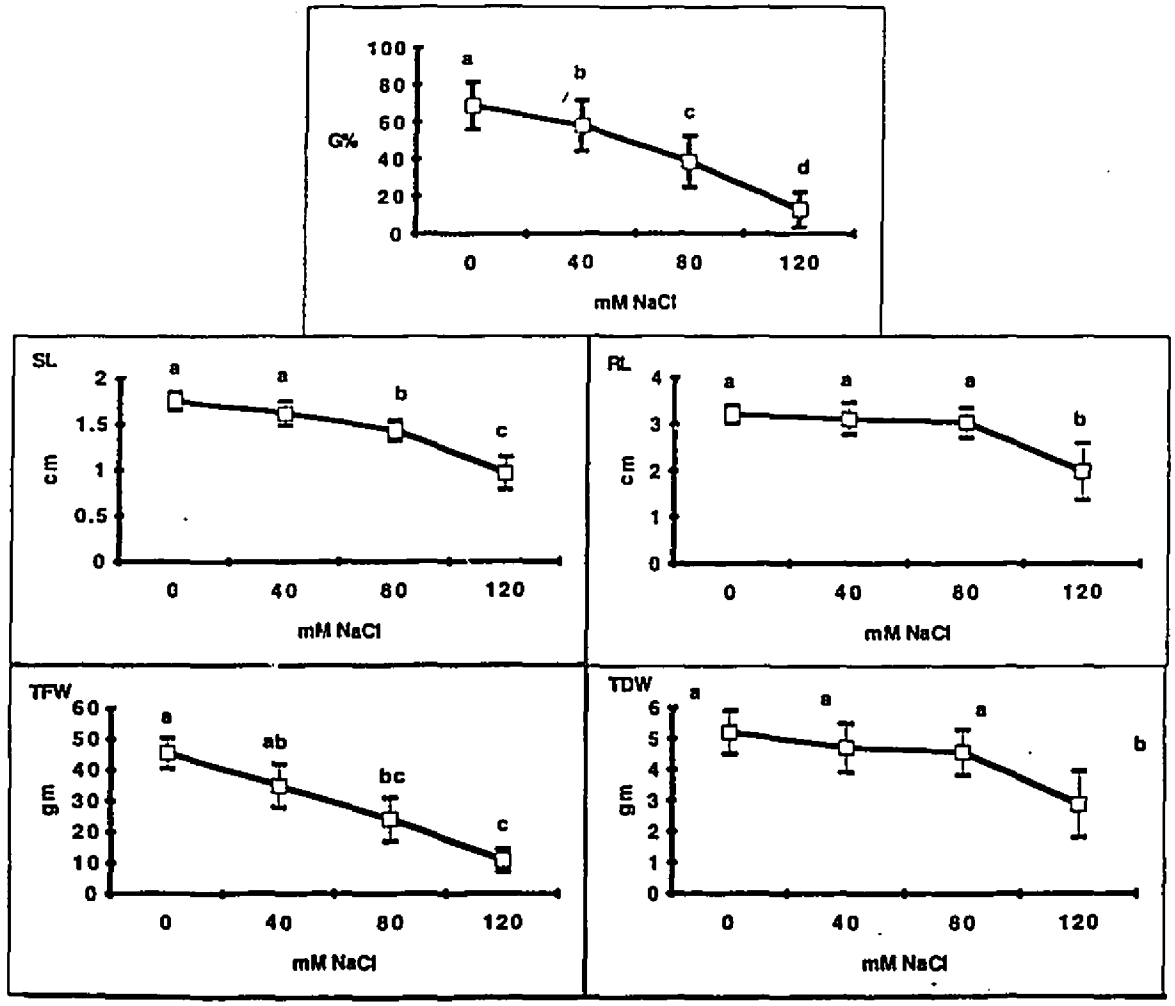


Figure 5.1 The effect of the increase in NaCl concentrations on final germination percentage, shoot length (cm), root length(cm), total fresh weight (gm), and total dry weight (gm) of 13 *Solanum chacoense* accessions in the seedling bioassay. Values followed by the same letter do not differ by the Scheffe's test at the 0.05 level. Bars show S.E. [G% = final germination percentage, SL = shoot length, RL = root length, TFW = total fresh weight, TDW = Total dry weight]

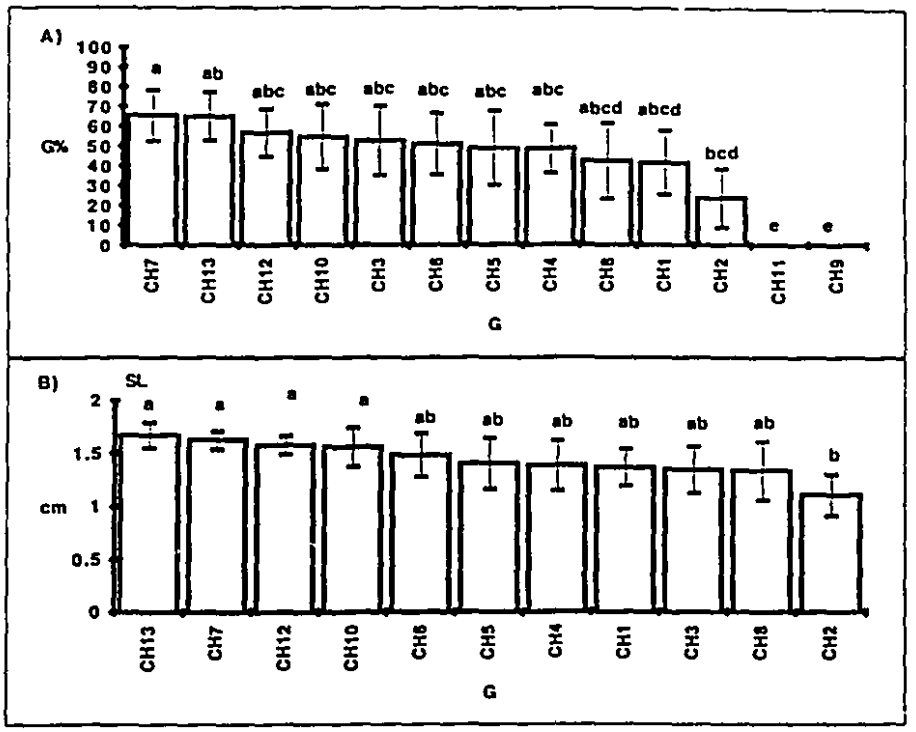
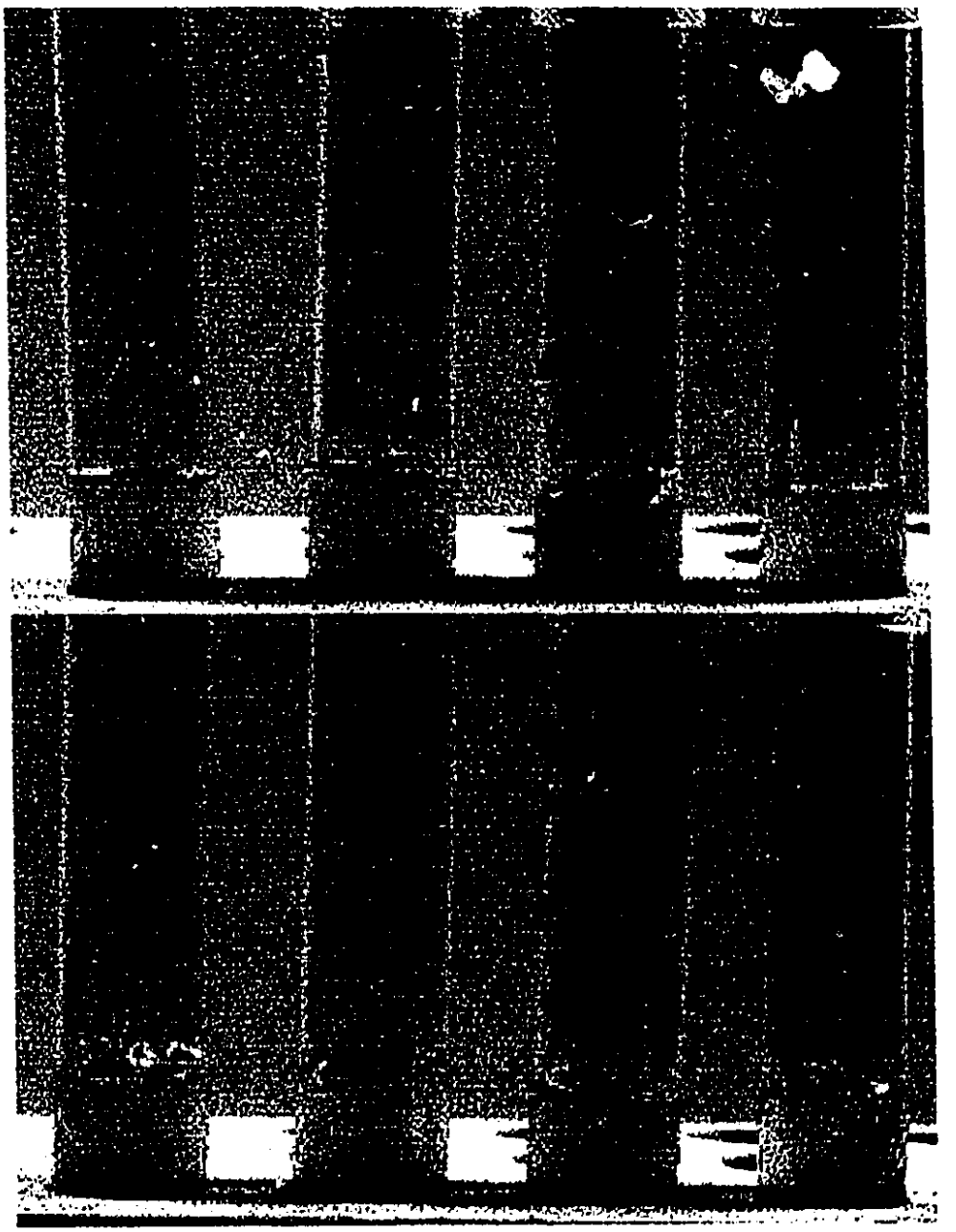


Figure 5.2 A) Mean final germination percentage of 13 *S. chacoense* accessions (B) Mean shoot length (cm) of 11 *S. chacoense* accessions. Values are the means of 120 treated plantlets, in 2 trials averaged over NaCl levels 40, 80, and 120 mM NaCl. Bars show S.E. of the treated plantlets. [G% = final germination percentage, SL = shoot length]

Figure 5.3 Morphological differences between a salt tolerant and a salt sensitive *S. chacoense* accession (CH10, and CH2 respectively), in the seedling bioassay at 4 weeks.


