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DEVELOPMENT OF IN VITRO BIOASSAYS FOR DETERMINATION OF SALINITY TOLERANCE IN POTATO (*SOLANUM* SPP.)

By

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

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May 1998

TO MY BELOVED FATHER AND MOTHER
ZHUTING AND WENSHEN
ZHANG, AND MY FAMILIES

DEVELOPMENT OF IN VITRO BIOASSAYS FOR DETERMINATION OF SALINITY TOLERANCE IN POTATO (*SOLANUM* SPP.)

Ph.D. Dissertation
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ABSTRACT

Salinity problems seriously affect agricultural production by reducing crop yield and arable land. The evaluation of potato genotypes (*Solanum* spp.) for their salinity (NaCl) tolerance in conventional field trials is time consuming and labour intensive. The results are often confounded by many field and environmental variations. In vitro bioassays can overcome some of these difficulties by providing faster, more convenient and dependable methods for screening and selection of salt tolerant potato genotypes. The objective of this research was to develop in vitro bioassay methods for screening and selection of salt tolerant potato. Under in vitro NaCl stress conditions, seed germination, early seedling growth, and single-node cutting bioassays were used to evaluate salinity tolerance. The selected genotypes were further tested with three in vitro bioassays (single-node cuttings, root tip segments, and microtuberization). The rankings of potato cultivar salinity tolerance were similar in these bioassays. The single-node cutting bioassay was recommended because it was simpler to perform than the root tip segment and microtuberization bioassays and did not exclude certain genotypes as did the microtuberization bioassay. The in vitro bioassay rankings were compared with yield ranking in field lysimeters. In both the in vitro and in vivo saline stress experiments, cvs. Kennebec and Russet Burbank were more salt tolerant than Norland. The tubers and microtubers harvested from previous experiments were tested in the greenhouse to investigate salinity carry-over effect for seed tuber production. There was no apparent residual carry-over effect found. Microtuber yield increase in the presence of low NaCl concentration was induced primarily by specific ion (Na^+), and not osmotic effects. This research clearly indicated that in vitro bioassays are relatively simple, rapid, convenient, repeatable, and agree with the field lysimeter results. They can be used to substitute for field trials to screen and select salt tolerant genotypes of potato. We recommend future field lysimeter trials to confirm in vitro bioassay results using a wider range of potato genotypes under a range of environmental conditions. We also advise future field evaluations in saline, arid areas for further confirmation of our in vitro bioassays.

DÉVELOPPEMENT D'ESSAIS *IN VITRO* POUR LA DÉTERMINATION DE LA TOLÉRANCE DE LA POMME DE TERRE (*SOLANUM* SPP.) À LA SALINITÉ

RÉSUMÉ

Des problèmes de salinité affectent sérieusement les productions agricoles en réduisant le rendement des cultures et les terres arables. L'évaluation des génotypes de pomme de terre (*Solanum* spp.) pour leur tolérance à la salinité (NaCl) dans des essais conventionnels en champ demande beaucoup de temps et de travail intensif. Les résultats sont souvent influencés par plusieurs variations environnementales. Des essais *in vitro* peuvent surmonter certaines de ces difficultés en fournissant des méthodes plus rapides, pratiques et sûres.

L'objectif de ce projet de recherche était donc de développer des méthodes d'essais *in vitro* pour le criblage et la sélection de génotypes de pomme de terre tolérants au sel. En conditions *in vitro*, la germination de semences de pomme de terre, la croissance précoce des semis et le bouturage de segment nodal unique ont été utilisés pour évaluer la tolérance des pommes de terre à la salinité. Les génotypes sélectionnés ont ensuite été testés dans trois essais *in vitro*, soit le bouturage de segment nodal unique, la segmentation des extrémités racinaires et la microtubérisation. Les classements des cultivars de pomme de terre face à la tolérance à la salinité ont été similaires lors de ces essais. Le bouturage de segment nodal unique a été recommandé parce qu'il était le plus simple à faire et qu'il n'excluait pas certains génotypes comme le faisait la microtubérisation. Les résultats de ces essais *in vitro* ont été comparés avec des résultats de mesure de lysimètre en champ. Dans les deux cas des expériences de stress de salinité *in vitro* et *in vivo*, les cultivars Kennebec et Russet Burbank ont été plus tolérants au sel que le cultivar Norland. Les tubercules et les microtubercules récoltés d'expériences antérieures ont été testés en serre pour étudier les effets de transmission de la salinité pour la production de semence de tubercule. Aucun effet de transmissibilité residuelle apparente n'a été trouvé.

Cette recherche a clairement indiqué que les essais *in vitro* sont relativement simples, pratiques et rapides. Ils peuvent être utilisés pour remplacer les essais en champ pour le criblage et la sélection de génotypes de pomme de terre tolérants au sel. Nous recommandons que de futurs essais de mesure de lysimètre en champ soient effectués pour confirmer les résultats des essais *in vitro* avec une plus grande variété de génotypes de pomme de terre sous différentes conditions environnementales. Nous recommandons aussi que de futurs essais en champs soient faits dans des conditions arides et de haute salinité pour plus de confirmations de nos essais *in vitro*.

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The following paragraph concerning the authorship of manuscripts is cited from Guidelines Concerning Thesis Preparation published by the Faculty of Graduate Studies and Research:

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Chapter 3 was published in the Proceedings of the 13th International Congress of Biometeorology, Calgary, AB, Canada (1994). The authors were Y. Zhang, M. Brault, V. Chalavi, and D.J. Donnelly (thesis supervisor). Ms. M. Brault (lab technician) and Ms. V. Chalavi (volunteer) initiated the project preparation under the supervision of Dr. D.J. Donnelly. Mr. Y. Zhang started research after Ms. M. Brault left. He did the lab experiment, data collection and analysis, and collaborated with Dr. D.J. Donnelly in the manuscript preparation.

Chapters 4 and 5 consist of a manuscript published in Potato Research (Zhang and Donnelly, 1997). The authors were Y. Zhang and D.J. Donnelly. Mr. Y. Zhang did the in vitro research, data collection and analysis, and field lysimeter trials with field help from Mr. R. Patel, a Ph.D candidate of the Agricultural and Biosystems Engineering Department, Mr. J. Abdulnour, a Ph.D candidate from the Natural Resource Sciences Department and Mr. D. Shateryan, a former graduate student in the Plant Science Department. He

collaborated with Dr. D.J. Donnelly in the manuscript preparation.

Chapter 6 consists of a manuscript in preparation for submission to HortScience. The authors were Y. Zhang, D.J. Donnelly and J. Abdulnour. Mr. Y. Zhang designed and managed the experiment and shared the greenhouse work with Mr. J. Abdulnour. Mr. Y. Zhang did the data analysis and collaborated with Mr. J. Abdulnour and Dr. D.J. Donnelly in the manuscript preparation.

Chapter 7 consists of a manuscript in preparation for submission to HortScience. The authors were Y. Zhang, J. Abdulnour, and D.J. Donnelly. Mr. Y. Zhang managed the experiment with the participation of Mr. J. Abdulnour. Mr. Y. Zhang did the data analysis and collaborated with Mr. J. Abdulnour and Dr. D.J. Donnelly in the manuscript preparation.

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LIST OF THE ABBREVIATIONS USED IN THE THESIS:

Abbreviation	Full description
approx.	approximately
BAP	6-benzylaminopurine
CA	cluster analysis
CCC	cubic clustering criterion
cm	centimetre
CORR	correlation
CRD	complete randomized design
cv.	cultivar
cvs.	cultivars
D/N	day/night
dSm^{-1}	decisiemens per metre
EC	electrical conductivity
FAO	Food and Agricultural Organization
Fig.	figure
g	gram
GA_3	gibberellic acid
$g\ l^{-1}$	gram per litre
GLM	general linear model
h	hour
ha	hectare
K	potassium
kg	kilogram
l	litre
LSD	least square difference
mg	milligram
mm	millimetre
mM	millimole
mo	month
MS	Murashige-Skoog medium (1962)
mTD	microtuber-derived plants
N	nitrogen
p	probability
P	phosphorus
R. Burbank	Russet Burbank
CRBD	complete randomized block design
RDW	root dry weight
RFW	root fresh weight
RL	root length
SAS	statistical analysis system

SDW	shoot dry weight
SFW	shoot fresh weight
SL	shoot length
SN	shoot number
SPT	specific pathogen tested
STD	seed tuber-derived plants
t	metric ton
TDR	Time Domain Reflectometry
μmol $m^{-2}s^{-1}$	micro mole per square metre per second
w/w	weight/weight
wk	week

CHAPTER 1 INTRODUCTION

1.1 General introduction

Excess soil salinity is a common problem in agriculture affecting one-third of the world's land area (Epstein, 1976; Epstein and Rains, 1987). Salinity problems usually result from excessive soil concentrations of soluble salts (e.g., NaCl, Na_2SO_4, KCl, K_2SO_4, $MgCl_2$, and $MgSO_4$). In general, salt tolerance can be defined as sustained plant growth in an environment containing a toxic level of NaCl or a combination of mixed salts (Levitt, 1977). Plant salinity tolerance is evaluated based on physiological injuries or yield reduction under salinity stress. The mechanism of salinity stress injury to plants involves osmotic effects followed by specific-ion effects after prolonged exposure (Munns, 1993; Munns et al., 1995).

Genetic variation in salt tolerance within and among plant species can be utilized to screen and select breeding candidates (Epstein and Rains, 1987). This screening and selection is usually done under field conditions (Shannon, 1984). Conventional field trials are not only time consuming and labour intensive, but also difficult to replicate since the whole plant response to salinity stress is complex and varies with environmental conditions (e.g., season, light, climate, and soil type) (Flowers and Yeo, 1989). Until now, the most effective method to cope with soil salinity problems is through the selection and use of salt tolerant crop plants (Ashraf, 1994; Tal, 1993 and

1994). In consideration of the environmental variations in conventional field trials, in vitro conditions can provide faster and more precise evaluations of plant growth under saline stress.

Potato (*Solanum* spp.) is considered as moderately salt sensitive in comparison with other crops since potato tuber yield could be adversely affected at field salinity EC levels of 2.0 - 3.0 dSm^{-1} (approx. 22 - 33 mM NaCl) (Maas and Hoffman, 1977). This may only apply to the genotypes tested since in some exceptional cases only 20 % potato tuber yield was decreased with saline irrigation water at an EC level of 6.0 dSm^{-1} (approx. 66 mM NaCl) (Nadler and Heuer, 1995).

Evaluation of salinity stress on potato plant growth and tuber yield has been conducted under field (Ahmad and Abdullah, 1979; Levy, 1992), greenhouse (Bilski et al., 1988a,b; Levy et al., 1988), and in vitro conditions (Arslan et al., 1987; Morpurgo, 1991; Morpurgo and Rodriguen, 1987; Naik and Widholm, 1993). These evaluations of potato responses under salinity stress were focussed on the shoot and/or root parameters or tuber yield in the field, the haulm growth in the greenhouse or plantlet growth in in vitro experiments.

Single-node cutting and in vitro stem layering methods have been used for potato in vitro propagation (Espinosa et al., 1986; Wang, 1977). These culture methods were utilized to supply in vitro experimental materials in the evaluation of potato salinity stress tolerance (Arslan et al., 1987; Morpurgo,

1991). Arslan et al. (1987) cultured single-node cuttings of wild potato species and cultivars under NaCl (0 - 120 mM) stress and tested genotype salinity tolerance. Morpurgo (1991) correlated saline stress effects on vegetative growth of five-node stem cutting culture and tuber yield in the field. He found a positive correlation between field tuber yield and plantlet root fresh weight, but not other growth parameters.

Using in vitro bioassays to evaluate plant salinity tolerance, a large genotype population can be tested within a limited space. This avoids the difficulties encountered in conventional field trials, while using less labour and time. In vitro evaluation and ranking of genotype salinity tolerance will be convincing and useful only if the selected salt tolerant genotypes are correlated positively with yield under saline field conditions (Jones, 1986). The correlation of in vitro bioassays for salinity tolerance with field studies can be best determined in our area (eastern Canada) using lysimeters irrigated with defined water. Once salinity tolerant genotypes have been identified, these may have uses in potato breeding programs or for crop production under saline growing conditions.

Potato production has been of increasing importance in Africa and the Middle East (FAO, 1995). Seed tuber production in these areas is hampered primarily by environmental stresses, e.g., saline soil, heat, and insect vectored diseases. Introducing salt tolerant cultivars to produce seed tubers I will reduce the cost of seed tuber importation in these areas. Local

agricultural production, of necessary, must be carried out on salinized land or irrigated with salinized water (Levy, 1992). If saline stress carry-over effect is proved minimal, moderately salinized soil may not be the limiting factor and utilized to produce potato tubers and seed tubers. There are no reports on the salinity carry-over effect on yield performance of seed tubers derived from saline environments.

It is understood that saline stress is composed of two phases; osmotic and specific-ion effects on plant growth and yield (Munns, 1993; Munns et al., 1995). These effects were evaluated in some crops but not potato under in vivo, e.g., Prunus (*P. Persica* L.) (Rajashekar et al., 1995) or in vitro, e.g., tobacco (*Nicotiana tabacum*) (Sweby et al., 1993) conditions. Observations by Zhang and Donnelly (1997) on the stimulatory effect of low NaCl concentrations on the yield of some cultivars lead to an investigation of which effect (osmotic or specific-ion) was responsible.

1.2 Thesis objectives

The objectives of this study were to:

1) Develop in vitro bioassays (seed germination and early seedling growth, single-node cutting, root tip segment, and microtuberization) to evaluate relative salinity tolerance of different potato (*Solanum* spp.) genotypes.

2) Use field lysimeters with saline water irrigation to determine the impact

of salinity on potato cultivar tuber yields and correlate these with the rankings of the in vitro bioassays.

3) Test the carry-over effect (if any) on yield performance under salt stress of plants derived from seed tubers and microtubers originally grown in saline or non-saline environments.

4) Test osmotic and specific-ion (Na^+) effects on potato microtuber yield to determine which factor increased yield under NaCl stress.