0
40
80
120
mM NaCl

Chapter 6 - General Discussion and Conclusions

In the nodal cutting bioassay, the shoot and root parameters decreased progressively with the increase in the NaCl levels from 0 to 40, 80, and 120 mM, at the vegetative stage. The vegetative growth in the control media (0 mM) was only correlated with the vegetative growth at the lowest NaCl level (40 mM), but the vegetative growth among the 3 NaCl levels 40, 80, and 120 mM was positively correlated.

In the seedling bioassay, the increase in NaCl levels affected the various measured parameters quite differently. A progressive decrease was observed in the final germination percentage. Shoot length only decreased at NaCl levels of 80 and 120 mM. Root length was only inhibited at 120 mM. There was no reduction in total fresh weight at 40 mM, compared with the control (0 mM), and at 80 mM compared with 40 mM. This was also indicated by the absence of differences in total dry weight among cultures growing on the 3 NaCl levels (0, 40, and 80 mM).

Potato cvs. were found to be a useful potential genetic source for the improvement of salt tolerance in potato, with their already improved agronomical characteristics. Among the 131 cvs. assayed in the present study, 20 were promising in salt tolerance at the vegetative stage. The North American cvs. Atlantic, Coastal Chip, Chipeta, Norqueen, and Onaway, and the European cvs. Bintje, and Erntestolz, were

particularly recommended, due to previous reports of their drought or salinity tolerance, or wide adaptability. The parentage records of a limited number of cvs. indicating that salt tolerance might be a recessive trait, as a related species tomato *(Lycopersicum esculentum)* (Ashraf, 1994). This was indicated by the salt tolerant by the salt tolerant cv. Green Mountain and its sensitive progenies Keswick and LaChipper, and the salt sensitive parents and progenies: Russet Burbank and Coastal Russet, Blue Mac and Ac Domino, Keswick and Fundy, Norchip and Islander.

The salt tolerance of *S. chacoense* was confirmed in this study, but differences were detected in salt tolerance among *S. chacoense* accessions, during germination and early seedling growth, and at the later vegetative stage. Seven accessions showed similar response in salt tolerance in both stages. CH7 and CH10 were outstanding accessions during germination and early seedling growth and at the later vegetative stage.

Within the 32 *Solanum* spp. genotypes in the nodal cutting bioassay, the wild species *S. chacoense* seemed more salt tolerant than the hybrid *S. chacoense* x *S. tuberosum*. The primitive cultivated species *S. phureja* and/or *S. stenotomum* seemed more salt tolerant than the clones of the hybrid *S. phureja/S. stenotomum* x *S. tuberosum*. Perhaps crossing *S. tuberosum* with these genotypes reduced salt tolerance. This observation was supported by the finding that the hybrid *S. tuberosum* x *S. tuberosum* was the lowest in salt tolerance in

both the multiple comparison method and in cluster analysis. Two clones of *S. chacoense* x *S. tuberosum* were superior to their progenies in salt tolerance in both the multiple comparison method and cluster analysis. However, two clones of the hybrid *S. phureja/S. stenotomum* x *S. tuberosum* and the hybrid *S. tuberosum* x *S. tuberosum* were surpassed by their progenies.

Based on this research, a better protocol for large scale screening can be recommended. The nodal cutting bioassay can be accomplished in one trial only, since a positive correlation occurred between the growth in the seven trials of the nodal cutting bioassay of the 131 cvs. At the same time, the differences in the growth among the 32 genotypes in the second nodal cutting bioassay were only indicated at NaCl levels of 0 and 40 mM. The concentrations of 40, 80, and 120 are recommended for testing the genotypes, since this range indicated a progressive reduction in growth with the increased salt levels in both the present research and the report by Elhag, 1991. One dosage of a high salt concentration, such as 80 or 120 mM might result in no growth to quantify in many potentially useful genotypes of *Solanum* spp. The lowest NaCl level (40 mM) is equivalent to the lowest level at which soils are classified as salt-affected (about 44 mM NaCl). It is also the lowest NaCl level at which yield was reported to decrease *in vivo* (40-45 mM NaCl) (Bruns and Caesar 1990; Levy, 1992). The intermediate level of 80 mM NaCl in this

present study, was similar to the levels of salinity used *in vivo* to determine salt tolerance (about 90 mM) (personal communication Zhang Yanling, 1995). The level of 120 mM NaCl where some growth resulted in *Solanum* spp. genotypes (Elhag, 1991). The importance of the control level of 0 mM, was not apparent in the nodal cutting bioassay used in the present study. Positive correlation was found between the growth at 40, 80 mM, and 120 mM NaCl. Shoot length could be used as an easy non destructive parameter in assessing the salt tolerance of the genotypes. If a positive correlation occurred between growth at the different levels, ranking could be done either averaged over the levels, or at the intermediate level of 80 mM NaCl. If a positive correlation did not occur, then ranking should be done at the level corresponding with the levels *in vivo*. Cluster analysis is suggested to group the genotypes into different categories of salt tolerance, based on a single parameter, as previously described by Horst and Dunning (1989). These authors used cluster analysis to group fifty cvs. of perennial ryegrass (*Lolium perenne* L.) by using leaf blade length, and total seedling fresh and dry weight, individually.

In the present study, among the eleven *S. chacoense* accessions, a similar ranking at the early seedling and later vegetative stage was found only among seven accessions. This suggests the importance of conducting both the seed germination tests and the nodal cutting bioassays to confirm

the salt tolerance of the wild species at both ontogenic stages. Shoot length could be used as an easy non destructive parameter in assessing the salt tolerance. A control of the control 0 mM NaCl as well as the level of 80 mM are recommended for the seedling bioassay. The control level is important to distinguish the differences between low viability and salt sensitivity at this stage. The NaCl level of 80 mM is suggested for the ranking in the seedling bioassay and for the comparison with the results in the nodal cutting bioassay at the same level, since 80 mM was the level at which shoot length started to decrease in the present seedling bioassay.

In the present research, the use of cluster analysis was found beneficial to overcome the obvious disagreement between the reported morphological parameter recommendations for ranking potato cvs. in the literature (Elhag, 1991; Morpurgo, 1991; Morpurgo and Rodriguez, 1987), and at a stage in the research when *in vivo* field tests were still being undertaken to validate the correlation between *in vitro* parameters and the yield *in vivo*. Cluster analysis had the advantage of presenting the ranking of a large number of cvs. in a single dendrogram, based on the collected morphological parameters, that had two different measurement units. Ranking was based on absolute values. Ranking could be also based on the relative values corrected for growth at the control level, or the use of the regression slope, which was not covered by the statistical analysis of the present study. Later on, a

positive correlation was found between the yield *in vivo* and shoot length, shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight *in vitro* (personal communication Yanling Zhang, 1995), verifying the use of the different morphological parameters in the analysis.

Among the 32 *Solanum* spp. genotypes, and among *S. chacoense* accessions in the seedling bioassay, cluster analysis was used as a complementary rather than an alternative procedure to the multiple comparison method. The multiple comparison method detected significant differences among *S. chacoense* accessions for more morphological traits in the nodal cutting bioassay than it did for the seedling bioassay. Significant differences among the accessions were detected only in the multiple comparison of final germination percentage and shoot length for the seedling bioassay, while there were significant differences in all the morphological parameters in the nodal cutting bioassay. Statistically, multiple comparison methods have the advantage of decreasing the false significant differences that might arise in using pairwise comparisons (e.g. LSD), but the power to detect real differences might be decreased (Jolliffe et al. 1989), which might be the case in the seedling bioassay. Cluster analysis was used as a statistical tool to assist in ranking *S. chacoense* accessions at both the early seedling growth in the seedling bioassay, and the later vegetative stage in the nodal cutting bioassay. Also, cluster analysis assisted in

providing an overall picture of the ranking of the simple hybrids, along with their complex progenies.

An apparent limitation of the present study is the absence of a further check on the salt tolerant cvs. *in vivo*. This does not under estimate the importance of using the nodal cutting bioassay as a quick screening procedure, since the cultivars that are salt tolerant at the vegetative stage are expected to continue their salt tolerance to further crop ontogeny stages. *In vivo* experimemts should be conducted on the salt tolerant cvs., in a homogeneous inert medium (e.g. rockwool). It is suggested that the experiment be conducted in a step-wise manner. The cvs. should be tested at equivalent levels to the 3 NaCl levels used *in vitro* (40, 80, and 120 mM). Top performing cvs. at the vegetative stage *in vivo*, should be evaluated for their salt tolerance at the onset and advanced stages of tuberization. This would give a rough estimate of the levels of salt that could be used in the irrigation of these salt tolerant cvs., at the different stages of crop ontogeny, before recommendations are made for growers in salt-affected areas of the world.

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Appendices

Appendix 1 -A Alphabetical list of the 131 European and N American potato cv, the crop use (C), the year of cv. release (YR), the relative maturity date (M), and their known tolerance to abiotic stresses (New Brunswick Department of Agriculture, 1993). a+ abiotic stress tolerance, DT = drought tolerant, DS = drought sensitive, E = early, EMS = early to midseason, ES = export seed, L = late, ME = medium early, ML = medium late, MS = midseason, P = processing, T = table, VL = very late, VE = very early, W = widely adapted

CV	SYM	C	YR	M	A
Acadia Russet	CV1	T	1981	L	
AC Brador	CV2	ES	1991	VL	
AC Domino	CV3	T	1990	L	
Adora	CV4				
Agria	CV5				
Allegany	CV6	P&T	1989	VL	
Amanda	CV7				
Aminca	CV8				
Amisk	CV9	P	1990	MS	
Annika	CV10				
Anosta	CV11				
Atlantic	CV12	P&T	1976	MS	W
Ausonia	CV13				
Avanti	CV14				
Belleisle	CV15	T	1974	L	
Belmont	CV16				
BelRus	CV17	T	1978	ML	DS
Bintje	CV18	ES&T	1910	L	W
Blue Mac	CV19	T	1979	L	

Campbell-13	CV20	P&T	1977	MS	
Cardinal	CV21				
Caribe	CV22	T	1984	VE	W
Castile	CV23	P&T	1990	L	DS
Chaleur	CV24				
Cherokee	CV25	T	1954	MS	
Chieftan	CV26	S&T	1966	MS	W
Chipeta	CV27	P	1993	ML	W
Coastal Chip	CV28	P&T	1990	MS-ML	W
Coastal Russet	CV29	T	1987	MS	
Conestoga	CV30	P&T	1982	E	
Concorde	CV31				
Cupids	CV32	T	1986	MS-L	
D195-24	CV33				
Dark Red Norland	CV34				
Delcora	CV35				
Delta Gold	CV36				
Denali	CV37				
Diamant	CV38				
Disco	CV39				
Donna	CV40	ES	1986	MS-L	
Draga	CV41				
Dundrod	CV42				
Dundrum	CV43				
Estima	CV44				
Eide Russet	CV45	T	1990	MS-L	
Erntestolz	CV46				

Fontenot	CV47	T	1992	ML	
F83065	CV48				
Foreston Russet Burbank	CV49				
Frontier Russet	CV50	P&T	1990	MS	
Fundy	CV51	T	1958	E	
Gemchip	CV52	P	1989	ML	
Gloria	CV53				
Goldrush	CV54	P&T	1992	MS	DT
Green Mountain	CV55	T	1885	L	
Hampton	CV56				
Hertha	CV57				
Hilite Russet	CV58	P&T	1987	E	DS
Hudson	CV59	T	1972	L	
Hulda	CV60				
Idole	CV61				
Irish Cobbler	CV62	T		E	W
Islander	CV63				
Jemseg	CV64		1978	VE	
Junior	CV65				
Kanona	CV66	P	1988	MS	
Katahdin	CV67	T	1932	L	DT, W
Kennebec	CV68	ES&P&T	1948	MS	DT, W
Keswick	CV69	T	1951	MS	
LaChipper	CV70	P&T	1962	MS	

LaRouge	CV71	T	1962	MS-ML	
Lenape	CV72				
Lesita	CV73				
Lily	CV74				
Mainechip	CV75	P	1991	MS	
Marfona	CV76				
Matilda	CV77				
Mirton Pearl	CV78	T	1975	EMS	
MN 12567	CV79				
MN 9632	CV80				
Monona	CV81	P	1964	MS	
Mouraska	CV82	P&T	1990	EMS	
ND 860-2	CV83				
Nemarus	CV84				
New Red Norland	CV85				
Norchip	CV86	P	1968	MS	DS
NorQueen	CV87	P&T	1993	MS	DT
NY 73	CV88				
Ocenia	CV89				
Ofelia	CV90				
Onaway	CV91	T	1956	E	DT
Premiere	CV92				
Prior	CV93				
Radosa	CV94				
Red Gold	CV95	ES & T	1987	EMS	

Red LaSoda	CV96	T	1952	MS	DT
Red Pontiac	CV97	ES	1954	MS	DT, W
Redsen	CV98	T	1983	ML	
Rideau	CV99	ES&T	1979	L	
Rhinered	CV100				
Ropta I-1234	CV101				
Ropta F815	CV102				
Ropta J418	CV103				
Rose Gold	CV104	ES&T	1987	ML	
Russet	CV105				
Russet Burbank	CV106	P&T	1874	VL	
Russet Norkotah	CV107	T	1987	MS	
Russet Nugget	CV108	P&T	1988	VL	DT
Rubinia	CV109				
Saginaw Gold	CV110	P	1987	ML	
Sante	CV111				
Sebago	CV112	SE	1938	VL	
Sierra	CV113	T	1986	L	
Snowden	CV114	P	1990	L	
Somerset	CV115				
Spartan Pearl	CV116				
Spunta	CV117				
Suncrisp	CV118				
Sunrise	CV119	T	1984	ME	
Superior	CV120	P&T	1961	EMS	

Tejon	CV121				
Tobique	CV122	P&T	1976	E	
Tolaas	CV123	P&T	1984	MS	
Trent	CV124	P&T	1978	MS	
Ulster Sceptre	CV125				
Ute Russet	CV126	T	1986	VL	
Viking	CV127	T	1963	MS	DT
Vital	CV128				
WF31-4	CV129				
Yankee Chipper	CV130	P&T	1983	ML	
Yukon Gold	CV131	T	1980	MS	

Appendix 1 - B List of the 131 European and N. American cultivars, their salt tolerance based on cluster analysis (CA), their parentage and origin [T:tolerant, S:sensitive].

Cv	CA	Parentage	Origin
Acadia Russet	T	Bake King x Norgold Russet	Ag. and Agrifood Canada, NB
AC Brador	S	F61101 x F60034	Ag. and Agrifood Canada, NB
AC Domino	S	Blue Mac x S.V.P. AM66-42	Ag. and Agrifood Canada, Nfld
Adora *2	S		
Agria *2	S		
Allegany	S	M297-17 x bulk of golden nematode susc. clones	Cornell
Amanda *4	S		
Aminca *2	S		
Amisk	T	A66102-13 x Targhee	USDA
Annika *4	S		
Anosta *1	S		
Atlantic	T	Wauseon x B5141-6	USDA, Maine, Florida, and New Jersey
Ausonia *1	S		
Avanti *1	S		
Belleisle	T	834C(29) x F47024	Ag. and Agrifood Canada, NB
Belmont	S		
BelRus	S	Penobscot x W39-1	USDA, Florida and Maine

Bintje *2	T	Munstersen x Fransen	K.L. de Vries, The Netherlands
Blue Mac	S	Arran Victory x And 5-142	Ag. and Agrifood Canada, Nfld
Campbell-13	S	Wauseon x B5042-2	Campbell Institute
Cardinal *5	S		
Caribe	S	F55066 x USDA 96-56	Ag. and Agrifood Canada, NB
Castile	S	Peconic x F107-30	USDA
Chaleur	S		
Cherokee	S	USDA X 96-56x USDAX528-170	Iowa and USDA
Chieftan	S	la1027-18 x La1354	USDA
Chipeta	T	WNC 612-13 X Wischip	USDA
Coastal Chip	T	Wauseon x B5141-6	USDA
Coastal Russet	S	Russet Burbank x B8281-5	USDA
Conestoga	S	G7063 x G6652	Ag. and Agrifood Canada, Univ. Guelph
Concorde *1	S		
Cupids	S	N150-3 x Wauseon	Ag. and Agrifood Canada, Nfld
D195-24	S		
Dark Red Norland	S		
Delcora *2	S		
Delta Gold	S	USDA S45208 x Earlaine	USDA and Maine
Denali	S	B5141-6 x AKI-62-90-64	USDA and Alaska
Diamant *5	S		
Disco *5	S		

Donna	S	Raritan x Agitato	Ag. and Agrifood Canada, NB
Draga *2	S		
Dundrod *3	S		
Dundrum**	S		
Estima *1	S		
Eide Russet	T	WC325-1 x Norgold Russet	Minnesota
Erntestolz *2	T		
Fontenot	S	La42-38 selfed	
F83065	S		
Foreston Russet Burbank	S		
Frontier Russet	S	A66102-16 x WN330-1	USDA
Fundy	S	Keswick x USDAx96-56	Ag. and Agrifood Canada, NB
Gemchip	S	BR5960-9 XND5737-3	USDA
Gloria *2	S		
Goldrush	S	Lemhi Russet X ND450-3 Russ	N. Dakotah
Green Mountain	T	Dunmore x Excelsior	Vermont
Hampton	S	NY48 x NY51	New York
Hertha	S		
Hilite Russet	S	Mixture from Butte Field	N.W. Potato Sales, Inc.
Hudson	S	NIF-1X 56N18-4	New York
Hulda *4	S		
Idole *5	S		
Irish Cobbler	S	Mutant of Early Rose	Massachusetts

Islander	S	Chipbelle x Norchip	Maine and L.I. Research Lab
Jemseg	S	Sable x F55069	Ag. and Agrifood Canada, NB
Junior *5	T		
Kanona	S	Peconic x bulk of golden nematode susc. clones	Cornell
Katahdin	S	USDA 40568 x USDA 24642	USDA
Kennebec	S	B127xUSDA 96-56	USDA & Maine
Keswick	S	F1020-1 x Green Mountain	Ag. and Agrifood Canada, NB
LaChipper	S	Green Mountain x Cayuga	Louisiana
LaRouge	S	'(LaSoda x Progress) Self'	Louisiana
Lenape	S	Delta Gold(47156) x B3672-3	USDA & Pennsylvania
Lesita *2	S		
Lily *4	S		
Mainechip	S	AF186-2x AF84-4	Maine
Marfona *1	S		
Matilda *4	S		
Mirton Pearl	S	Mira x F5318	Ag. and Agrifood Canada, Nfld
MN 12567	S		
MN 9632	S		
Monona	S	B1268-46 x B1299-15	Frito-Lay /USA
Mouraska **	S	Hudson x F59103	Agr. and Agrifood Canada,NB
ND 860-2	S		
Nemarus	S		USDA

New Red Norland	S		
Norchip	S	Nd4631-1 x M5009-2	N. Dakota
NorQueen **	T	Wash 330 X ND9567-2 Russ	N. Dakota
NY 73	S		
Ocenia	S	DT5997-1R x B5283-5	USDA, Florida, Virginia, New Jersey, and Maine
Ofelia *4	S		
Onaway	T	USDA96-56 XKatahdin	USDA
Premiere *1	S		
Prior *1	S		
Radosa *1	S		
Red Gold	S	G68211 X G6521-4RY	Ag. and Agrifood Canada, Univ. Guelph, O.M.A.F
Red LaSoda	S	Mutation of La Soda (Triumph x Katahdin)	Louisiana Agr. Exp. Station
Red Pontiac	S	Mutation of Pontiac (Triumph x Katahdin)	USDA
Redsen	S	ND8978-3R x ND9403-20R	N. Dakota
Rideau	S	Viking x P177-13R	Ag. and Agrifood Canada, Univ. Guelph, O.M.A.F
Rhinered	T	Norchief x W639	Wisconsin
Ropta I-1234	S		
Ropta F815	S		
Ropta J418	S		
Rose Gold	S	Abanki x G6521-4RY	Ag. Canada, Univ. Guelph, and O.M.A.F.