CHAPTER 2 LITERATURE REVIEW

Potato (*S. tuberosum*) is the only globally cultivated species among the 162 tuber-bearing species of the *Solanum* family (Hawkes, 1992). In terms of global crop yield, potato is ranked fourth, following rice (*Oryza sativa*), wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) (Woolfe, 1986). Potato crops supply food for over one billion people around the world. Potato production has increased 120 % from the 1960's to 1986 in developing market economies (FAO, 1990). North America, Europe, and the former USSR have the major potato production areas in the world (FAO, 1990). Countries in Asia, Africa, and Latin America produce about 30 % of world's total potato crop (FAO, 1990). The planting areas were 18×10^6 ha on average with yields of 15 t ha^{-1} in these developing countries (FAO, 1995). Canada produced 3.26×10^6 t potato on 123,000 ha which yielded an average of 27 t ha^{-1} in 1993 (FAO, 1995).

2.1 In vitro propagation

Single-node cuttings are routinely used to propagate potato in vitro (Wang and Hu, 1982 and 1985). Single-node cuttings are approximately 1-cm-long with one leaf and a lateral bud. They are maintained by subculturing on Murashige and Skoog (1962) (MS) basal salt medium containing 30 g l^{-1} sucrose, 100 mg l^{-1} inositol, 2 g l^{-1} Ca-pantothenate

(Estrada et al, 1986; Hussey and Stacey, 1984), vitamins (Murashige and Skoog, 1962), 6 - 7 g l^{-1} agar, without growth regulators (Austin and Cassels, 1983; Hussey and Stacey, 1984). The medium pH is adjusted to 5.7 before autoclaving. The culture conditions are 16/8 h (D/N) period with ≥ 40 μmol $m^{-2}s^{-1}$ photosynthetic photon flux (PPF) (cool white fluorescent light) and 23 ± 2 °C incubation temperature.

Microtubers can be produced from different parts of potato plants, such as excised buds (Barker, 1953; Chapman, 1955; Gregory, 1956), or stolon segments (Garcia-Torrez and Gomez-Campo, 1973; Palmer and Smith, 1970; Stallknecht and Farnsworth, 1982a,b), or single-node cuttings (Wattimena, 1983). A two-step microtuberization system (via stem layering followed by microtuber induction) was developed by Estrada et al. (1986) and Meuleman et al. (1986) and later revised by Leclerc (1994) for more efficient microtuber production.

Microtuberization is one method (single-node cutting is another) to increase specific pathogen tested (SPT) materials (Jones, 1988; Wiersema et al., 1987). Microtuber disks can provide a different resource in vitro for *Agrobacterium*-mediated gene transformation (Ishida et al., 1989). Microtuberization is also used to screen for early maturity (Lentini et al., 1988), heat tolerance (Huang et al., 1988; Nowak and Colborne, 1989), and tuber protein gene expression (Bourque et al., 1987).

2.2 Mechanisms of plant growth under saline stress

2.2.1 What is salinity

Excessive minerals in soil result in soil salinization. Salinized soils exist in every continent on earth and the amount of agricultural soils affected by salinity is increasing on a global basis. There are 14 billion ha of cultivated land on earth and 1.0 billion ha land are affected by excessive minerals (Christiansen, 1982). NaCl, which is a dominant mineral in salinized soils, occupied 70 % of mineral affected land areas (Epstein, 1976; Massoud, 1974), while the balance were affected by other minerals (Lewis, 1984).

Soil salinization is induced when surface soil accumulates toxic ions through evaporation of surface irrigation water or is invaded by underground saline water. Salinized soil reduces plant growth and yield (Epstein, 1976). The excessive minerals imposing salinity stress are mainly soluble salts present in the soil such as KCl, K_2SO_4, $MgCl_2$, $MgSO_4$, NaCl, and Na_4SO_4 (Lewis, 1984). In this thesis, salinity stress is mainly equated with NaCl stress for two reasons: a) it is one of the dominant salt components in the soil; b) it has been the focus of most salinity stress studies both in vivo and in vitro. The unit usually used to describe soil salinity level is EC (dSm^{-1}), while in vitro levels are expressed as mM, %, mg l^{-1} or ppm, which are inter-convertible but not readily converted to dSm^{-1} (Table 2.1). An EC of 1 dSm^{-1} is approximately 11 mM or 640 mg l^{-1} NaCl (Lewis, 1984).

Table 2.1 Different units used to describe salinity stress with the expression of exchange in NaCl concentration (mg l^{-1})

Unit	(NaCl, mg l^{-1})
mM	58.44
meq l^{-1}	58.44
dSm^{-1}	640.00
$mmhoscm^{-1}$	640.00
ppm	1.00
sea water (≈0.4% of NaCl)	(0.1% NaCl) ≈ 17.11 mM NaCl)

2.2.2 Plant responses to soil salinity

Salinized soil is a harsh environment for plant growth. It imposes a stressful selection pressure on plants. Some plants have been more successful than others in adapting to salinized soils. For example, halophytes (salt lovers, e.g., mangrove) can live in soils with salinity levels in excess of 300 mM NaCl (Flowers et al., 1977). Conversely, glycophytes (non-halophytes, such us potato) have reduced productivity in soils with salinity levels at EC of 2.0 - 3.0 dSm^{-1} (approx. 22 - 33 mM NaCl) (Maas and Hoffman, 1977).

Salinity stresses on plants have been hypothesized to involve a two-phase response; osmotic and specific-ion effects (Munns, 1993; Munns et al., 1995). Osmotic stress occurs first and induces an equal growth reduction in both salt sensitive and tolerant plants. Then specific-ion stress leads to the build-up of toxic ion levels in salt-sensitive but not tolerant plants because salt sensitive plants are less capable of excluding or compartmentalizing salts from plant cells.

Halophytes can survive on saline soils by a) excluding the salts from their roots and/or secreting them through specialized salt glands on the leaf surfaces; b) compartmentalizing the salts they take up into cell vacuoles (Ashraf, 1994; Shannon et al., 1994). These two mechanisms enable halophytes to avoid cellular ion toxicity so that they can tolerate salinity stress (Ashraf, 1994). Under saline conditions these plant usually develop

succulent leaves with thick cuticles.

Most glycophytes can not survive on salinized soil. Under low soil osmotic potential, their cells dehydrate and loss of turgor occurs. Then either by taking up mainly Na^+ and Cl^- from the soil, or producing different osmolytes such as glycerol, sucrose, or proline in the cytoplasm, plant cells accumulate large amounts of toxic ions in their vacuoles to resist the external osmotic pressure (Serrano and Gaxiola, 1994). These accumulated toxic ions reduce enzyme activities and inhibit other ion uptake, e.g., K^+ and Ca^{+2} (Serrano and Gaxiola, 1994). The more salt tolerant glycophytes can either exclude salt (Na^+ and/or Cl^-) from their cells through ion pumps, or produce different osmolytes to compensate for the external osmotic stress (Shannon et al., 1994; Tal, 1984). Glycophytes injured by saline stress appear stunted and darker green with fewer nodes and shorter internodes (Shannon et al., 1994).

In detecting the two-phase saline stress effects on plants, NaCl effects are contrasted with the effects of mannitol or other osmotic agents (Sweby et al., 1994; Zekri, 1993). When the tobacco plantlets were cultured under NaCl or isoosmotic mannitol stress, vegetative growth was equally affected. Osmotic stress was the major negative NaCl effect on tobacco plantlet growth under in vitro condition (Sweby et al., 1994). Mannitol-induced osmotic stress reduced seed germination and seedling emergence of citrus rootstocks while NaCl induced specific-ion effects reduced the percentage of

final seedling emergence and seedling growth (Zekri, 1993). There are no reports to distinguish osmotic or specific-ion effects on potato growth or yield responses under NaCl stress condition in vivo or vitro.

2.3 Strategies to cope with saline soils

2.3.1 In vivo or in vitro screening and selection methods to improve plant salinity tolerance

The ability of plants to tolerate salt stress is genetically controlled; in most cases by multiple genes (Epstein and Rains, 1987; Tal, 1994). Plant salt tolerance varies within as well as among plant species (Epstein and Rains, 1987; Maas, 1984). Different screening and selection methods can distinguish salt tolerant from sensitive genotypes. Tolerant genotypes can be utilized in plant breeding programs or assigned directly to crop production.

Whole plant responses to saline stress are complex and vary with field environmental conditions (e.g., season, light, climate, and soil type). These variables interact with plant productivity to confound salt tolerance expression (Flowers and Yeo, 1989). Significant progress is slow in plant salt tolerance improvement even though many different plant breeding methods have been tried (Shannon, 1996). The screening and selection of salt tolerant plants is still a commonly used and problematic activity (Ashraf, 1994; Shannon, 1979 and 1996; Tal, 1994).

The following criteria must be satisfied to establish an efficient and valid

screening and selection method for the improvement of salt tolerance: a) genetic variation for salinity tolerance must exist within the test group; b) selected salinity tolerance should be heritable; c) ranking parameters should be correlated with yield in the field under saline stress or with a yield-based stress-resistance index; and d) the selection method as well as the ranking parameters should be economical, simple, and rapid to assess (Acevedo and Fereres, 1990).

The term "bioassay", in a broad sense, refers to a test in which the quantity or strength of material is determined by the reaction of a living organism to it (Sparague, 1973). A plant bioassay for salinity (NaCl) tolerance quantifies the impact of NaCl on the growth process of the tested plant(s) and ranks them either in vivo or in vitro.

Many authors have evaluated salinity stress effects on plant growth and productivity. These experiments were performed either in vivo (Ahmad and Abdullah, 1979; Bilski et al., 1988a,b; Levy, 1992; Levy et al., 1988; Paliwal and Yadav, 1980), or in vitro (Arslan et al., 1987; Morpurgo, 1991; Morpurgo and Rodriguez, 1987; Naik and Widholm, 1993) in potato, and in other crops such as alfalfa (*Medicago sativa*) (Shah et al., 1993), citrus rootstock (*Poncirus trifoliata*) (Beloualy and Bouharmont, 1993), tobacco (*N. tobacum*) (Nabors et al., 1980), or wheat (Maddock et al., 1983), etc..

In field screening and selection, plant salt tolerance is ranked based on the degree of inhibition of plant vegetative growth or yield (Levy, 1992;

Morpurgo, 1991; Ponnamperuma, 1977 and 1984; Zurayk et al., 1993). Field saline stress studies have met with several common problems which influence the ranking results. Soil salinity varies substantially with time, location, and soil depth (Shannon, 1984). Plant genotypes have large interactions with the environment (Ekanayake and Dodds, 1993). Plant reactions under saline stress also vary with ontogeny (Levy, 1992). All these factors lead to low repeatability, as well as large investments in monitoring devices in the field trials (Saxena et al., 1994; Shannon, 1984). Obviously the field trial does not meet the ideal criteria to evaluate crop salinity tolerance for economical and efficient screening and selection (Acevedo and Fereres, 1990).

Screening and selection for salinity tolerance has been accomplished under more controlled conditions. A) Greenhouse or growth chambers were used for testing crops at the seed germination or early seedling growth stages (Jones, 1986; Nieman and Shannon, 1976; Saxena et al., 1994). Controlled greenhouse conditions eliminated the environmental variants which exist in the field trials, including soil type, climate, and field location (Zeroni, 1988). Sand culture, solution culture, and controlled climate rooms were all used to evaluate crop salt tolerance. It is convenient to control both salinity and nutritional conditions in solution culture (Nieman and Shannon, 1976). B) Plant tissue culture systems were used to culture plants at the cell or organ (node, shoot or root) levels (Tal, 1993). Compared with field or

greenhouse trials, in vitro cell or organ culture can test large populations within a relatively small space (Wenzel and Foroughi-Wehr, 1990) and provide a relatively homogenous experimental unit (Tal, 1984). C) Field lysimeter trials were used to test plant responses under more controlled soil salinity environments (Milbocker, 1988; Saxena et al., 1994).

Since plants will eventually be grown under field conditions for agricultural production, it must be emphasized that the validity of screening and selection for salinity tolerance using greenhouse or in vitro bioassay methods depends on the correlation of these trials or bioassays with yield performance of the selected plants under field conditions. This means that plants selected in the greenhouse or in vitro for their salt tolerance should exhibit similar salt tolerance in saline fields. For many crops, this information is unavailable (Wenzel and Foroughi-Wehr, 1990).

Lysimeters are soil-filled containers which can be located in the field. Soil parameters can be monitored and adjusted in these units to provide better control of salinity. Standardized field screening conditions using lysimeters reduce soil environmental variations (Milbocker, 1988; Saxena et al., 1994) and make the estimation of salinity tolerance for a particular plant more accurate and quantitative (Jones, 1986).

The field lysimeters are irrigated with non-saline or artificially salinized water (NaCl and/or $CaCl_2$). The EC levels of saline water are 4.7, 9.4, and 14.0 dSm^{-1} (approx. 52, 103, and 154 mM NaCl) for a moderately tolerant

crop. A steady soil salinity level is maintained and checked periodically during crop growth by measuring the EC levels in the root zone. Data are collected at critical plant developmental stages to assess crop performance (Nieman and Shannon, 1976). Although field lysimeter trials were used to test the plant salt tolerance (Picchioni and Miyamoto, 1990), there were no reports about using lysimeters to verify the in vitro evaluation of plant salt tolerance, particularly in potato.

In summary, salt tolerant genotypes can be screened and selected under different saline stress situations, e.g., field, greenhouse, or in vitro. In practice screening and selection should be economical and efficient. In vitro bioassays are efficient and can avoid many variables encountered in the field or greenhouse conditions. The in vitro bioassay results are useful only if they correlate with the relative amount of growth or yield produced under salt stress in the field (Jones, 1986). This field trial will be the ultimate test of the value and validity of any in vitro screening and selection method (Miller et al., 1991). The verification of in vitro bioassay results can be best done under controlled field conditions using field lysimeters.

2.3.2 Salinity tolerance evaluation during seed germination and early seedling growth stage

Salt tolerant genotypes exist between and within plant species, e.g., wild species, hybrids and cultivars. Bernstein and Hayward (1958) indicated that

salt tolerant traits presented in wild germplasm resources could be incorporated into plant breeding programs.

Salinity tolerance evaluations can be performed during seed germination and early seedling growth stages. Salinity stress decreases the seed germination rate and germination percentage, as well as seedling early growth rates. Germination rate is a useful criterion to evaluate crop salt tolerance in cases where the tolerance does not diminish at some later growth stage (Ramage, 1980). Seed germination tests are rapid, non-destructive, and can easily quantify saline stress effects (Scott et al., 1984). Seedling shoot or root vegetative growth in a saline environment is often used to indicate salt tolerance (Bernstein, 1974; Bernstein and Hayward, 1958; Khan and Ungar, 1984).

Crop responses to salt stress vary throughout plant ontogeny (Greenway, 1965; Levitt, 1977). Some species or cultivars within a species can germinate under high salinities, but their subsequent growth may be severely restricted (Kumar, 1985). The reverse was also true in faba bean (*Vicia faba* L. var. Minor) (Hamid and Talibuddin, 1976). A tolerant plant at one growth stage may become sensitive at another or vice versa (Ayers et al., 1952; Nieman and Shannon, 1976; Norlyn, 1980).

After pre-selecting at germination and seedling growth stages in sand culture with 0, 30, and 60 mM NaCl, Ashraf and Waheed (1993) assessed lentil (*Lens culinaris* Medic.) salt tolerance at the adult plant stage. Not all

salt tolerance was positively correlated between the different growth stages. The positive correlations were recorded in three out of five tolerant accessions, two out of three moderately tolerant accessions, and all three sensitive accessions.

2.3.3 In vitro salt tolerance research in callus and cell cultures

Salt tolerant cell lines have been successfully obtained in many plant species through in vitro selection (Tal, 1993). Among salt-tolerant cell lines selected from 24 genera of 12 families, 90 % of them (18 out of 20 reports) were stable for salt tolerance; 40 % (8 out of 20 reports) of regenerated plants were verified as salt tolerant; the salt tolerance in 20 % (4 out of 20 reports) of regenerated plants was inherited by the R_1, R_2 or R_3 generations (Reviewed by: Ram and Nabors, 1985; Tal, 1990).

In vitro cell selection methods can be divided into two categories: a) one-step or short-term and b) stepwise or long-term. One-step selection methods involve exposing vigorously growing callus to sublethal salt concentrations, usually from 80 to 200 mM NaCl. Callus which is able to grow normally (similar to control) on this medium is transferred to regeneration medium with no salt. By using this method, stable, salt tolerant plants were obtained from callus cultures of alfalfa (*Medicago sativa* L.) (Winicov, 1991), Indian mustard (*Brassica juncea*) (Kirki et al., 1991), flax (*Linum usitatissimum* L.) (Rowland et al., 1989), and sugar beet (*Beta*

valgaris L.) (Freytag et al., 1990).

Stepwise selection methods involve challenging cells at relatively high salt levels, usually from 150 to 300 mM NaCl. Using this method, 50 - 95 % of cells were killed and the regenerated plants were selected from the surviving cells (Bressan et al., 1985). After 50 subcultures, at least 25 of which had cells exposed to 400 mM NaCl, R_1 plants of tetraploid tobacco (*N. tabacum* cv. W38) were selected with higher relative growth rates than non-selected plants growing in medium containing NaCl.

Plantlets were regenerated from callus cultures of a hybrid potato (*S. tuberosum* L. X *S. chacoense* Bitt.) exposed to 200 mM NaCl stress (Burgutin et al., 1996). The hybrid explants and the regenerated plantlets were cultured for 60 generations in medium containing 7 % NaCl (approx. 120 mM). Shoot vegetative growth in regenerated plantlets was significantly better than that of the hybrid. The authors did not test and confirm the salt tolerance of regenerated plantlets under the field conditions.

2.3.4 Potato in vivo salt tolerance research

Potato was ranked as a moderately salt sensitive crop compared with other species (Maas and Hoffman, 1977). Plant growth and tuber yield were adversely affected by soil EC levels at 2.0 - 3.0 dSm^{-1} (approx. 22 - 33 mM NaCl). Field salinity trials later revealed that some potato cultivars had increased yield at low soil salinity levels (0.2 - 0.4 % mixed salts; approx. 34

- 68 mM NaCl) (Ahmad and Abdullah, 1979), and others could grow at an EC level of 6.0 dSm^{-1} (approx. 66 mM NaCl) with 20 % (Paliwal and Yadav, 1980) or without yield reduction (Nadler and Heuer, 1995).

Potato salinity tolerance was evaluated in several field experiments. The tuber yields of cv. Kufri Chandramukhi were progressively decreased under saline stress (4, 20, 40 and 80 meq l^{-1}) (approx. 4, 20, 40, and 80 mM NaCl) in a field micro-plot (sandy-loam soil) experiment (Paliwal and Yadav, 1980). Potato could grow normally when soil was irrigated with saline water (40 meq l^{-1}, equal to 40 mM NaCl). Yield was reduced 20 % at an soil EC level of 6.0 dSm^{-1} at harvest. Increased salinity levels had a more adverse effect on potato tuber size than number.

Potato cvs. Cardinal, Chieftain, Multa, Norland, Patrones, Red Bed, and Red Lasoda were grown in a pot trial using sandy loam and saline irrigation water containing 0.2 - 1.0 % of mixed salts (NaCl, $MgSO_4$, $CaCl_2$, and $NaHCO_3$; approx. 34 - 171 mM NaCl) (Ahmad and Abdullah, 1979). Low salt concentrations during irrigation of up to 0.4 % (approx. 68 mM NaCl) increased tuberization in cvs. Cardinal (178 %), Multa (132 %), and Patrones (184 %) but decreased tuberization in cv. Red Bed (67 %) compared with the control (100 %). Higher salt (1.0 %) NaCl concentrations during irrigation inhibited tuberization in cvs. Cardinal (72 %), Multa (80 %), Patrones (59 %), and Red Bed (10 %) compared with the control. Total tuber glycoalkaloid (TGA) content was decreased as irrigation salt

concentration increased but TGA increased slightly in cv. Red Bed (108 %) at irrigation salt concentration of 1.0 %. Based on tuber fresh weights, cvs. Norland, Patrones, and Red Lasoda were described as salt tolerant and cv. Red Bed as salt sensitive.

Fourteen potato cultivars were field grown in sandy soil and drip-irrigated with saline water at EC levels between 1.0 - 1.4, 3.8 - 4.3, and 6.1 - 6.9 dSm^{-1} (approx. 11 - 15, 42 - 47, and 67 - 77 mM NaCl) (Levy, 1992). Saline stress retarded plant emergence, enhanced haulm senescence, and reduced both haulm fresh weight and tuber number and weight. Salinity treatments applied at different potato growth stages had different effects on tuber yields. In the field trials, the tuber yields were decreased by 22 - 31 % when saline stress (saline water irrigation) was applied after plants were well established. Yields were decreased 21 - 54 % when saline stress was applied after tuber emergence. Yields were depressed by 21 - 79 % when saline stress was applied soon after planting. The 14 potato cultivars used had different maturity times (early or late). There was no interaction between cultivar maturity time and salinity treatments.

Potato (*Solanum* spp.) salinity (NaCl) tolerance was tested in greenhouse experiments (Bilski et al., 1988a,b; Levy et al., 1988). Cvs., Norchip, Norgold Russet, Red Pontiac, and Russet Burbank, were challenged with NaCl at 0, 40, 80, and 120 mM and Na_2SO_4 at 0, 20, and 40 mM respectively in the greenhouse (Bilski et al., 1988a). Cv. Norgold Russet was

more salt tolerant than other cultivars based on leaf and stem dry weight criteria. The salt tolerance of 11 accessions of 6 wild *Solanum* species (*S. bulbocastanum, S. chacoense, S. gourlayi, S. microdontum, S. papita,* and *S. sparsipitum*) were tested based on parameters such as: percentage seed germination, percentage seed survival, and haulm dry weights (Bilski et al., 1988b). Growth parameters in surviving seedlings were decreased by NaCl (40, 80, and 120 mM) stress. *S. chacoense* was the most tolerant based on seed survival. NaCl tolerance among or within potato species was varied. There was no apparent relationship between accessions with known heat or drought resistance and salt resistance.

Levy et al. (1988) reported on greenhouse pot trials of potato cvs. Alpha, Banca, Cara, and Desiree growing under NaCl stress (20.5, 34.2, and 51.3 mM). NaCl stress reduced tuber yield, water and osmotic potentials in leaves and tubers, but increased the total proline content and tuber dry matter. Cv. Alpha was moderately tolerant, with a 15 % tuber dry weight reduction at 34.2 mM NaCl.

Seed germination, plant vegetative growth, or tuber yield were commonly used to evaluate salinity tolerance of potato wild species and cultivars (not hybrids) in the field and greenhouse experiments. The number of genotypes or cultivars tested in these experiments were limited; from 1 (Paliwal and Yadav, 1980) to 14 (Levy, 1992) in the field and from 4 (Levy et al., 1988) to 11 (Bilski et al., 1988b) in the greenhouse experiments. These in vivo

tests were space and labour constrained, time consuming, and expensive. Evaluation of larger potato populations, which is a formidable task in field trials, can be performed using in vitro bioassays under controlled conditions requiring limited space and relatively short time intervals. This is a potentially valuable and economically efficient alternative choice for screening and selection of salt tolerant germplasm.

2.3.5 Potato in vitro salt tolerance research

In order to overcome some of the problems inherent in the field trials, in vitro culture methods were used to evaluate potato salinity tolerance. The single-node cutting (Arslan et al., 1987), stem cutting (Kim et al., 1995; Morpurgo, 1991; Morpurgo and Rodriguez, 1987; Naik and Widholm, 1993), axillary bud (Potluri and Prasad, 1993), root tip segment (Naik and Widholm, 1993), and microtuberization (Kim et al., 1995) cultures were used to test potato salinity tolerance. Salinity tolerance evaluations at the cell level were also reported in callus (Burgutin et al., 1996) and suspension cultures (Naik and Widholm, 1993).

Two potato cvs. Fruhbote and Hansa and three wild species *S. chacoense, S. phureja,* and *S. sparsipilum* were micropropagated using the single-node cutting method under 0, 4, 8, 12, and 16 mmhoscm^{-1} NaCl stress (approx. 0, 44, 88, 132, and 176 mM NaCl) (Arslan et al., 1987). Based on plantlet growth after 6 wk culture, cv. Fruhbote was more salt

tolerant than Hansa, and among the wild species *S. sparsipilum* was more tolerant than *S. phureja*, while *S. chacoense* showed no distinct tendency.

A similar screening method was used to test 20 potato varieties under NaCl stress (0, 50, 100, 150, 200, and 250 mM) (Kim et al., 1995). Based on the growth reduction in total fresh weight, root and shoot lengths in stem segments, and potato microtuber yield, 10 of 20 cultivars were judged to be relatively salt resistant.

Six tropical low-land potato cultivars were assessed with axillary bud culture exposed to crude sea salts (Potluri and Prasad, 1993). Salt stress (0.4 - 0.8 %; approx. 68 - 136 mM NaCl) inhibited axillary bud growth in all cultivars. A lower salt concentration (0.2 %) (approx. 34 mM NaCl) was beneficial and increased shoot dry weight in most of the cultivars. The K^+/Na^+ ratio of plantlet leaves was positively ($p<0.01$) correlated with plantlet dry weight yield.

The salt tolerance of six potato cvs., Kennebec, Norchip, Red Pontiac, Russet Burbank, Russet Norkotah, and Superior were tested using NaCl (0.0 - 250 mM) stress with several tissue culture methods and a greenhouse experiment (Naik and Widholm, 1993). Data were collected from plantlet rooting pattern in the single-node cutting culture, root extension growth in the root tip segment culture, harvested cell fresh weight from cell suspension culture, and the whole plant haulm fresh weight in the greenhouse experiment. Only the root extension growth from the root tip

segment bioassay was positively correlated with the greenhouse whole plant haulm fresh weight, but not other growth parameters. Based on this information they suggested that root tip segment culture could be used as a preliminary screening method.

Five-node stem cuttings of 8 potato clones were cultured in MS (1962) basal liquid medium incorporated with 120 mM NaCl (Morpurgo and Rodriguez, 1987). After 1 mo culture they evaluated the clones using parameters including shoot and root fresh weight, shoot height, shoot number, node number and dry weight per plantlet. Among the eight clones tested, growth parameters, e.g., shoot length, shoot fresh weight and root fresh weight, were less inhibited in 'BR 69.84' and 'Dozier' under NaCl stress. Root fresh weight was the parameter most affected by NaCl stress in all the clones.

Using the same methodology, Morpurgo (1991) tested the salt tolerance of 10 potato cvs. DTO-33, G-1, LT-1, LT-5, LT-6, Mariva, P-3, Rosita, Serrana, and Yungay under 154 mM NaCl stress. He also conducted a field trial in sandy, salty soil. The soil salinity levels varied greatly between EC 4.0 - 7.0 dSm^{-1} (approx. 44 - 77 mM NaCl) during the growing season. Root fresh weight but not the other in vitro growth parameters was positively correlated with tuber yield in saline soil. This is the only in vitro and in vivo correlation reported for potato salinity tolerance evaluations. Despite the limited NaCl treatments in vitro and the huge salinity range applied in the

field variations, this research showed the possibility of utilizing in vitro screening and selection methods for potato.

Salt tolerant cell lines derived from a hybrid potato (*S. tuberosum* L. X *S. chacoense* Bitt.) in callus culture were maintained under 1.0 or 1.5 % NaCl stress (approx. 171 or 257 mM NaCl) (Burgutin et al., 1996). Microcuttings of regenerated plantlets were cultured in MS medium containing 0.7 % NaCl (approx. 120 mM). Five out of 38 cell lines significantly surpassed the hybrid in shoot growth. Salt tolerance was present even after more than 60 vegetative subcultures. Plantlet salt tolerance was not tested under saline field conditions.

These preliminary tests described potato response under in vitro NaCl stress, sometimes only at one NaCl level (Morpurgo, 1991; Morpurgo and Rodriguez, 1987). When this thesis was initiated, it was still unclear which NaCl level was the most effective for screening and selection in vitro and which in vitro culture methods were most applicable for screening and selection bioassays. Most potato in vitro salinity studies used only one culture method to test potato salinity tolerance. Some authors used two culture methods, e.g., stem segment and microtuberization cultures (Kim et al., 1995) but did not compare them. Stem cutting, root segment, and cell suspension cultures were employed by Naik and Widholm (1993) to test potato NaCl tolerance. In root tip segment culture (Naik and Widholm, 1993), seed tuber buds were aseptically dissected and cultured. The newly

formed 1-cm-long root tip was cut and used as the explant. This method was slow and labourious, and could be improved.