Russet	S	B5141-6 x W245-2	USDA et al.
Russet Burbank	S	A mutant of Burbank (seed ball from Early Rose)	
Russet Norkotah	T	ND9526-4 Russ x ND9687-5 Russ	Luther Burbank
Russet Nugget	S	Krantz x AND71609-1 Ru	Corolado and Texas Agr. Exp. Stations
Rubinia *2	S		
Saginaw Gold	T	MS 321-38 cx MS 709	Michigan
Sante *1	S		
Sebago	S	Chippewa x Katahdin	USDA
Sierra	T	A66110-39 x Targhee	USDA
Snowden	S	B5141-6 x Wischip	Wisconsin
Somerset	S		
Spartan Pearl	T		
Spunta *2	S		
Suncrisp	S		
Sunrise	S	Wauseon x B6563-2	Maine
Superior	S	B96-56 x M59.44	Wisconsin
Tejon	S		
Tobique	T	F45019 x Cariboo	Ag. and Agrifood Canada, NB
Tolaas	S	Neb.16.55 -1 x MN1106.64-1	Minnesota
Trent	T	B5141-6 x Nordak	Ag. and Agrifood Canada, Univ. Guelph, O.M.A.F.
Ulster Sceptre	S		
Ute Russet	S	W12-3 x Nooksack	USDA

Viking	S	Redskin x Nordak	N. Dakota
Vital *2	S		
WF31-4	S		
Yankee Chipper	S	B6987-148 x BR6864-8	Maine
Yukon Gold	S	W5279-4 x Norgleam	Ag. and Agrifood Canada, Univ. Guelph, O.M.A.F

*1 'AGRICO Holland'.
*2 'Hettema Zonen'.
*3 'Seed Potato Promotions (N.I.)Limitted'.
*4 'Svalof Weibull Seed Ltd'.
*5 'Wolf and Wolf'.

Appendix 2 - List of *S. chacoense* Bitt. accessions (PI) from Argentina, available form (AVL), site of collection (STAT), and reputed tolerance for abiotic stresses, compiled from (Bamberg et al., 1986; Bamberg et al., 1994; Bamberg and Martin, 1993; Hanneman and Bamberg, 1986. CH = *S. chacoense*, Hyb S = intraspecific hybrid seed, NA = not available, OP S = open-pollinated seed, PI = plant introduction number used in the US plant germplasm repository, as the primary identifier, R = resistant, Sf S = self seed, S = sensitive, Sib S = sib seed.

PI	AVL	STAT	FROST HEAT &	DROUGHT	SYMB	
133663	Hyb S	NA	NA	NA	CH1	**
175402	Hyb S	NA	NA	S	CH2	**
197760	OP S	NA	NA	NA	CH3	**
201846	Hyb S	NA	S	NA	CH4	**
209411	Sf S	NA	NA	NA	CH5	**
275138	Sib S	Tucuman	NA	NA	CH6	
275139	Sib S	Salta	NA	NA	CH7	**
320282	Sib S	San Lois	S	R	CH8	
320283	Sib S	San Lois	S	R	CH9	**
320285	Sib S	Cordoba	S	R	CH10	**
320289	Sib S	Tucuman	S	R	CH11	**
320290	Sib S	Salta	S	R	CH12	**
458309	Sib S	Jujuy	NA	NA	CH13	**

Appendix 3 - List of the simple diploid hybrids, and their tetraploid complex hybrid progenies [personal communication Dr. H. DE Jong, 1993).

A. The hybrids:

	Clone	SYM	Crossed parents
1	9787-04	PA1	*S. chacoense* x *S. tuberosum*
	9787-07	PA2	
	9787-01	PA3	
	9507-04	PA4	
	9507-05	PA5	
2	8661-02	PB1	*S. phureja/S. stenotomum* x *S. tuberosum*
	BPH32-03	PB2	
	9751-03	PB3	
	9901-01	PB4	
	10304-01	PB5	
	9479-05	PB6	
3	9126-01	PC	*S. tuberosum* x *S. tuberosum*
4	9113-11	PD1	*S. phureja /S. stenotomum*
	9595-03	PD2	

B. The Progenies:

	SYM	CROSSED PARENTS
1. 9113-11 x 9787-07	HB1	PD1 x PA2
2. 9479-05 x 9787-07	HB2	PB6 x PA2
3. 9507-04 x BPH 32-	HB3	PA4 x PB2
4. 9507-04 x 8661-02	HB4	PA4 x PB1
5. 9507-05 x 8661-02	HB5	PA5 x PB1
6. 9751-03 x 9787-07	HB6	PB3 x PA2
7. 9787-01 x 9126-01	HB7	PA3 x PC
8. 9787-04 x BPH 32-	HB8	PA1 x PB2
9. 9787-04 x 8661-02	HB9	PA1 x PB1
10. 9787-07 x 9126-01	HB10	PA2 x PC
11. 9787-07 x 9495-04	HB11	PA2 x PD2
12. 9787-07 x 9901-01	HB12	PA2 x PB4
13. 10304-01 x 9787-	HB13	PB5 x PA2

** Screened in both the NCB and SB.

Appendix 4 - A. The nodal cutting bioassay procedure, and B. the culture conditions for the seedling bioassay and the nodal cutting bioassay:

A. The nodal cutting bioassay utilized in this study was described by Zhang et al. (1993). Single node cuttings 1 cm in length were obtained from *in vitro* micropropagated plantlets. Each cutting consisted of one leaf and an axillary bud. All nodal positions were used, except the apical and basal nodes. One nodal cutting was cultured per 25 x 150 mm glass test tube, containing 15 ml of medium (Appendix 5). The nodal cuttings were grown under the conditions described below, NaCl concentrations of 0, 40 ,80, and 120 mM were tested. Each NaCl level had five replicates (nodal cuttings)/genotype/trial. The treatments were distributed in a completely randomized design.

B. Culture conditions for the SB and the NCB 16/8 hr light /dark period, 40 μMOL m^{-2} s^{-1} photon flux density (cool white fluorescent light), 23-25 ± 2°C.

Appendix 5 - The composition of Murashige and Skoog Basal Medium (1962) utilised in the NCB, SB, and for micropropagation. SS = stock solution.

SS	Constituent	Concen. SS (gl^{-1})	Volume of SS in medium (ml)	Concen. medium (ml)
A	NH_4NO_3	82.50	20	1650.00
B	KNO_3	95.00	20	1900.00
C	H_3BO_3	1.24	5	6.20
	KH_2PO4	34.00		170.00
	KI	0.16		0.83
	$NA_2MoO_4.2H2O$	0.05		0.25
	$CoCL_2.6H2O$	0.01		0.02
D	$CaCl_2$. 2H2O	88.00	5	440
E	$MgSO_4.7H2O$	74.00	5	370.00
	$MnSO_4$. 4H2O	4.46		22.30
	$ZnSO_4$. 7H2O	1.72		8.60
	$CuSO_4.5H2O$	5.00		0.02
F	Na_2.EDTA	7.45	5	37.35
	$FeSO_4.7H2O$	5.57		27.85
G	thiamine.HCl*	0.20	5	1.00
	nicotinic acid	0.10		0.50
	pyrodoxine. HCl	0.10		0.50
	glycine	0.40		2.00

* The MS (1962) concentration of thiamine.HCl was raised to 1.0 mgl^{-1} as suggested by Linsmair and Skoog (1965). One litre of the MS basal medium contains the assigned volumes (20, or 5 ml) of the stock solution, sucrose (30 gl^{-1}), myo-inositol (100 mgl^{-1}), and Ca pantothenate (2 mg l^{-1}), dissolved in double distilled water. After pH adjustment to 5.8 Anachaemia agar (7 gl^{-1}) was added. The medium was autoclaved at 121°C for 20 min.