Several other problems existed in previous salinity studies. The huge field salinity variations (EC levels of 4.0 - 7.0 dSm^{-1}) in Morpurgo's correlation test (1991) make the experiment difficult to replicate. The in vivo and in vitro correlation established by Naik and Widholm (1993) was not based on yield parameters in the field but on the greenhouse plant haulm fresh weight. Early foliage growth does not necessarily correlate with potato tuber yield (Vayda, 1990). Potato haulm growth was less affected than yield parameters under saline stress (Levy, 1992). It is necessary to find more convincing in vitro and in vivo correlations with different in vitro culture methods when NaCl stress is applied.

2.3.6 Application of potato seed tubers produced under saline stress environments

Potato production is of increasing importance globally, and especially in Africa and the Middle East (FAO, 1995). Potato seed tuber production technology has not been well developed in these areas (Leclerc, 1993). Potato seed tubers are usually imported from other countries (e.g., Europe or Canada) where remote areas with reduced disease pressure are available to the seed potato industry (Leclerc, 1993). The establishment of national potato seed tuber certification programs would reduce the reliance on

imported seed tubers in countries such as Egypt (Leclerc, 1993). However, in some parts of potential production areas cultivation must be carried out under saline soil conditions (Hamdy et al., 1993; Levy, 1992; Rowley, 1993; Satti et al., 1994) or using saline irrigation water (Levy, 1992). Where disease pressure will permit this (i.e., in desert areas), saline soil can be used for potato seed tuber production. But the question, "is there any carry-over effect for seed tubers produced in saline environments", has to be answered first. To date there are no reports on the evaluation of potato tubers obtained from saline conditions. We tried to quantify the yield effects of plants derived from seed tubers obtained from saline soils. This information is potentially important in improving potato production in saline soil areas.

PREFACE TO CHAPTER 3

This Chapter reports on preliminary investigations of potato (*Solanum* spp.) salinity tolerance under in vitro NaCl stress conditions. The objective of this research was to observe the response to salinity in three different potato genotype groups using the single-node cutting and seedling bioassays.

CHAPTER 3 IN VITRO SCREENING FOR SALINITY TOLERANT POTATO

Abstract

Salinity is an increasingly serious and widespread agricultural problem that contributes to lost crop yield and arable land. Plant breeding efforts to improve the salinity tolerance of plants must now supplement expensive soil reclamation and water desalination programs. The cultivated potato (*S. tuberosum* L.) is a globally important crop with moderate salt sensitivity. The in vitro screening tests for salt tolerant germplasm employed in this study are potentially faster and more reliable than traditional field assessment. In vitro single-node cutting bioassays were used to rank seven potato cultivars and six hybrids. Seedling bioassays were used to compare three wild *Solanum* species. Cvs. Russet Burbank and Spunta outperformed five other cultivars based on shoot and root lengths and oven-dried weights in vitro growth parameters. Hybrids derived from *S. chacoense* outperformed hybrids derived from *S. gourlayi* and *S. microdontum*. *S. chacoense* seedlings performed better when challenged with salt in vitro than did the other two wild species. In vitro screening of germplasm, utilizing single-node cutting and seedling bioassays, holds promise in the development of more salinity tolerant potato plants.

3.1 Introduction

Salinity, usually excess sodium chloride (NaCl), is among the most serious and widespread of agricultural problems contributing to lost crop yield and arable land. At least 25 % of the world's cultivated land area is occupied by salt-affected soil, primarily in semi-arid and arid areas (Nabors, 1990). Under more favourable environments, where land is intensively cultivated, salinity problems may develop where quality irrigation water is limited or mismanaged or drainage is lacking (Morpurgo, 1991). In the past, major efforts to circumvent salinity were directed to soil reclamation and water desalination. Modifying the environment to suit the plant is increasingly expensive, which provides strong impetus to the development of plants with increased salinity tolerance (Nabors, 1990).

When the diversity of salt tolerance among cultivars (or their wild relatives) is extensive, conventional breeding techniques can be used to improve their salt resistance. In species with little diversity of salt resistance or where conventional breeding progress has been slow, tissue culture techniques could be used for selection of variants for salt resistance (Ram and Nabors, 1985; Tal, 1984). Progress in exploiting genetic variability to develop crops with increased salt tolerance is still in its infancy. The mechanisms of salt tolerance, the effect of plant growth stage and the effect of environmental factors on salt tolerance are not well understood. It is known that salt tolerance is under polygenic control mechanisms. In

sugarcane (*Saccharum* spp.), 29 proteins were regulated by salinity; a multitude of mechanisms at the transcriptional and post-transcriptional levels contributed to the control of gene expression in salt tolerant cells (Ramagopal and Carr, 1991).

The cultivated potato (*S. tuberosum* L.) is an internationally important, staple food crop. It is grown in 79 % of all countries, and in terms of volume of production, ranks fourth globally after rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) (Woolfe, 1986). Potato is a crop with moderate salt sensitivity (Maas, 1987). Tolerance to irrigation with saline water has been evaluated on several potato cultivars and species in pot (greenhouse, screenhouse) or field trials in Pakistan (Ahmad and Abdullah, 1979), India (Paliwal and Yadav, 1980), Tunisia (Bouaziz, 1980), Israel (Levy et al., 1988), United States (Bilski et al., 1988a,b), and other countries. Differences in salinity tolerance existed among cultivars and species; salinity depressed yield and generally had a greater effect on tuber weight than tuber number. Assessment of salinity tolerance in the field is seasonally constrained, affected by climate, and may lack reliability due to the combined salinity and water stress problems. Variable salt composition and uneven distribution in the field may also tend to confound field screening (Yeo et al., 1990). More reliable and time-saving selection techniques have been developed using tissue culture technology.

In vitro determination of salinity tolerance, utilizing single-node cuttings of

tissue culture propagated (micropropagated) plants enabled ranking of potato cultivars and wild species (Arslan et al., 1987; Morpurgo and Rodriguez, 1987). A highly significant correlation was found between in vitro growth parameters and the field performance of 10 potato clones exposed to a high level of NaCl (Morpurgo, 1991).

The objective of this research was to utilize the in vitro single-node cutting bioassay to determine the salt tolerance of seven potato cultivars and six hybrids maintained in culture. The effect of salinity on germination and early seedling growth in vitro of accessions of three wild species was also investigated.

3.2 Materials and methods

Tissue cultured cvs. Atlantic, Kennebec, Norland, Russet Burbank, Shepody, Spunta and Superior were obtained from the La Pocatière (QC, Canada) germplasm repository (Appendix 1A). Six hybrids of S. *tuberosum* X wild species (*S. chacoense, S. gourlayi* or *S. microdontum*) (9787-07; 9506-04; 10908-06; 10910-08; 10911-02; 10911-04) (Appendix 1B) were obtained from Dr. H. De Jong, Agriculture and Agri-food Research Station, Fredericton, NB, Canada. Potato clones were micropropagated from single-node cuttings, 1-cm-long with 1 axillary bud, in a modified Murashige and Skoog (1962) basal salt medium plus (mg l^{-1}): Ca-pantothenate (2.0), glycine (2.0), myo-inositol (100), niacin (0.5), pyridoxine·HCl (0.5),

(0.4), 3 % sucrose and 0.7 % agar (Anachemia, Lachine, QC, Canada; added after pH adjustment to 5.7). The medium was autoclaved at 121 °C, 103.46 KPa for 20 min. Culture conditions were 16/8 h D/N photoperiod, 40 μmol $m^{-2}s^{-1}$ photosynthetic photon flux (PPF) (cool white fluorescent light) and 23$\pm$2 °C.

True potato seeds of the three wild species mentioned above, with putative salinity tolerance, were obtained from the United States Department of Agriculture, Agricultural Research Service, Inter-Regional Potato Introduction Station, Sturgeon Bay, WI, USA (Appendix 1C). These were surface sterilized in 10 % household bleach (5.25 % sodium hypochlorite), rinsed in sterile water, soaked in a 2 g l^{-1} solution of gibberellic acid (GA_3) for 24 h to break dormancy (if any), again rinsed in sterile water and transferred, 1 per 25 cm test tube containing 10 ml of medium, for the germination trials. They spent the first 4 d in the dark followed by 3 wk in the light.

The cultivars and hybrids were tested in the single-node cutting bioassay at a different time. The wild species were tested in seed germination and early seedling growth bioassays. The salinity stress bioassays lasted 3 wk and were conducted with a Complete Randomized Block Design (CRBD), each with three treatments - 0, 80, and 120 mM NaCl added to the micropropagation medium and 5 (single-node cutting bioassays) or 10 (seed germination bioassays) replicates and repeated 3 times. The single-node cutting bioassays were conducted twice and the seedling experiments were

conducted three times. For the single-node cutting bioassays, and in addition to tabulation of percentage germination for the seedlings, data were collected on shoot and root lengths and oven-dry weights (48 h at 60 °C). Due to the inheritance diversity in potato wild species, seeds are genetically different from each other. Data collected from two accessions of each wild species were pooled together and the average was reported for the salinity tolerance evaluation of that wild species. Data were analysed with the general linear model (GLM) (SAS, 1989). The treatment differences were tested with the least square differences (LSD) comparison at the 5 % level of significance. Data were expressed as percentage of control values.

3.3 Results and discussion

Shoot and root lengths and root but not shoot dry weights were significantly decreased ($p<0.001$) in the single-node cuttings challenged with NaCl for the seven cultivars (Fig. 3.1 A-C; please see Appendix 3 for actual data). Salinity exposure depressed these parameters from 30-80 %, with cvs. Russet Burbank and Spunta least and Norland most affected compared with the other cultivars. All parameters were significantly decreased ($p<0.001$) in the single-node cuttings challenged with NaCl for the six hybrids (Fig.3.2 A-D; please see Appendix 4 for actual data). Salinity exposure depressed these parameters from 60-100 %. Hybrids 9787-07 derived from *S. chacoense* X *S. tuberosum* and 9506-04, derived from

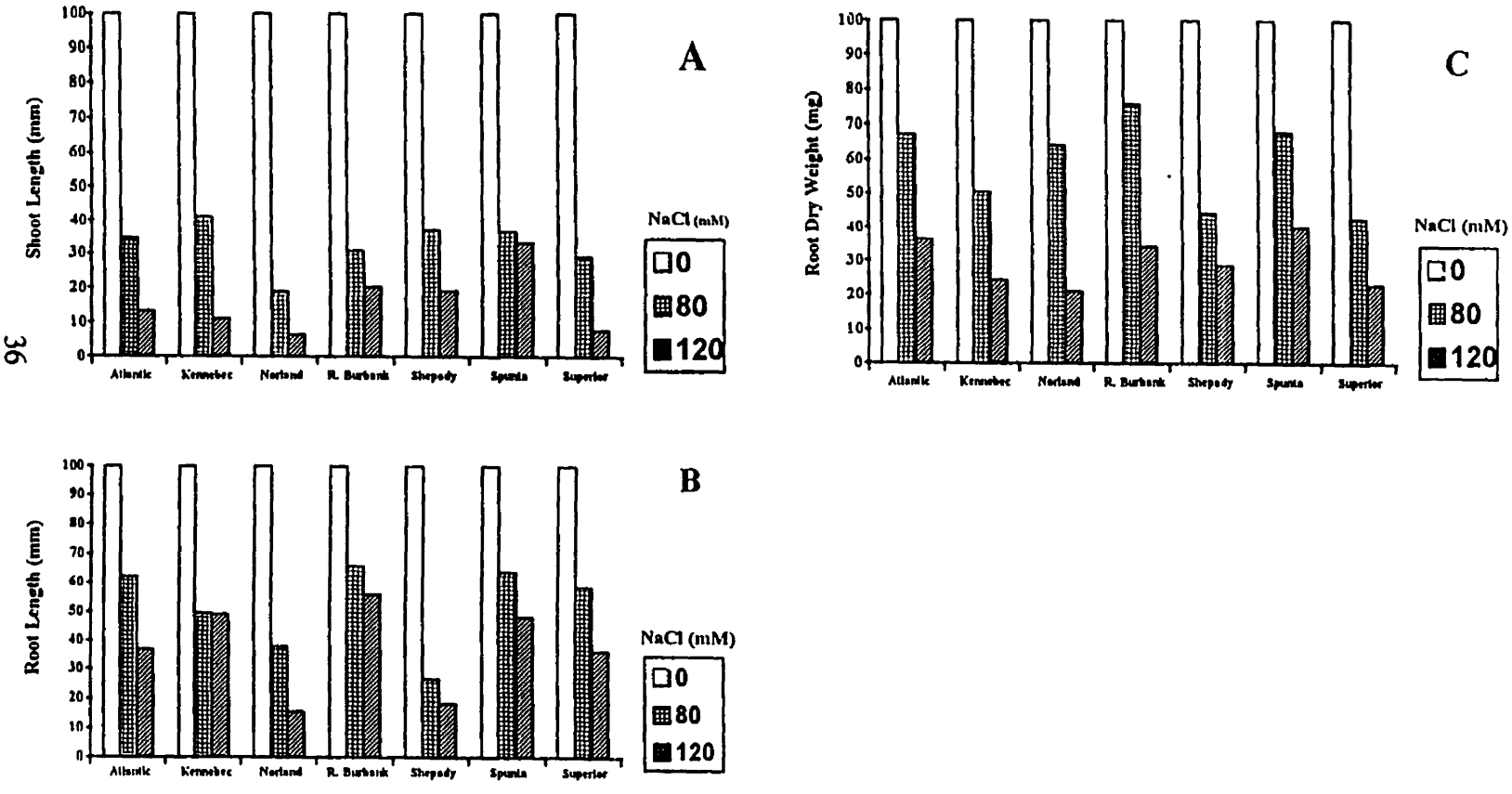


Fig. 3.1 The effect of NaCl (0, 80, and 120 mM) on single-node cuttings of seven potato cultivars

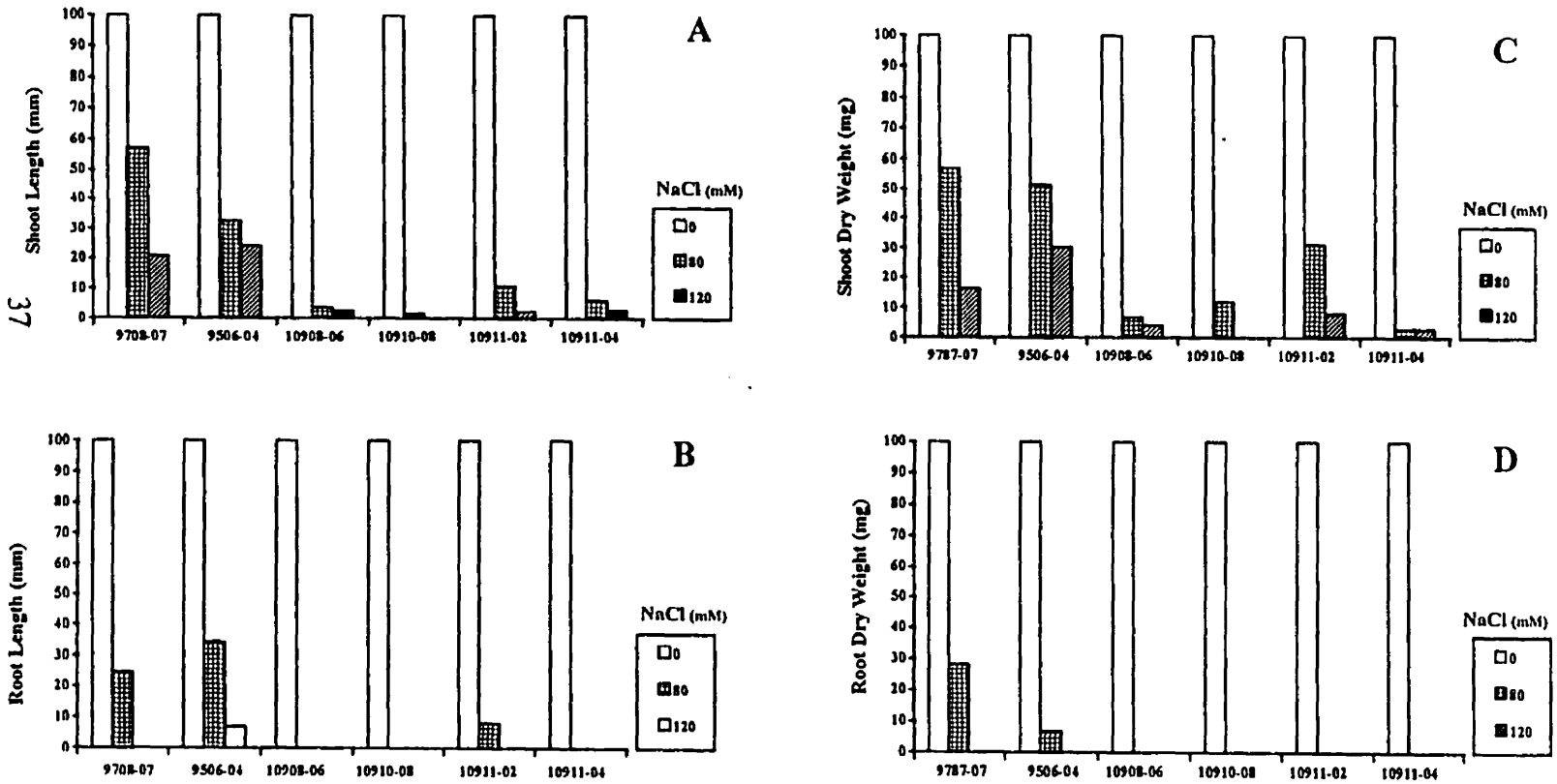


Fig. 3.2 **The effect of NaCl (0, 80, and 120 mM) on single-node cuttings of potato hybrids**

croses among *S. chacoense, S. microdontum,* and *S. tuberosum* were more tolerant to salinity stress than hybrids derived from the backcross hybrids between Argentinian materials (*S. gourlayi* or *S. microdontum*) and *S. tuberosum* (hybrids: 10908-06, 10910-08, 10911-02, and 10911-04). The relative salinity tolerance of the cultivars and hybrids was not obtained. However, preliminary comparisons of Figs. 3.1 and 3.2 indicated that the hybrids were not more salinity tolerant than the cultivars tested.

The germination rate of true potato seed varied with the species ($p<0.01$); *S. chacoense* had the highest germination rate and *S. gourlayi* had the lowest (Fig. 3.3 A; please see Appendix 5 for actual data). The germination rate was significantly reduced ($p<0.001$) for all species when seed was exposed to NaCl in the medium, compared with the control medium. Salinity exposure depressed the shoot and root lengths and the root but not shoot dry weights (Fig. 3.3 B-D; please see Appendix 5 for actual data). Least affected was *S. chacoense* and most affected was *S. gourlayi,* with intermediate response, for most parameters, by *S. microdontum.*

In conclusion, of the seven cultivars tested, cvs. Russet Burbank and Spunta were relatively more salinity tolerant than cvs. Atlantic, Kennebec, Norland, Shepody, and Superior. Germination percentage and seedling growth parameters were less affected by salinity in seedling accessions of *S.chacoense* compared with *S. gourlayi* and *S. microdontum.* Two hybrids derived from *S. tuberosum* X *S. chacoense* (9708-07, 9506-04)

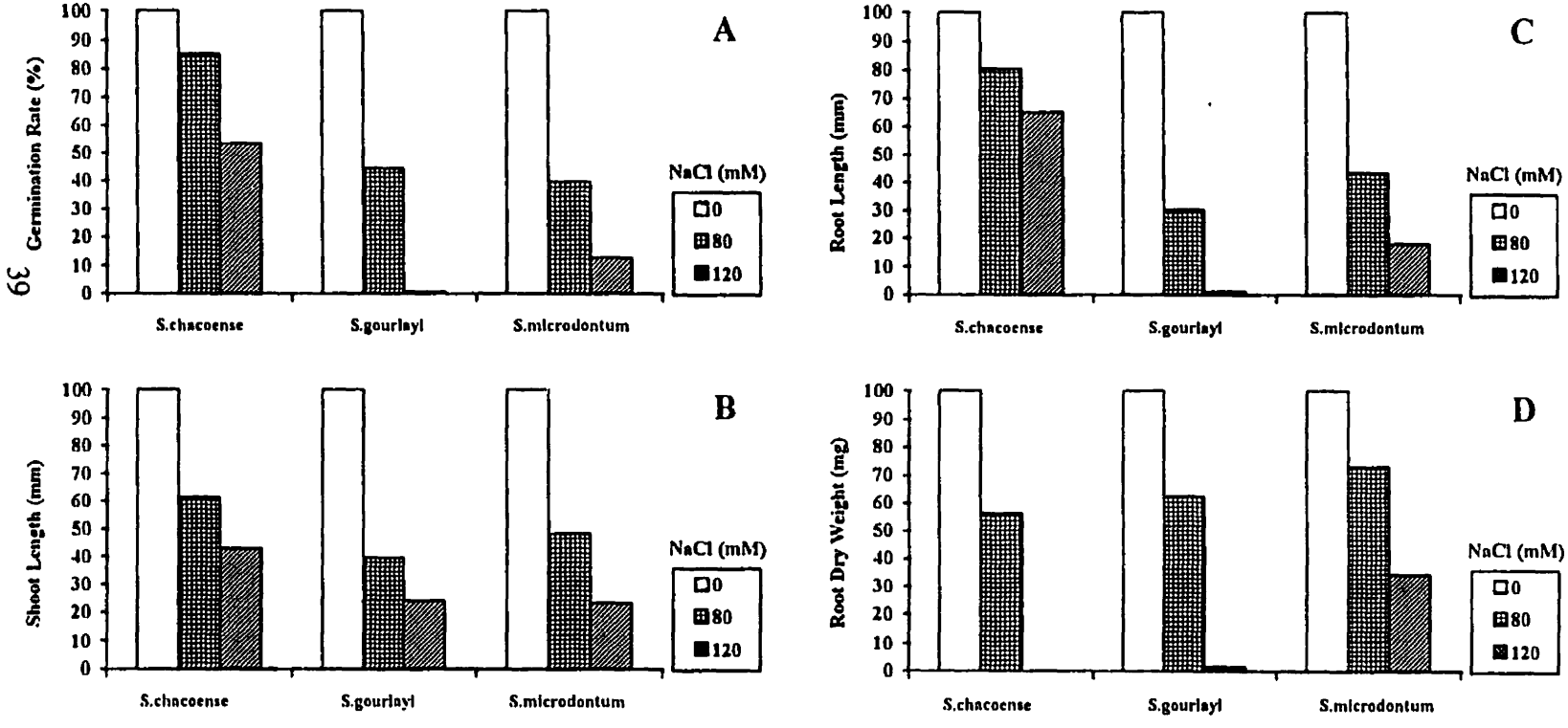


Fig. 3.3 **The effect of NaCl (0, 80, and 120 mM) on three potato species seed germination rate and early seedling growth in vitro**

outperformed hybrids derived from the other two wild species in the single-node cutting bioassay. Although the six hybrids were not directly compared with the seven cultivars, preliminary evidence showed that their performance in the single-node cutting bioassay was similar. Although these results need to be tested under field conditions, they suggested that application of tissue culture technology for in vitro screening of germplasm, utilizing single-node cutting and seedling bioassays, holds promise to develop more salinity tolerant potato plants and help meet the breeding challenges caused by salinization of cultivated land. These bioassay methods were used to evaluate the in vitro NaCl tolerance of more cultivars and potato genotypes (*Solanum* spp.) (Khrais, 1996).

PREFACE TO CHAPTER 4

This Chapter reports on evaluation of different potato genotypes tested in Chapter 3 for their salinity tolerance under NaCl stress conditions using three in vitro bioassays, the single-node cutting, root tip segment, and microtuberization culture. A range of potato genotypes of wild species, hybrids and cvs. were tested in the same bioassay experiment to develop an efficient in vitro bioassay method for screening and selection of salt tolerant genotypes.

CHAPTER 4 IN VITRO BIOASSAYS FOR SALINITY TOLERANCE SCREENING OF POTATO

Abstract

Three in vitro bioassays (single-node cutting, root tip segment, and microtuberization), gave similar results in ranking the salinity (NaCl) tolerance of several potato genotypes (*Solanum* spp.). Salinity stress consistently depressed plantlet growth in the single-node cutting and root tip segment bioassays and reduced microtuber yield in the microtuberization bioassay. The single-node cutting bioassay was simpler to perform than the root tip segment and microtuberization bioassays and did not exclude certain genotypes as did the microtuberization bioassay. The single-node cutting bioassay is recommended as a substitute for more labour-intensive and costly field assessments of salinity effects on yield.

4.1 Introduction

Soil salinity stress is a critical environmental constraint to crop productivity. Six of the 14 billion ha of arable land available in the world are located in arid or semi-arid areas, and of this, about 1 billion ha are affected by excess salt (Christiansen, 1982). Potato is considered moderately salt sensitive compared with other crops, since a salinity level of 2.0 - 3.0 dSm^{-1} in soil reduced plant growth and tuber yield up to 50 % (Maas and Hoffman,

1977).

Despite the global importance of the potato crop, the salinity tolerance of only a small number of potato genotypes has been evaluated under field, greenhouse, or in vitro conditions. Field trials (Ahmad and Abdullah, 1979; Bouaziz, 1980; Levy, 1992; Paliwal and Yadav, 1980) and outdoor pot trials (Levy et al., 1988) were primarily focussed on NaCl (or mixed salt) effects on tuber yields. Greenhouse pot trials were used to examine genotype salinity tolerance under NaCl or mixed sodium salt irrigation solutions based on either plant tuber yield (Bruns and Caesar, 1990; Heuer and Nadler, 1995; Nadler and Heuer, 1995) or relative reduction of foliage dry weight (Bilski et al., 1988a,b), or haulm fresh weight (Naik and Widholm, 1993).

In vitro evaluations of NaCl or mixed salt stress effects on potato genotypes were recently proposed as alternatives to the costly, labour-intensive, and sometimes problematic field-based evaluations. These used single-node cuttings (Arslan et al., 1987; Naik and Widholm, 1993; Zhang et al., 1993), five-node stem cuttings (Morpurgo, 1991; Morpurgo and Rodriguez, 1987), root tip segments or suspension cultures (Naik and Widholm, 1993) and measured one or more growth parameters at one or more salinity levels in vitro. Morpurgo (1991) found a correlation between root fresh weights of in vitro plantlets derived from five-node stem cuttings and tuber fresh weights under saline irrigation in the field. However, the relatively high NaCl levels used for screening resulted in little growth in vitro.

The only group to compare more than one in vitro bioassay (Naik and Widholm, 1993) found a correlation between in vitro root tip segments, but not suspension cultures exposed to salinized medium, and haulm fresh weight in greenhouse pot trials. However, early foliage growth does not necessarily correlate with yield (Vayda, 1990) and haulms were generally less affected than yield parameters by salinity stress (Levy, 1992).

The objectives of this study were to assess the potential and develop recomendations for in vitro salinity screening of potato. Genotypes included three different groups: wild species, a hybrid, and cultivars. These were tested using three in vitro bioassays (single-node cuttings, root tip segments, and microtuberization), at a range of salt levels in vitro. These rankings were evaluated based on crop yield in field lysimeters under saline irrigation conditions (Chapter 5).

4.2 Materials and methods

4.2.1 Plant materials

True potato seeds of two *S. chacoense* (PI#: 458309, 473403) and one *S. microdontum* (PI#: 458353) accessions were received from the United States Department of Agriculture, Agricultural Research Service, Inter-Regional Potato Introduction Station, Sturgeon Bay, WI, USA in March, 1993. Seeds were disinfested for 20 min in 10 % household bleach (5.25 % sodium hypochlorite), rinsed 3 times with sterile distilled water, soaked for

24 h in a 2 g l^{-1} filter-sterilized gibberellic acid (GA_3) solution to break dormancy (if any), and again rinsed with sterile distilled water. Seeds were aseptically transferred to micropropagation medium and germinated in vitro.

Five potato cvs. Atlantic, Kennebec, Norland, Russet Burbank, and Spunta were received as in vitro plantlets from the Potato Propagation Centre, Fredericton, NB, Canada in March, 1993. The in vitro seedlings, and the plantlets received in vitro, were micropropagated using single-node cuttings.