Appendix 6 - Mean squares for salt tolerance sources of variation for 131 potato cvs. and 4 NaCl levels (0, 40, 80, and 120 mM).

a. Dependent variable SL

Source	DF	Mean Square	F Value
G	130	14.51	8.09 ***
CNC	3	1971.28	1099.82 ***
TIME	6	20.40	11.38 ***
G*CNC	523	17.12	9.48 ***
G*TIME	124	2.28	1.27 ***
CNC*TIME	18	17.94	10.01 ***

R-Square	C.V.
0.86	40.61

b. Dependent variable SFW

Source	DF	Mean Square	F Value
G	130	42437.42	9.25 ***
CNC	3	1983766.27	432.27 ***
TIME	6	25224.49	5.50 ***
G*CNC	523	27786.61	8.72 ***
G*TIME	124	5830.61	1.27 *
CNC*TIME	18	19180.35	4.18 ***

R-Square	C.V.
0.90	46.68

c. Dependent variable SDW

Source	DF	Mean Square	F Value
G	130	210.10	5.28 ***
CNC	3	10345.87	260.04 ***
TIME	6	323.90	8.14 ***
G*CNC	523	154.20	3.69 ***
G*TIME	124	65.43	1.64 ***
CNC*TIME	18	199.17	5.01 ***

R-Square	C.V.
0.70	50.28

d. Dependent variable RL

Source	DF	Mean Square	F Value
G	130	20.84	4.06 ***
CNC	3	3291.29	640.84 ***
TIME	6	8.37	1.63 NS
G*CNC	523	29.84	10.12 ***
G*TIME	124	3.95	0.77 NS
CNC*TIME	18	7.28	1.42 NS

R-Square	C.V.
0.77	42.15

e. Dependent variable RFW

Source	DF	Mean Square	F Value
G	130	4911.34	4.47 ***
CNC	3	633806.03	577.20 ***
TIME	6	23632.41	21.52 ***
G*CNC	523	154.20	3.69 ***
G*TIME	124	1177.95	1.07 NS
CNC*TIME	18	4729.55	4.31 ***

R-Square	C.V.
0.77	43.89

f. Dependent variable RDW

Source	DF	Mean Square	F Value
G	130	20.11	2.65 ***
CNC	3	2216.40	292.27 ***
TIME	6	33.32	4.39 ***
G*CNC	523	1578.96	277.72 ***
G*TIME	124	7.08	0.93 NS
CNC*TIME	18	22.91	3.02 ***

R-Square	C.V.
0.75	44.40

Appendix 7 - Mean squares for salt tolerance sources of variation for 32 *Solanum* spp. genotypes and 4 NaCl levels (0, 40, 80, and 120 mM).

a. Dependent variable SL

Source	DF	Mean Square	F Value
G	32	27.30	49.34 ***
CNC	3	461.73	834.31 ***
TIME	1	12.62	22.80 ***
G*CNC	96	4.88	8.81 ***
CNC*TIME	3	4.00	7.22 ***
G*TIME	32	1.45	2.61 ***

R-Square	C.V.
0.98	20.65

b. Dependent variable SFW

Source	DF	Mean Square	F Value
G	32	43572.84	42.98 ***
CNC	3	395592.36	390.10 ***
TIME	1	20517.41	20.24 ***
G*CNC	96	11223.73	11.07 ***
CNC*TIME	3	6528.76	6.44 ***
G*TIME	32	1575.63	1.55 NS

R-Square	C.V.
0.97	30.03

c. Dependent variable SDW

Source	DF	Mean Square	F Value
G	32	491.99	95.44 ***
CNC	3	2189.89	424.79 ***
TIME	1	110.44	21.42 ***
G*CNC	96	171.87	33.34 ***
CNC*TIME	3	46.53	9.03 ***
G*TIME	32	9.40	1.82 *

R-Square	C.V.
0.98	24.10

d. Dependent variable RL

Source	DF	Mean Square	F Value
G	32	18.89	16.01 ***
CNC	3	336.62	285.24 ***
TIME	1	6.59	5.58 *
G*CNC	96	4.15	3.52 ***
CNC*TIME	3	3.04	2.58 NS
G*TIME	32	1.70	1.44 NS

R-Square	C.V.
0.95	7.13

e. Dependent variable RFW

Source	DF	Mean Square	F Value
G	32	2358.98	18.97 ***
CNC	3	53477.00	429.97 ***
TIME	1	3534.13	28.42 ***
G*CNC	96	615.20	4.95 ***
CNC*TIME	3	882.42	7.09 ***
G*TIME	32	250.57	2.01 **

R-Square	C.V.
0.96	36.50

f. Dependent variable RDW:

Source	DF	Mean Square	F Value
G	32	32.03	5.33 ***
CNC	3	367.19	61.09 ***
TIME	1	3.35	0.56 NS
G*CNC	96	14.09	2.34 ***
CNC*TIME	3	1.83	0.3 NS
G*TIME	32	6.7	1.12 NS

R-Square	C.V.
0.865	60.18

Appendix 8- Mean squares for salt tolerance sources of variation for 13 accessions of *Solanum chacoense* and 4 salt levels (0, 40, 80, and 120 mM).

a. Dependent variable SL

Source	DF	Mean Square	F Value
G	10	0.21	6.09 ***
CNC	3	2.60	75.26 ***
TIME	1	0.05	1.30 NS
G*CNC	30	0.06	1.76 NS
CNC*TIME	3	0.06	1.81 NS
G*TIME	10	0.07	1.92 NS

R-Square	C.V.
0.92	12.88

b. Dependent variable RL

Source	DF	Mean Square	F Value
G	10	1.35	2.81 *
CNC	3	7.27	15.13 ***
TIME	1	0.086	0.18 NS
G*CNC	30	0.65	1.36 NS
CNC*TIME	3	0.14	0.28 NS
G*TIME	10	0.63	1.3 NS

R-Square	C.V.
0.81	24.43

c. Dependent variable TFW

Source	DF	Mean Square	F Value
G	10	767.93	2.47 *
CNC	3	4835.10	15.54 ***
TIME	1	204.53	0.66 NS
G*CNC	30	472.95	1.52 NS
CNC*TIME	3	1029.51	3.31 NS
G*TIME	10	215.45	0.69 NS

R-Square	C.V.
0.82	61.10

d. Dependent variable TDW

Source	DF	Mean Square	F Value
G	10	7.56	4.86 ***
CNC	3	22.45	14.40 ***
TIME	1	7.34	4.72 *
G*CNC	30	2.34	1.50 NS
CNC*TIME	3	1.18	0.76 NS
G*TIME	10	6.42	4.13 **

R-Square	C.V.
0.861	28.8

D. Dependent variable G% (untransformed data)

Source	DF	Mean Square	F Value
G	12	0.00008001	9.29 ***
CNC	3	0.0004816	55.93 ***
TIME	1	0.00003483	4.05 NS
G*CNC	36	0.00001306	1.52 NS
CNC*TIME	3	0.00001889	2.19 NS
G*TIME	12	0.00000749	0.87 NS

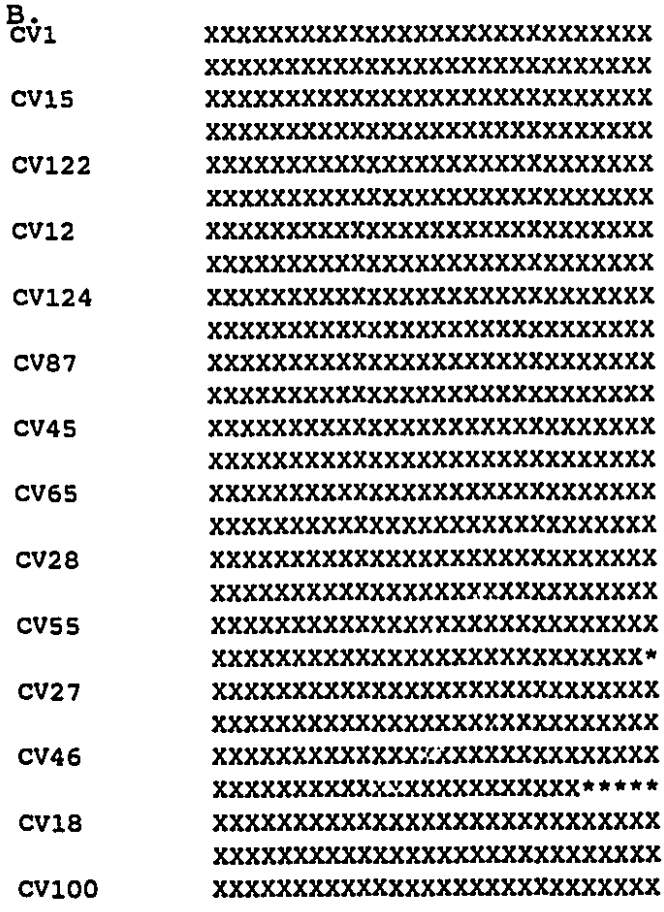
R-Square	C.V.
0.91	37.91

Appendix 9 - CA of 131 potato cvs. using Ward's Minimum Cluster Analysis. A. The drop of the pseudo t^2 statistic(t**2), the rise of the pseudo F statistic (F) and the cubic clustering criterion (CCC), divided the cvs. into two clusters (NCL). CA was performed on mean SL, SFW, SDW, RL, RFW, and RDW for each genotype across the salt levels 40, 80, and 120 mM NaCl. B. The dendrogram.

A.

NCL	RSQ	CCC	F	t**2
4	0.78	24.91	66.40	16.00
3	0.76	17.99	56.20	71.60
2	**0.74**	**13.61**	**53.30**	**52.10**
1	0.00	0.00	.	53.30

B.



```
                XXXXXXXXXXXXXXXXXXXXXXXXXXX
CV110           XXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXX
CV107           XXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXX*
CV9             XXXXXXXXXXXXXXXXXXXXXXXXXXX.
                XXXXXXXXXXXXXXXXXXXXXXXXXXX*
CV113           XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV91            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXX***
CV116           XXXXXXXXXXXXXXXXXXXXXXXXXXX...
                X****************************
CV10            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV112           XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV33            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV123           XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV47            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV74            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX*
CV103           XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV5             XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV32            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV54            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX*
CV11            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV53            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV56            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV71            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV111           XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV90            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV60            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV62            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX*
CV117           XXXXXXXXXXXXXXXXXXXXXXXXXXXX.
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                XXXXXXXXXXXXXXXXXXXXXXXXXXXXXX**
CV120           XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV126           XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV26            XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXX******
CV101           XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV73            XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV13            XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV68            XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV114           XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV67            XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV129           XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV38            XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV115           XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV98            XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV121           XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXXXX*
CV104           XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV70            XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV78            XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV128           XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV43            XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV57            XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV99            XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXXXX*
CV108           XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV52            XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV125           XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV2             XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
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```
                XXXXXXXXXXXXXXXXXXXXXXXXX***
CV109           XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV36            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV80            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV40            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX*
CV17            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV30            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV83            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX*