Tubers of a hybrid cross between *S. chacoense, S. microdontum*, and *S. tuberosum* came from the Agriculture and Agri-food Canada Research Station, Fredericton, NB, Canada in March, 1993. Hybrid tubers were planted in the greenhouse. Shoot tips were then explanted to culture and the resulting plantlets were micropropagated.

In Naik and Widholm (1993) procedure, explants used for the root tip segment bioassay were collected from buds of germinated seed tubers. These were disinfested and cultured on MS (1962) medium and after 1 wk in vitro root tip segments were collected. The method was modified for use in our experiment. This involved collecting root segment from 1-wk-old nodal cuttings. This represented a significant saving in time and labour.

4.2.2 Culture conditions and medium

Cultures were incubated at 25 ± 2 °C with 16/8 h D/N at 40 μmol m^{-2} s^{-1}

photosynthetic photon flux (PPF) (cool white fluorescent light). For micropropagation, a modified MS (Murashige and Skoog, 1962) basal salt solution was used, supplemented with (mg l^{-1}): Ca-pantothenate (2.0), glycine (2.0), myo-inositol (100), niacin (0.5), pyridoxine·HCl (0.5), thiamine·HCl (1.0), 3 % sucrose, and 0.6 % agar (Anachemia, Lachine, QC, Canada). The medium was adjusted to pH 5.7 prior to autoclaving at 121 °C, 103.46 KPa for 20 min.

4.2.3 Single-node cutting bioassay

Single-nodes were cut from micropropagated plantlets of the two wild species, the hybrid, and the five cultivars. To achieve the most consistent response to salinity, nodes were selected from the middle of each plantlet and the shoot apex and base were discarded (Naik and Widholm, 1993). Each single-node cutting, 1-cm-long with one leaf and one axillary bud, was individually cultured in a 25 x 150 mm Pyrex glass culture tube. Tubes contained 15 ml of potato micropropagation medium with NaCl at 0, 40, 80, or 120 mM. There were five single-node cuttings of each genotype at each salinity level. The experiment lasted 4 wk and was conducted 3 times. Plantlets were harvested for growth data collection which included shoot and root lengths (SL, RL), fresh (SFW, RFW), and dry (60 °C oven for 48 h) weights (SDW, RDW) at each salinity level. Growth data were expressed as true or relative (percentage of the growth in control medium) values.

4.2.4 Root tip segment bioassay

One-cm-long root tip segments were cut directly from 1-wk-old single-node cuttings growing in potato micropropagation medium and then cultured either on solid or in liquid potato micropropagation medium containing NaCl at 0, 40, 80, or 120 mM. Root tip segments of the three accessions of the two wild species, the hybrid, and the five cultivars were placed into 9-cm-diameter petri dishes containing 20 ml solid medium. Root tip segments of the hybrid and three of the cvs., Kennebec, Norland, and Russet Burbank, were placed into 125 ml flasks with 25 ml liquid medium and agitated on a rotary platform (Innova 2300 Platform Shaker, New Brunswick Scientific) at 60 rpm. There were five root tip segments per petri dish or flask for each genotype at each salinity level tested. Incubation occurred in the dark at 25 ± 2 °C. Each experiment lasted 1 wk and was performed 3 times. The data collected included root extension growth (final length minus initial length), relative to growth in control medium, for both solid and liquid medium.

4.2.5 Microtuberization bioassay

Micropropagation was carried out using the two-step procedure described by Leclerc et al. (1994). In step-1, 3 plantlets, with root and apex severed, trimmed to 5 nodes each, were layered in 50 ml of liquid potato micropropagation medium, containing (mg l^{-1}) 6-benzylaminopurine (0.5),

GA_3 (0.4) and with sucrose reduced to 2 %, in each 400 ml plastic container (Better Plastics, Kissamee, FL, USA). These were incubated for 4 wk at 25 ± 2 °C and with 16/8 h D/N cycle. Step-2 involved draining off the residual medium and replacing it with 50 ml of a similar medium without growth regulators, with sucrose increased to 8 % and with NaCl at 0, 80, or 160 mM. Four more weeks of incubation occurred at a decreased temperature (15 ± 1 °C) and with 8/16 h D/N cycle. There were 6 containers at each salinity level for each genotype. The experiment was performed twice. Data were collected on microtuber yield (number and fresh weight) and size distribution per container.

4.2.6 Data analysis

Data analysis included SAS procedures of the general linear model (GLM), correlation (CORR), and Ward's minimum-variance cluster analysis (CA) (SAS, 1989). The difference among treatments was tested with least square difference (LSD) comparison at the 5 % level of significance.

4.3 Results and discussion

4.3.1 Single-node cutting bioassay

Plantlet growth parameters were not affected by 40 mM NaCl, while 80 and 120 mM NaCl significantly reduced all plantlet growth parameters, compared with the control (0 mM) and with each other (Table 4.1). The only

Table 4.1 Relative values (true values) of each of six growth parameters, for all the genotypes, in response to NaCl treatment in the single-node cutting bioassay.

NaCl (mM)	Growth parameters						Average
	Shoot			Root			
	length (mm)	fresh weight (mg)	dry weight (mg)	length (mm)	fresh weight (mg)	dry weight (mg)	
0	100.00a[a] (60.02)	100.00a (244.00)	100.00a (16.30)	100.00a (69.01)	100.00a (70.40)	100.00a (4.80)	100.00
40	94.75a	84.71a	86.47a	99.63a	93.34a	77.41a	89.14
80	59.81b	44.40b	51.46b	79.06b	48.83b	43.86b	54.57
120	28.51c	20.68c	21.70c	64.76b	23.85c	22.87c	30.11
means[b]	59.96	49.93	53.21	81.14	55.34	48.05	57.94

Significance[c]	SL	SFW	SDW	RL	RFW	RDW
Treatment (T)	***	***	***	**	***	***
Cultivar (C)	**	***	**	**	***	***
C x T	**	**	***	**	**	***

[a]Values in each column with the same letter are not significantly different at LSD $p < 0.05$.
[b]Treatment means are the relative values at 40, 80, and 120 mM NaCl.
[c]*, **, or *** indicate significance at the 0.05, 0.01 or 0.001 probability levels respectively.

exception to this was RL, which was similarly affected by 80 and 120 mM NaCl, and less affected by salinity than the other growth parameters. At 40, 80, and 120 mM NaCl, the average plantlet growth parameter was 89, 55, and 30 % of control levels, respectively (Table 4.1). Correlation analysis among NaCl levels showed that all the growth parameter true values were correlated between 0 and 40 mM NaCl, with only partial correlation at higher NaCl levels (Table 4.2). Correlation analysis for the relationship of the relative values among the six growth parameters at 40, 80, and 120 mM NaCl showed no consistent correlation across the range of salinity levels (Table 4.3). This infers that growth (vigour) in the control medium or relative growth data at 40 or 80 mM cannot be used to predict genotype tolerance at higher salinity levels in vitro, and that these must be tested. At 80 mM NaCl, all the growth parameters were correlated except RL. However, at 40 or 120 mM NaCl, only a few growth parameters were correlated to each other and these were not consistent between salinity levels. It was therefore not possible to recommend the use of any one of the six growth parameters over any other for salinity tolerance ranking. Differential growth responses, observed at different salinity levels in vitro, are consistent with similar observations found in vivo, and suggest the involvement of different genes (Tal, 1994).

Cluster analysis was chosen to evaluate genotype salinity tolerance based on the relative values of all six growth parameters at each salinity

Table 4.2 Correlation analysis on the true values of each of six growth parameters, for all the genotypes, in response to NaCl treatment in the single-node cutting bioassay.

NaCl (mM)	40	80	120
0	SL, SFW, SDW RL, RFW, RDW	SFW, RFW, RDW	SFW, SDW,
40		SFW	SL, SFW, SDW RDW
80			SL, SFW

Note: The growth parameters included shoot and root length (SL, RL), fresh (SFW, RFW) and oven-dry (SDW, RDW) weights.

Note: The growth parameters listed in the table are significantly ($r^2 > 0.9$, $p < 0.001$) correlated between the two NaCl treatments.

Table 4.3 Correlation analysis on the relative values of each of six growth parameters, for all the genotypes, in response to NaCl treatment in the single-node cutting bioassay.

		RL	SFW	SDW	RFW	RDW
SL[a]	40	ns[b]	0.75**	ns	0.70*	ns
	80	ns	0.90***	0.83*	0.88**	0.78**
	120	ns	0.93***	ns	0.95***	ns
RL	40		ns	ns	0.87**	ns
	80		ns	ns	ns	ns
	120		ns	0.72**	ns	0.88**
SFW	40			ns	ns	ns
	80			0.93***	0.93***	0.82**
	120			0.70*	0.98***	ns
SDW	40				ns	0.9***
	80				0.95***	0.72**
	120				0.68*	0.9***
RFW	40					ns
	80					0.80**
	120					ns

[a]The growth parameters included shoot and root length (SL, RL), fresh (SFW, RFW) and oven-dry (SDW, RDW) weights.
[b]ns, *, **, *** refers to correlations which are not significant, or significant at the 0.05, 0.01, or 0.001 probability levels, respectively.

Table 4.4 Potato genotype response to NaCl stress in the single-node cutting bioassay after grouping by cluster analysis using the relative values of six growth parameters (shoot and root length, fresh and dry weights) at each salinity level.

NaCl (mM)	First cluster	Second cluster
40	Atlantic Norland R. Burbank Hybrid (9506-04) *S. microdontum* (PI# 458353) *S. chacoense* (PI# 473403)	Kennebec Spunta *S. chacoense* (PI#[a] 458309)
80	Atlantic Kennebec R. Burbank Hybrid (9506-04) *S. microdontum* (PI# 458353)	Norland Spunta *S. chacoense* (PI# 458309) *S. chacoense* (PI# 473403)
120	Kennebec R. Burbank Hybrid (9506-04)	Atlantic Norland Spunta *S. chacoense* (PI# 458309) *S. chacoense* (PI# 473403) *S. microdontum* (PI#458353)

[a]PI# = Plant Introduction number, Inter-Regional Potato Introduction Station, Sturgeon Bay, WI, USA

level (Table 4.4) (Zhang and Donnelly, 1995). The increase in the cubic clustering criterion and the pseudo F statistic, and the decrease in the t^2 statistic, were used to partition the cluster groups. Two cluster groups were apparent at every NaCl level. As the salinity level increased the number of genotypes in the first cluster group decreased. For each cluster group, the relative values of each six growth parameters in all the genotypes were averaged to express their response at each NaCl level (Table 4.5) (Snowdon and Wheeler, 1993; Zhang and Donnelly, 1995). The hybrid, and cvs. Kennebec and Russet Burbank were consistently found in the first group. These three genotypes and cv. Norland, selected from the second group, are shown in Fig. 4.1.

4.3.2 Root tip segment bioassay

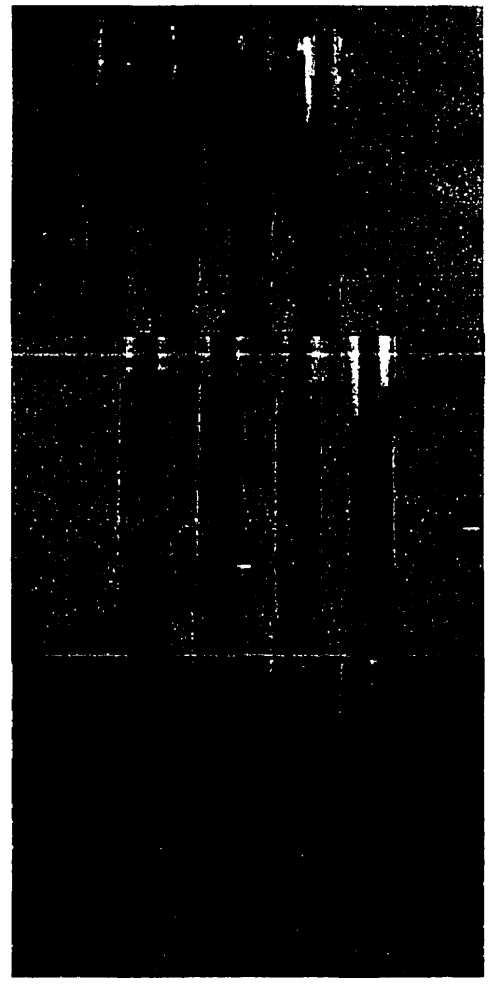
Root tip segment growth on solid medium was inhibited by 40 mM NaCl in all genotypes except the hybrid and *S. chacoense* (PI #473403) and at 80 and 120 mM NaCl growth was inhibited in all genotypes (Table 4.6 A). Root tip segment growth in liquid medium was not affected at 40 mM NaCl, but was inhibited at higher NaCl concentrations (Table 4.6 B). The impact on root growth was less in liquid (94.42 %, 70.11 %) than in solid medium (73.45 %, 48.29 %) in the 40 and 80 mM NaCl treatments respectively, but similarly drastic in the 120 mM NaCl treatments (Table 4.6 A,B). For cultivars used in the lysimeter field trial (Chapter 5), inconsistency occurred

Table 4.5 The means of the relative values, for each six growth parameters, in all the genotypes of each cluster group in the single-node cutting bioassay under NaCl stress at 40, 80 or 120 mM.

NaCl (mM)	Growth parameters						Average
	Shoot			Root			
	length (mm)	fresh weight (mg)	dry weight (mg)	length (mm)	fresh weight (mg)	dry weight (mg)	
First group							
40	92.96[a]	103.42	91.94	92.31	90.47	76.70	91.3
	11.68[b]	8.22	7.47	13.50	8.95	11.72	
80	79.85	93.52	67.62	69.20	81.25	64.21	75.94
	16.72	15.10	18.22	16.80	21.28	14.20	
120	49.37	76.82	41.06	36.42	46.48	31.38	46.92
	15.16	8.50	10.04	5.07	13.74	4.26	
Second group							
40	85.75	92.83	74.26	82.07	79.93	70.58	80.91
	9.83	2.65	2.18	3.15	7.51	3.76	
80	41.08	72.16	28.59	37.26	35.61	31.07	40.96
	7.28	8.20	4.78	6.75	5.40	7.32	
120	16.45	60.04	11.84	17.62	15.38	17.10	23.07
	18.16	23.49	15.10	10.84	16.99	9.70	

[a]Averaged relative values of parameter among the genotypes in that cluster group.
[b]Standard error.

Fig. 4.1 The single-node cutting bioassay showing two cluster groups.