CV119           XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV31            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV63            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV69            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV76            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX**
CV118           XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV77            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV35            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV89            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV92            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV88            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV19            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV61            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV97            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV75            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX*
CV127           XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV64            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV7             XXXXXXXXXXXXXXXXXXXXXXXXXXXXy
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                 XXXXXXXXXXXXXXXXXX**********
CV102            XXXXXXXXXXXXXXXXXXXXXXXXXXX
                 XXXXXXXXXXXXXXXXXXXXXXXXXXX
CV44             XXXXXXXXXXXXXXXXXXXXXXXXXXX
                 XXXXXXXXXXXXXXXXXXXXXXXXXXX
CV50             XXXXXXXXXXXXXXXXXXXXXXXXXXX
                 XXXXXXXXXXXXXXXXXXXXXXXXXXX
CV20             XXXXXXXXXXXXXXXXXXXXXXXXXXX
                 XXXXXXXXXXXXXXXXXXXXXXXXXXX
CV41             XXXXXXXXXXXXXXXXXXXXXXXXXXX
                 XXXXXXXXXXXXXXXXXXXXXXXXXXX
CV8              XXXXXXXXXXXXXXXXXXXXXXXXXXX
                 XXXXXXXXXXXXXXXXXXXXXXXXXXX*
CV105            XXXXXXXXXXXXXXXXXXXXXXXXXXX
                 XXXXXXXXXXXXXXXXXXXXXXXXXXX
CV130            XXXXXXXXXXXXXXXXXXXXXXXXXXX
                 XXXXXXXXXXXXXXXXXXXXXXXXXXX
CV34             XXXXXXXXXXXXXXXXXXXXXXXXXXX
                 XXXXXXXXXXXXXXXXXXXXXXXXXXX
CV24             XXXXXXXXXXXXXXXXXXXXXXXXXXX
                 XXXXXXXXXXXXXXXXXXXXXXXXXXX
CV93             XXXXXXXXXXXXXXXXXXXXXXXXXXX
                 XXXXXXXXXXXXXXXXXXXXXXXXXXX
CV79             XXXXXXXXXXXXXXXXXXXXXXXXXXX
                 XXXXXXXXXXXXXXXXXXXXXXXXXXX
CV85             XXXXXXXXXXXXXXXXXXXXXXXXXXX
                 XXXXXXXXXXXXXXXXXXXXXXXXXXX
CV58             XXXXXXXXXXXXXXXXXXXXXXXXXXX
                 XXXXXXXXXXXXXXXXXXXXXXXXXXX
CV72             XXXXXXXXXXXXXXXXXXXXXXXXXXX
                 XXXXXXXXXXXXXXXXXXXXXXXXXXX
CV106            XXXXXXXXXXXXXXXXXXXXXXXXXXX
                 XXXXXXXXXXXXXXXXXXXXXXXXXXX
CV49             XXXXXXXXXXXXXXXXXXXXXXXXXXX
                 XXXXXXXXXXXXXXXXXXXXXXXXXXX
CV6              XXXXXXXXXXXXXXXXXXXXXXXXXXX
                 XXXXXXXXXXXXXXXXXXXXXXXXXXX
CV86             XXXXXXXXXXXXXXXXXXXXXXXXXXX
                 XXXXXXXXXXXXXXXXXXXXXXXXXXX***
CV131            XXXXXXXXXXXXXXXXXXXXXXXXXXX
                 XXXXXXXXXXXXXXXXXXXXXXXXXXX
CV22             XXXXXXXXXXXXXXXXXXXXXXXXXXX
                 XXXXXXXXXXXXXXXXXXXXXXXXXXX
CV3              XXXXXXXXXXXXXXXXXXXXXXXXXXX
                 XXXXXXXXXXXXXXXXXXXXXXXXXXX
CV16             XXXXXXXXXXXXXXXXXXXXXXXXXXX
                 XXXXXXXXXXXXXXXXXXXXXXXXXXX
CV42             XXXXXXXXXXXXXXXXXXXXXXXXXXX
                 XXXXXXXXXXXXXXXXXXXXXXXXXXX
CV48             XXXXXXXXXXXXXXXXXXXXXXXXXXX
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                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV39            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV95            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV4             XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV29            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV37            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV51            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV23            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX*
CV14            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV94            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV66            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV84            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV81            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV21            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV25            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV96            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXXXXXXXXXXXXXXXXXXX
CV82            XXXXXXXXXXXXXXXXXXXXXXXXXXXX
                XXXXXXXXXXX*****************
CV59            XXXXXXXXXXX.................
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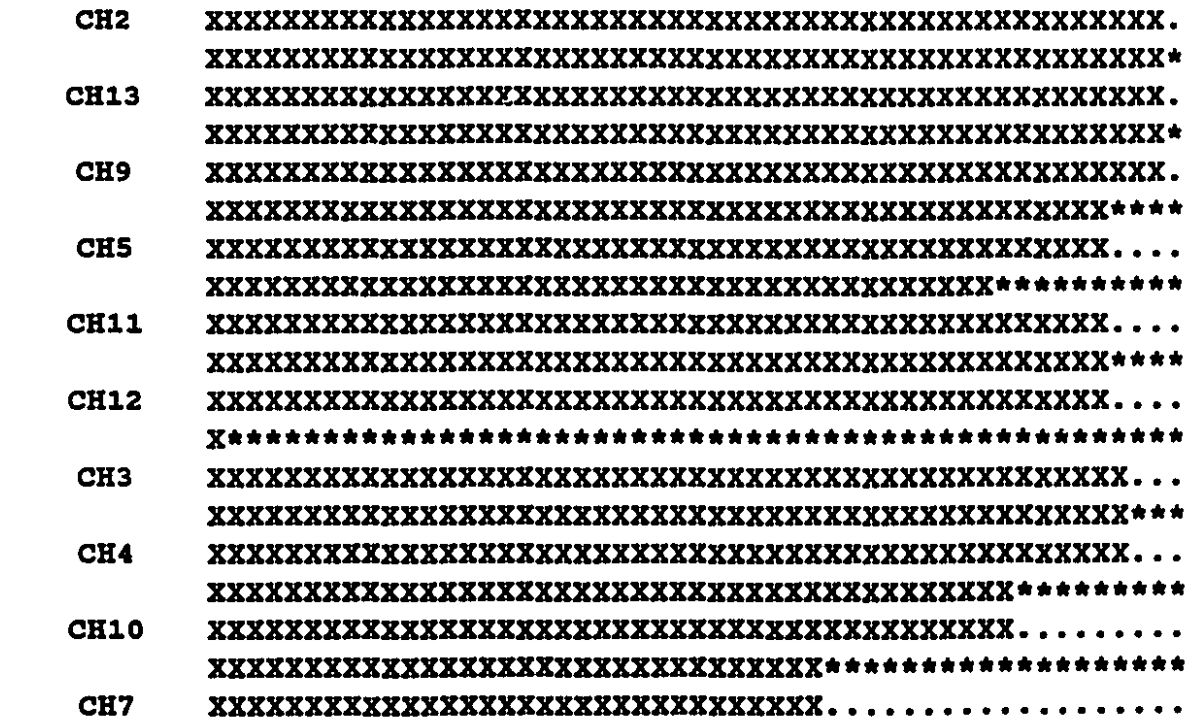
Appendix 10 - CA of 11 *S. chacoense* accessions using Ward's Minimum Cluster Analysis. A. The drop of the pseudo t^2 statistic (t**2), the rise of the pseudo F statistic (F) and the cubic clustering criterion (CCC), divided the accessionss into two clusters (NCL). CA was performed on mean SL, SFW, SDW, RL, RFW, and RDW for each genotype, across the salt levels 40, 80, and 120 mM NaCl. B) The dendrogram

A)

NCL	RSQ	CCC	F	t**2
4	0.92	.	7.10	3.50
3	0.87	.	7.60	3.90
2	0.78	2.09	7.90	3.10
1	0.00	0.00	.	7.90

B)

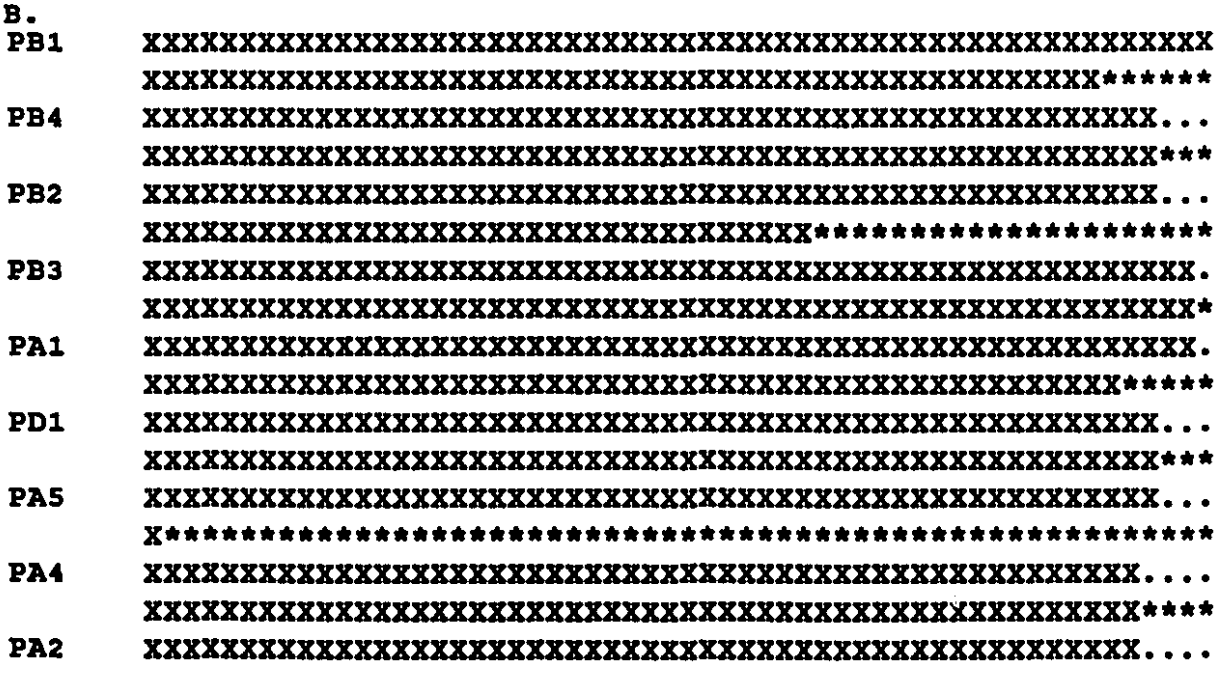


Appendix 11- CA of 9 hybrid clones of *S. tuberosum* crossed with either *S. chacoense*, *S. phureja* and/or *S. stenotomum*, and 1 accession of the primitive cultivated potatoes *S. phureja* and/or *S. stenotomum* using Ward's Minimum Cluster Analysis. A. The drop of the pseudo t^2 statistic (t2), the rise of the pseudo F statistic (F), and the cubic clustering criterion (CCC), divided the genotypes into three clusters (NCL). CA was performed on mean SL, SFW, SDW, RL, RFW, and RDW for each genotype across the salt levels 40, 80, and 120 mM NaCl B. The dendrogram**

A.

NCL	RSQ	CCC	F	T**2
4	0.82	.	9.30	2.20
3	0.76	3.50	11.20	3.30
2	0.55	3.02	9.90	6.30
1	0.00	0.00	.	9.90

B.

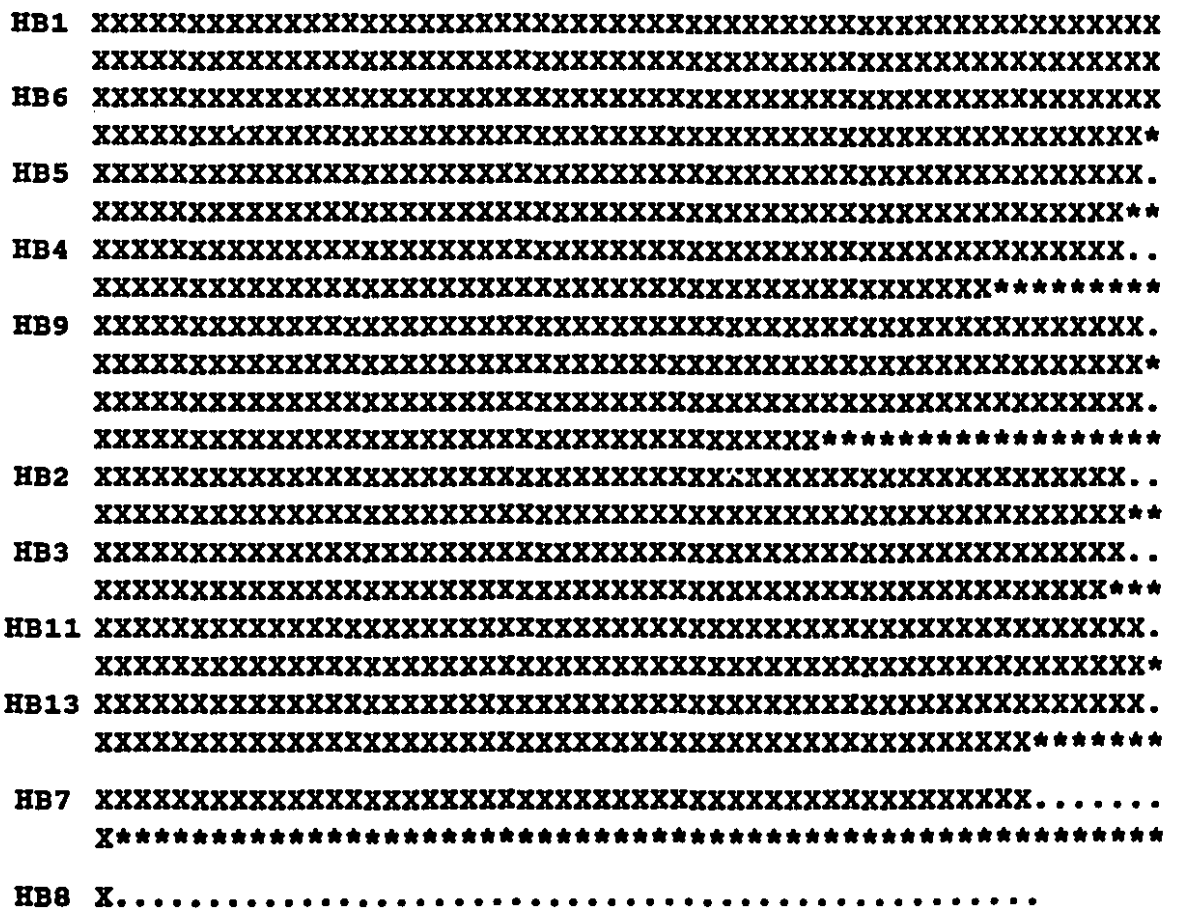


Appendix 12 - CA of 12 progenies using Ward's Minimum Cluster Analysis. A. The drop of the pseudo t^2 statistic (t**2), the rise of the pseudo F statistic (F), and the cubic clustering criterion (CCC), divided the progenies into two clusters (NCL). CA was performed on mean SL, SFW, SDW, RL, RFW, and RDW for each genotype across the salt levels 40, 80, and 120 mM NaCl. B. The dendrogram.

A.

NCL	RSQ	CCC	F	T**2
4	0.90	.	12.40	3.50
3	0.82	.	12.40	8.70
2	0.73	4.47	12.40	6.10
1	0.00	0.00	.	12.40

B.

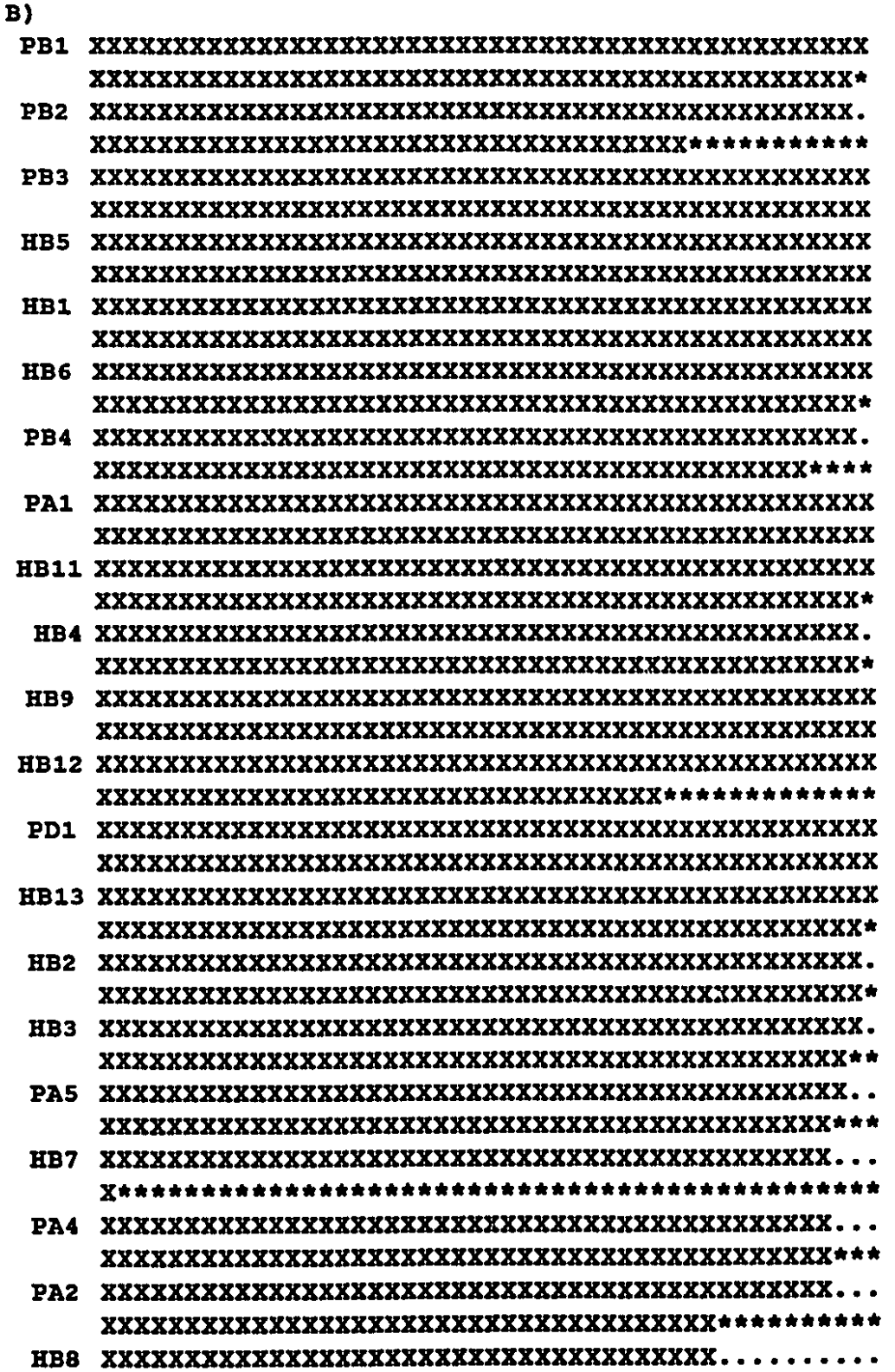


Appendix 13 - CA of 9 hybrid clones of S. tuberosum crossed with either *S. chacoense*, *S. phureja* and/or *S. stenotomum*, and one accession of the primitive cultivated potatoes *S. phureja* and/or *S. stenotomum*, and 12 of their progenies, using Ward's Minimum Cluster Analysis. A. The drop of the pseudo t^2 statistic (t2), and the rise of the pseudo F statistic (F), and the cubic clustering criterion (CCC), divided the genotypes into two clusters (NCL). CA was performed on mean SL, SFW, SDW, RL, RFW, and RDW for each genotype, across the salt levels 40, 80, and 120 mM NaCl. B. The dendrogram**

A)

NCL	RSQ	CCC	F	t**2
4	0.8	7.5	14.2	3.1
3	0.8	6.8	13.6	13.3
2	0.7	7.6	16.9	7.9
1	0.5	0.0	.	16.9

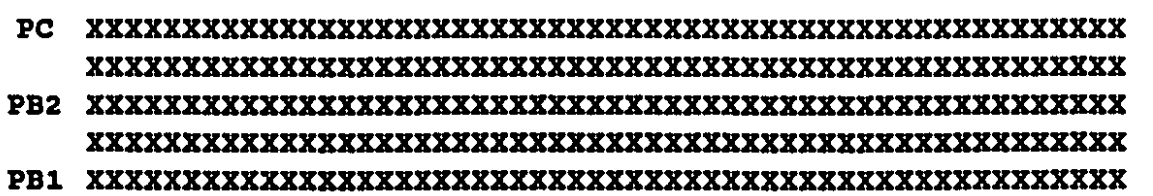
B)

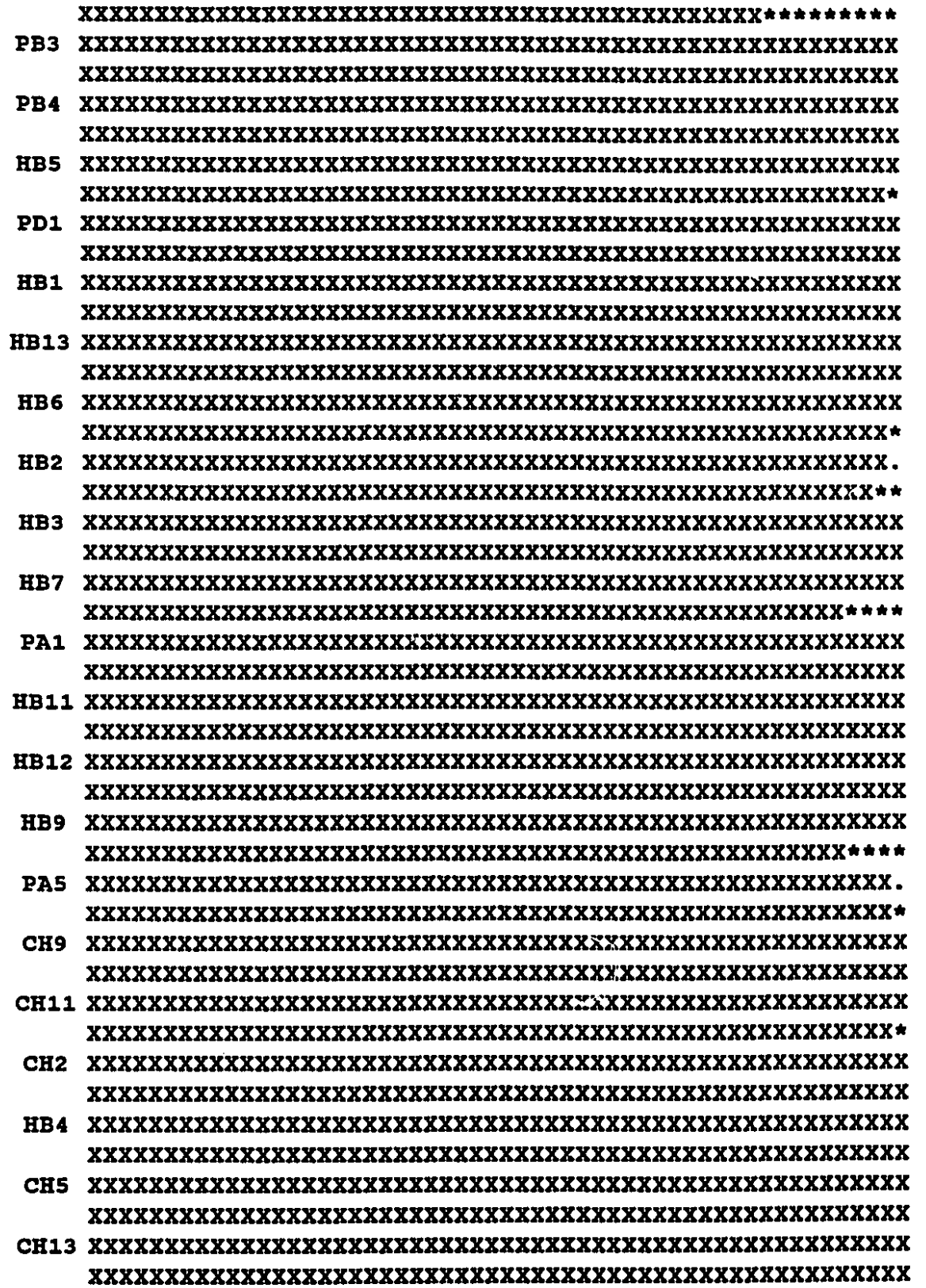

PB1
PB2
PB3
HB5
HB1
HB6
PB4
PA1
HB11
HB4
HB9
HB12
PD1
HB13
HB2
HB3
PA5
HB7
PA4
PA2
HB8

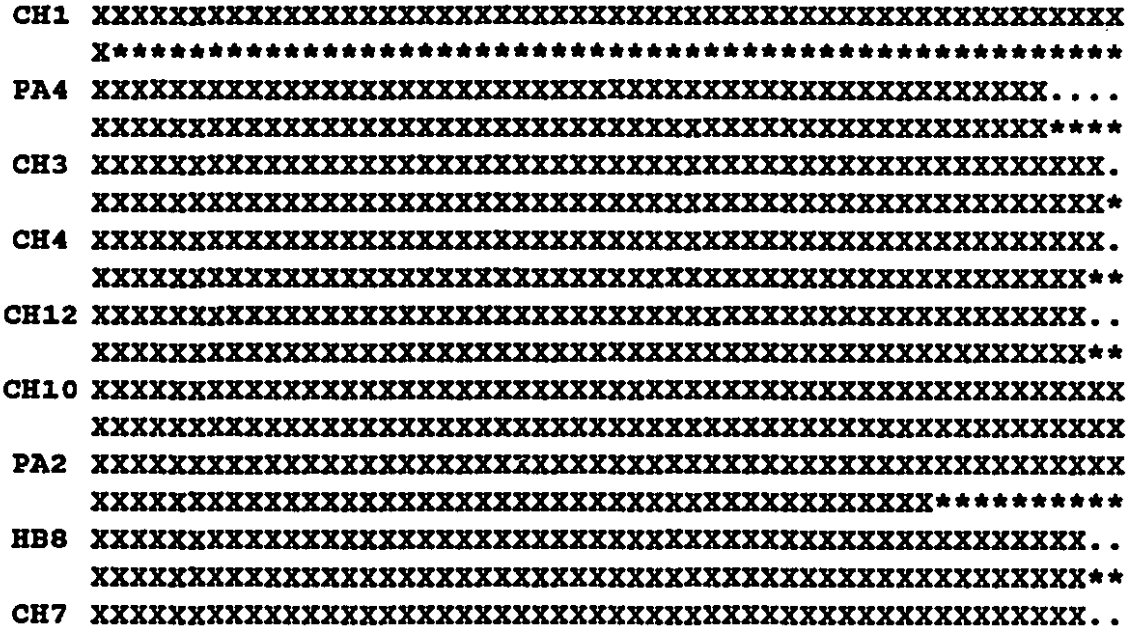
Appendix 14 - CA of 32 Solanum spp. genotypes using Ward's Minimum Cluster Analysis. A. The drop of the pseudo t^2 statistic (t**2), and the rise of the pseudo F statistic (F), and the cubic clustering criterion (CCC), divided the cvs. into two clusters (NCL). CA was performed on mean SL, SFW, SDW, RL, RFW, and RDW for each genotype across the salt levels 40, 80, and 120 mM NaCl. B. The dendrogram

A)

NCL	RSQ	CCC	F	T**2
4	0.81	14.57	25.40	3.70
3	0.77	14.31	26.00	12.90
2	0.72	17.26	35.50	5.30
1	0.00	0.00	.	35.50




PB3
PB4
HB5
PD1
HB1
HB13
HB6
HB2
HB3
HB7
PA1
HB11
HB12
HB9
PA5
CH9
CH11
CH2
HB4
CH5
CH13


CH1
PA4
CH3
CH4
CH12
CH10
PA2
HB8
CH7

Appendix 15 - CA of 11 *S. chacoense* accessions using Ward's Minimum Cluster Analysis. A) The drop of the pseudo t^2 statistic (t**2), and the rise of the pseudo F statistic (F), and the cubic clustering criterion (CCC), divided the cvs. into two clusters (NCL). CA was performed on mean SL, RL, TFW, and TDW for each genotype across the salt levels 40, 80, and 120 mM NaCl. B) The dendrogram

A)

NCL	RSQ	CCC	F	T**2
4	0.9	17.5	18	1.4
3	0.8	17.0	10.3	8.3
2	0.7	16.0	10.6	3.2
1	.	0.0	0.00	10.6

B)