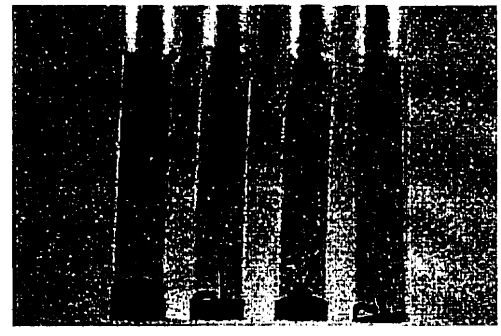


Hybrid

First Cluster

Kennebec

R. Burbank



Second Cluster

Norland

NaCl (mM) 0 40 80 120

Table 4.6 Relative values (true values) of root extension growth (mm) for potato genotypes in the root tip segment bioassay on A. solid growth medium and B. liquid growth medium.

A	NaCl Concentration (mM)					
Genotypes	0		40	80	120	Treatment means
Atlantic	100.00a[a]	(39.30)	54.33b	36.01c	34.50c	41.64[b]
Kennebec	100.00a	(43.53)	71.95b	63.31b	35.99c	57.08
Norland	100.00a	(31.48)	62.45b	42.47b	30.34c	45.09
R.Burbank	100.00a	(29.03)	75.06b	41.72c	36.96c	52.88
Spunta	100.00a	(55.22)	62.69b	51.67bc	44.29c	52.89
Hybrid (9506-04)	100.00a	(74.18)	89.58a	60.53b	37.92c	62.68
S. chacoense (PI# 458309)[c]	100.00a	(144.45)	72.14b	39.19c	20.18d	43.83
S. chacoense (PI# 473403)	100.00a	(117.75)	100.60a	67.18b	62.39b	76.71
S. microdontum (PI# 458353)	100.00a	(73.21)	79.87b	32.53c	29.44c	47.28
Average	100.00	(67.57)	74.30	48.29	35.77	

(continued on page 59)

(continued from the previous page)

B	NaCl Concentration (mM)					
Genotypes	0		40	80	120	Treatment means
Kennebec	100.00a	(14.94)	91.50a	68.94b	43.64c	68.03
Norland	100.00a	(13.78)	81.42a	59.80b	29.61c	56.94
R. Burbank	100.00a	(28.81)	91.67a	68.55b	24.60c	61.73
Hybrid (9506-04)	100.00a	(43.13)	113.10a	83.13b	29.70c	75.31
Average	100.00	(25.17)	94.42	70.11	31.98	

[a]Values in each column with the same letter are not significantly different at LSD $p<0.05$.
[b]Treatment means are the relative values at 40, 80, and 120 mM NaCl.
[c]PI# = Plant Introduction number, Inter-Regional Potato Introduction Station, Sturgeon Bay, WI, USA.

in the relative rankings of root extension growth at different salinity levels in liquid or solid medium (Table 4.6 A,B). When the treatment means for root extension growth in salinized medium were used for ranking, as done by Naik and Widholm (1993) for the same reasons, cvs. Kennebec and Russet Burbank outperformed Norland in both solid (57.08 %, 52.88 % and 45.09 %) and liquid (68.03 %, 61.73 %, and 56.94 %) medium (Table 4.6 A,B).

4.3.3 Microtuberization bioassay

The genotypes used in the single-node cutting bioassay were all tested in the microtuberization bioassay but of these only the five cultivars microtuberized. Unfortunately, this excluded the hybrid and the wild species. Total microtuber fresh weights per container were significantly decreased in all cultivars at both treatments levels, except for cvs. Kennebec and Russet Burbank which increased at 80 mM NaCl (Table 4.7).

For the cultivars used in the lysimeter field trial (Chapter 5), the relative total microtuber fresh weight, expressed as the averaged values for 80 and 160 mM NaCl treatments relative to the control, was 105.7 %, 43.58 %, and 123.13 % respectively in cvs. Kennebec, Norland, and Russet Burbank. This increase in cvs. Kennebec and Russet Burbank was primarily due to the increase in the $\geq$0.5 cm size class, which contributed more to total microtuber yield (Fig. 4.2). The total microtuber number, which was increased in the $\geq$0.5 cm size class in cv. Russet Burbank at 80 mM NaCl,

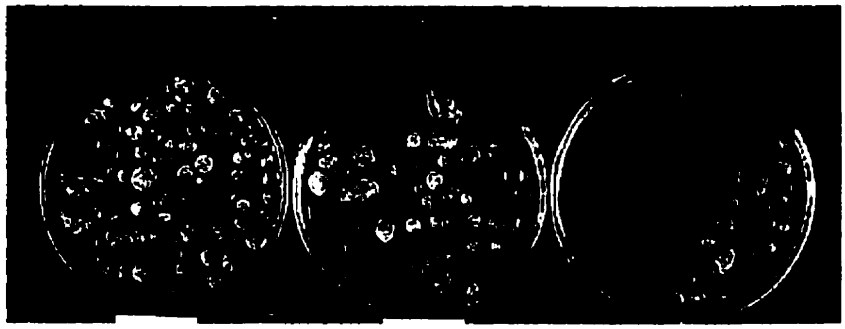
Table 4.7 Microtuber yield, including number (No.), fresh weight (FW) in grams, and size distribution per culture container, at 0, 80, and 160 mM NaCl.

		Microtuber size distribution (cm)					
		<0.5		≥0.5		Total	
Cultivar	NaCl (mM)	No.	FW	No.	FW	No.	FW
Atlantic	0	2.0b[a]	0.13b	4.0a	2.12a	6.0a	2.25a
	80	4.6a	0.30a	2.0a	0.44b	6.6a	0.74b
	160	1.3b	0.11b	2.8a	0.65b	4.1a	0.76b
Kennebec	0	17.2a	0.72a	3.8a	0.77b	21.0a	1.49b
	80	12.3b	0.66a	4.0a	1.41a	16.3ab	2.11a
	160	12.4b	0.68a	1.0b	0.33b	13.4b	1.04b
Norland	0	4.6a	0.21a	5.3a	0.36a	9.9a	2.57a
	80	4.4a	0.19a	5.1a	0.25b	9.5a	1.44b
	160	4.6a	0.26a	3.3a	0.54b	7.9a	0.80b
Russet Burbank	0	20.0a	0.34a	6.2b	0.74b	26.2a	1.08b
	80	10.5b	0.31a	10.3a	2.09a	20.8a	2.20a
	160	7.0b	0.18b	1.8c	0.28b	8.8b	0.46b
Spunta	0	7.9a	0.40a	2.3a	0.70a	10.2a	1.10a
	80	5.2a	0.32a	1.3a	0.46b	6.5a	0.78b
	160	5.1a	0.24a	1.7a	0.32b	7.4a	0.56b

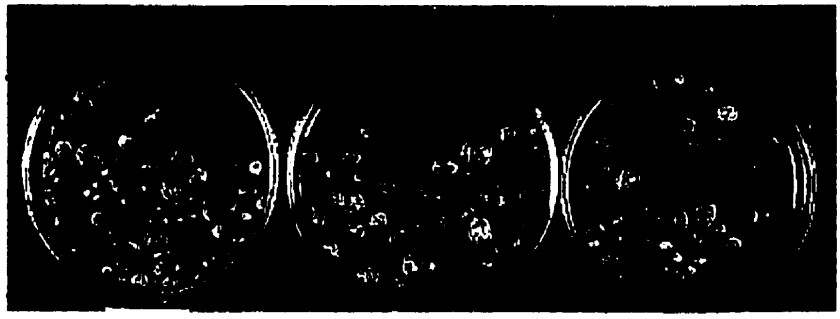
[a]Values in each column with the same letter are not significantly different at LSD $p<0.05$.

Fig. 4.2 The microtuberization bioassay in cvs. Kennebec (upper) and Russet Burbank (lower) showing yield of microtubers harvested after step-2 under NaCl stress (0, 80, and 160 mM)

Kennebec



R. Burbank



0 80 160

NaCl (mM)

was not affected in cv. Norland, and was significantly decreased in cvs. Kennebec and Russet Burbank at 160 mM NaCl (Table 4.7).

It is of potential commercial importance that the microtuber number and fresh weights of some cultivars were increased by 80 mM NaCl (Table 4.7). The presence of NaCl also stimulated potato axillary bud cultures (Potluri and Prasad, 1993). The single-node cutting bioassay was simpler to perform than the root tip segment or microtuberization bioassays and did not exclude certain genotypes such as the wild species and hybrids as did the microtuberization bioassay. On the negative side, data collection was more time-consuming for the single-node cutting bioassay. The longer duration of the single-node cutting, compared with the root tip segment bioassay, is not a disadvantage. Salinity exposure of 1 mo or more duration may distinguish more precisely between osmotic and specific ion effects (Munns, 1993; Munns et al., 1995). The single-node cutting bioassay is therefore recommended for in vitro screening for salinity tolerance.

The salinity levels suggested in the literature for in vitro screening of potato genotypes have varied widely, as have the growth parameters taken at harvest. There is merit in evaluation at a range of salinity levels, since different genes are apparently expressed at different stress levels in vitro as described in vivo (Tal, 1994). We recommend taking plantlet growth parameter measurements including shoot and root lengths, fresh and dry weights, at 0, 80, and 120 mM NaCl. Screening at 40 mM, where response

was generally similar to control levels, could be omitted. This should be followed by cluster analysis to partition the genotypes into groups.

PREFACE TO CHAPTER 5

This chapter reports on the evaluation of three potato cultivars used in the in vitro bioassays (Chapter 4) for their salinity tolerance under field lysimeter conditions with saline irrigation water. The objective of this experiment was to utilize the field lysimeter rankings of three cultivars of potato to evaluate the results of in vitro bioassays for salinity tolerance screening and selection in potato.

CHAPTER 5 THE EFFECTS OF SALINITY STRESS ON POTATO YIELD IN FIELD LYSIMETER TRIALS

Abstract

Field lysimeters were used to evaluate the salinity tolerance of three potato cultivars under control (averaged root zone EC level of 1.5 dSm^{-1}) and saline stress (averaged root zone EC level of 3.5 dSm^{-1}) conditions. Relative plant growth and tuber yield criteria used to rank the cultivar growth parameters, including shoot and root lengths, fresh and dry weights, and shoot number, were not significantly affected by saline water irrigation. Kennebec and Russet Burbank cvs. were more salinity tolerant than cv. Norland based on tuber yield criteria. In addition, cv. Norland foliage developed a bronze colour under saline stress conditions while Kennebec and Russet Burbank cvs. did not. This cultivar ranking for salinity tolerance was similar to that of the in vitro bioassays and supports the claim that in vitro bioassays can be used to evaluate the relative salinity tolerance of potato genotypes.

5.1 Introduction

In vitro methods to screen and select salt tolerant genotypes may provide faster and more precise estimations of plant growth and yield under salinity stress compared with field evaluation (Shannon, 1984). However, in vitro

screening and selection results must be verified under field conditions before their use can be advocated (Jones, 1986; Miller et al., 1991; Morpurgo, 1991). Among the several reports of in vitro screening experiments in potato (Arslan et al., 1987; Kim et al., 1995; Morpurgo, 1991; Morpurgo and Rodriguez, 1987; Naik and Widholm, 1993), only Morpurgo (1991) tried a field experiment to correlate the in vitro ranking results. This correlation first showed the feasibility of in vitro salinity tolerance screening procedures in potato. However, the field salinity levels described by Morpurgo (1991) varied from EC level of 4.0 to 7.0 dSm^{-1} during the experimental season. This large salinity variation is typical of field trials and difficult to replicate. It is necessary to set up a field trial with a more standardized stress environment. In a more environmentally controlled field facility (Milbocker, 1988; Saxena et al., 1994), plant growth in lysimeters was used to evaluate the results of rapid laboratory root leachate detection procedures to test salinity tolerance in *Pistachio* (Picchioni and Miyamoto, 1990 and 1991).

The objective of this study was to assess the relative salinity tolerance of three potato cultivars based on tuber yield in field lysimeters irrigated with saline water to determine the correlation with in vitro bioassay ranking results (Chapter 4).

5.2 Materials and methods

5.2.1 Plant materials

Potato seed tubers (cvs. Norland, Kennebec, and Russet Burbank) weighing 60-80 g each were received from the Bon Accord Elite Seed Farm, NB, Canada in May, 1995.

5.2.2 Lysimeter field trials

The field lysimeters were constructed by the Agricultural and Biosystems Engineering Department and were located on the Macdonald Campus of McGill University (Ste-Anne-de-Bellevue, QC, Canada). Lysimeters (120 cm tall, 50 cm in diameter) were filled with sandy soil (95.45 % sand:4.55 % silt clay) (Fig. 5.1). They were initially flushed with fresh water (EC level of 0.2 dSm^{-1}) until the drainage water reached the same EC level as that of the flushing water, then salinized and calibrated with diluted natural saline water.

The saline water was taken from a well on the farm of Mr. Charbonneau (St. Louis Sur Richelieu, QC, Canada). This water contained (g l^{-1}) Cl^{-} (6.5), Na^{+} (4.1), and other minor ions such as CO^{-2} (0.5), Ca^{+2} (0.37), K^{+} (0.09), Mg^{+2} (0.3), and NO^{-}_{3} (0.37). The saline water was then diluted with fresh tap water to make different salinity levels for lysimeter calibration and irrigation.

Lysimeters were flushed with diluted natural saline water to calibrate half of the lysimeters to EC level of 1.0 and the other half to 2.0 dSm^{-1}

Fig. 5.1 Field lysimeters used for potato salinity tolerance evaluations.

(approx. 11 and 22 mM NaCl) before the seed tubers of cvs. Kennebec, Norland, and Russet Burbank were planted. The lysimeters were then subirrigated (sub-surface irrigation) with diluted natural saline water at EC levels of 1.0 and 9.0 dSm^{-1} (approx. 11 and 99 mM NaCl), started right after tuber planting and stopped 1 wk before the tuber harvest, with the lysimeter water table at 40 cm. The salinity levels in the lysimeters were monitored with TDR (Time Domain Reflectometry) method (Appendix 6).

Potato seed tubers were individually planted into the lysimeters at a depth of 10 cm. Fertilizer at 200 kg ha^{-1} with NH_4NO_3 (36 % N), murat of potash (60 % K), and triple superphosphate (46 % P) was applied at the time of planting. Plants were hilled twice at the end of the second and fourth weeks after planting. Rain was excluded from the lysimeters with plastic shields through which the above-ground shoots projected. At harvest, data were collected on total tuber yield including number, fresh weight, and grade (categorized by diameter; undergrade = <3.0, grade B = 3.1-4.5, or grade A > 4.6 cm), shoot and root lengths, fresh and dry weights, and shoot number.

There were three blocks in the experiment. There were 36 treatments (2 EC levels in lysimeter calibration X 2 EC levels in subirrigation X 3 cvs. X 3 blocks). Thirty-six lysimeters were used. Treatments were arranged in a CRBD design. Potato seed tubers were planted on May 30 and plants were harvested on August 23, 1995. Data were collected on shoot length, fresh

and dry weights, and number (SN), as well as root fresh and dry weights.

5.2.3 Data analysis

Data were analysed using the general linear model (GLM) (SAS, 1989). The treatment differences were tested with the least square difference (LSD) comparison at the 5 % level of significance.

5.3 Results and discussion

Growth parameters were not significantly affected by any of the salinity treatments (Table 5.1). However, bronzed foliage was apparent in cv. Norland but not in cvs. Kennebec or Russet Burbank.

The salinity levels in the lysimeters fluctuated but increased slightly during the growing season and were monitored for 11 wk (Appendix 6). In the control lysimeters calibrated or flushed with an EC level of 1.0 dSm^{-1} and followed subirrigation with saline water at EC levels of 1.0 and 9.0 dSm^{-1} respectively, the actual root zone EC levels in the lysimeters were averaged 1.26 and 1.75 dSm^{-1} respectively throughout of the season (Appendix 6), which was averaged as EC of 1.5 dSm^{-1} in the control due to no treatment difference. In the same subirrigation treatments with the lysimeter EC level calibrated at 2.0 dSm^{-1}, the actual root zone EC levels in the lysimeters were averaged 3.13 and 3.89 dSm^{-1} respectively (Appendix 6), which was averaged as EC of 3.5 dSm^{-1} in the saline treatment due to no

Table 5.1 Potato plant vegetative growth (g) per field lysimeter under saline water irrigation at root zone EC levels of 1.5 and 3.5 dSm^{-1}.

Cultivars	EC (dSm^{-1})	SFW	SDW	SL	SN	RFW	RDW
Kennebec	1.5	326.5	39.2	46.7	3.3	37.2	4.0
	3.5	313.6	44.2	49.0	3.7	36.0	2.7
Norland	1.5	72.8	15.8	36.7	4.0	19.8	1.7
	3.5	44.7	11.7	37.2	4.2	12.0	0.8
R. Burbank	1.5	368.8	50.8	58.7	5.3	56.5	5.4
	3.5	343.8	47.5	47.5	4.2	45.0	4.3

SL*:All the parameters, e.g., SL, SFW, SDW, RFW, RDW, and SN were collected at plant harvest.

treatment difference. The salinity level variations in our lysimeter trials were very minimal. Compared with Morpurgo's (1991) field experiment in which salinity level varied from 4.0 to 7.0 dSm^{-1} during the experimental season, lysimeters clearly helped to standardize field environmental conditions for salinity stress, in agreement with Milbocker (1988) and Saxena et al. (1994).

Saline water subirrigation treatments (1 and 9 dSm^{-1}) yielded no significant effects on observed parameters both in potato plant vegetative growth and tuber yield (data not shown). The results were pooled into saline water calibration treatments. Lysimeters calibrated with saline water to EC 2.0 dSm^{-1} with averaged EC 3.5 dSm^{-1} at root zone significantly reduced relative tuber yield, both total number and weight, in cv. Norland, but not in cvs. Kennebec or Russet Burbank, in comparison with the control, calibrated with non-saline water to EC 1.0 dSm^{-1} with averaged EC 1.5 dSm^{-1} at root zone (Table 5.2). The total tuber number increase in cv. Norland exposed to saline water was due to increased undergrade (<3.0 cm) and grade B (3.0-4.5 cm) but not grade A (> =4.6 cm) tubers. The total tuber weight depression in cv. Norland was primarily due to weight reduction in grade A tubers. Similarly, microtuber fresh weight differences under NaCl stress were due to reduced weight of the larger size class of microtubers (Table 4.7).

The number of stems per plant is not usually affected by saline stress (Levy, 1992; Nadler and Heuer, 1995). Tuber yield was more sensitive to salinity than haulm growth. Our results agree with these reports. Tuber fresh

Table 5.2 Tuber yield (number (No.) and fresh weight (FW) in grams) and size distribution per field lysimeter under saline water irrigation at root zone EC levels of 1.5 and 3.5 dSm^{-1}.

Cultivar	EC	Tuber size distribution (cm)							
		<0.3		3.1-4.5		>4.6		Total	
	(dSm^{-1})	No.	Gram	No.	Gram	No.	Gram	No.	Gram
Kennebec	1.5	1.2a[a]	2a	2.0a	83a	2.2a	238a	5.3a	332a
	3.5	0.8a	8a	2.8a	138a	2.7a	270a	6.3a	416a
Norland	1.5	0.5b	7a	1.8b	82a	3.5a	379a	4.2b	468a
	3.5	1.8a	17a	3.5a	125a	2.0b	145b	5.7a	288b
Russet Burbank	1.5	0.8a	13a	2.5a	125a	2.2a	241a	5.5a	380a
	3.5	1.3a	15a	2.7a	111a	1.5a	148a	5.5a	275a

[a]Values with the same letter in each column are not significantly different at LSD $p<0.05$.

weight decrease in cv. Norland (38.56 % of the control) was similar to the reported observations (Maas and Hoffman, 1977).

The cultivars used in the field lysimeter trial were the early maturity, cv. Norland (90 d), and the mid- or later-season maturity, cvs. Kennebec (120 d) Russet Burbank (140 d). Fourteen cultivars including two early maturing cultivars were tested by Levy (1992). He found no interaction of potato yield with maturity differences under saline stress. The salinity tolerance variations observed in our three cultivars were probably due to their relative salt tolerance not their seasonality.

5.4 Conclusions

Relative salinity tolerance in field lysimeters indicated that cvs. Kennebec and Russet Burbank were more salinity tolerant than Norland based on total tuber fresh weight. This was consistent with the rankings in the single-node cutting bioassay using cluster analysis based on the relative means of the six growth parameters at three NaCl treatment levels (Tables 4.4 and 4.5), the root tip segment bioassay based on the relative means of root extension growth averaged over three NaCl treatment levels in liquid or solid medium (Table 4.6 A,B), and the microtuberization bioassay based on the relative means of total microtuber fresh weight for two NaCl treatment levels (Table 4.7). The similar in vitro and in vivo rankings support the hypothesis that potato salinity tolerance can be evaluated under in vitro saline conditions.

PREFACE TO CHAPTER 6

This chapter reports on the testing of potato microtubers and tubers obtained from previous salinity experiments (Chapters 4 and 5). These microtubers and tubers were used in greenhouse pot trials and irrigated with NaCl solutions to evaluate their growth and yield performance and study the salinity carry-over effect on their productivity.

CHAPTER 6 EFFECTS OF NaCl ON YIELD OF POTATO PLANTS DERIVED FROM PREVIOUSLY SALINE CONDITIONS

Abstract

Micropropagation and minituber production technology may enable the establishment of potato seed tuber certification programs in arid Middle Eastern and African countries and reduce their dependency on expensive seed tuber imports. In this experiment we investigated possible carry-over effects of salt stress on the next production cycle. The yields of seed tuber-derived (STD) and microtuber-derived (mTD) plants of potato (*Solanum tuberosum* L.) cvs. Norland and Russet Burbank, obtained from both saline (NaCl) or non-saline growing conditions (pre-treatment) were evaluated under saline irrigation conditions (0 or 60 mM NaCl) in a greenhouse pot trial. There was no apparent residual carry-over effect of salt stress on either plant growth or tuber yield of STD or mTD plants. Irrigation with a 60 mM NaCl solution reduced total tuber fresh weights in both cultivars, regardless of production history, but significantly increased the total number of tubers in cv. Norland. These results allay concern regarding salinity carry-over effects and indicate genotype-specific depressive or stimulative effects of relatively low salinity exposure.

6.1 Introduction

Cultivated potato is a major food crop in the world, with production steadily increasing in Africa and the Middle East since the 1960's (FAO, 1995). One major constraint to potato production in these areas is the availability of seed tubers which are usually imported from Europe or North America at high cost (Leclerc, 1993). Local production of good quality seed tubers is hindered by many factors, including arid environmental conditions, disease pressure, and saline water and soils. Using micropropagated plantlets and microtubers (Wang and Hu, 1982 and 1985), and greenhouse- or screenhouse-produced minitubers (Bryan, 1988), developing countries have the potential to establish their own seed tuber certification programs and to reduce their dependency on high-cost imports (Leclerc, 1993). Local production would be more feasible if the salinity carry-over effect in seed tubers obtained under saline growing conditions was minimal.

While salinity is known to depress the growth and productivity of many crop species including potato, which was moderately salt sensitive (Maas and Hoffman, 1977), low salinity levels have occasionally been reported to stimulate the growth of potato. For example, low salinity levels in vitro enhanced micropropagated plantlet growth in two of six cultivars (cvs.) (Potluri and Prasad, 1993), stimulated microtuber yield of two of five cvs. (Zhang and Donnelly, 1997), and increased tuber yield in two of four cvs. in pot trials (Ahmad and Abdullah, 1979) and six of ten cvs. in field trials (Kang

et al., 1995).

The productivity of tubers which were previously exposed to saline conditions during their development are not well known. The objective of this experiment was to determine whether there was a carry-over effect on plant growth and yield, and to evaluate the impact of salinity stress, in microtuber derived (mTD) and seed tuber derived (STD) plants.

6.2 Materials and methods

Seed tubers of cvs. Norland and Russet Burbank were obtained from plants grown in field lysimeters under saline and non-saline irrigation at electrical conductivity (EC) of 3.5 and 1.5 dSm^{-1} (40 and 17 mM NaCl) respectively (Zhang and Donnelly, 1997) and stored at 4 °C in the dark for 8 mo. Microtubers of these two cvs. were obtained from plantlets layered on a modified MS (Murashige and Skoog, 1962) basal salt medium with or without NaCl at 80 mM (Zhang et al., 1998a) and stored in the dark at 10 °C, in sealed petri dishes, for 4 mo.

Seed tubers (50 - 70 g) and microtubers (0.2 - 0.5 g) were planted 10 or 5 cm deep, respectively, into 10-liter pots filled with 8 liters of peatmoss-based potting mixture (Pro-Mix, Premier Brand, Riviere-du-Loup, QC, Canada), on May 21, 1996. The experiment was conducted in the Raymond building greenhouse of Macdonald Campus, McGill University. Slow release fertilizer (Osmocote, 19-6-12 at 50 g per pot) was applied twice: once at

planting time and a second application 6 wk later.

All pots were watered daily to run-off with 1.5 liters tap water per pot for 1 mo. Half of the pots were subsequently irrigated with NaCl solutions which were gradually increased in concentration over a 1 wk interval to reach 60 mM (3.51 g l^{-1} NaCl). During the course of the trial, the EC of the leachate from the saline water-irrigated pots was 6.0 - 6.3 dSm^{-1}, while it remained 0.2 - 0.4 dSm^{-1} in the control (tap water irrigated) pots.

Plants were grown at 26/20 °C D/N temperature under natural light conditions. Plants were hilled at 20 and 40 d after planting, by adding 0.75 liter of potting mixture per pot each time. The plants were harvested after 90 d and data was collected on percent sprouting, shoot number, and yield (tuber number (No.) and fresh weight (FW) in grams).

For both the standard seed tubers and microtubers, the treatments consisted of two cvs., two pre-treatments (saline and non-saline), and two imposed salinity stress levels (0 and 60 mM NaCl). Treatments for STD or mTD plants were arranged in a Complete Randomized Block Design (CRBD) with five blocks. Data were analysed with the general linear model (GLM) (SAS, 1989).

6.3 Results and discussion

The pre-treatment did not affect percentage sprouting, the mean number of plant stems (4.0 - 7.4) at harvest time, or the tuber yield (Table 6.1). The

Table 6.1 Tuber yield, number (No.) and fresh weight (FW) in grams per plant, from pre-treated (saline or non-saline) seed tuber-derived potato plants irrigated with 0 or 60 mM NaCl in a greenhouse pot trial.

Cultivar	Pre-Treatment	NaCl (mM)	Tuber size distribution (cm)					
			0.5 - 4.0		4.1 - 8.0		Total	
			No.	FW	No.	FW	No.	FW
Norland	Saline	0	5b[a]	33b	5a	455a	10b	488a
		60	17a	97a	2b	178b	19a	274b
	Non-saline	0	6b	56b	8a	492a	13b	548a
		60	20a	153a	3b	161b	23a	313b
Russet Burbank	Saline	0	7a	47a	4a	243a	11a	290a
		60	4a	27a	1b	23b	4b	50b
	Non-saline	0	8a	69a	4a	169a	12a	238a
		60	2a	20a	1b	50b	4b	70b

[a]Values with the same letter in each column are not significantly different at LSD $p<0.05$.

60 mM NaCl irrigation treatment reduced the No. and FW of large tubers (4.1 - 8.0 cm) in both cvs. Saline irrigation increased the No. and FW of small tubers (0.5 - 4.0 cm) in cv. Norland but not Russet Burbank. This resulted in increased total tuber No. but not FW for cv. Norland but not Russet Burbank.

The pre-treatment did not affect percentage sprouting, the mean number of stems (1.0), or tuber yield (Table 6.2). Saline irrigation decreased the No. and FW of large minitubers of both cvs. It also decreased the No. and FW of small minitubers in cv. Russet Burbank. However, for cv. Norland, irrigation with 60 mM NaCl significantly increased the No. of minitubers but did not affect the FW, resulting in a big increase in total No., but not overall yield, of microtubers.

The pre-treatments did not affect overall plant growth or tuber yield of either STD or mTD plants of either cv. (Tables 6.1 and 6.2) implying no apparent salinity carry-over effect when seed tubers or microtubers were harvested from saline environments. The yields of STD and mTD plants of both cvs. were affected by salt stress in a similar way. The yields of cv. Russet Burbank were more affected than cv. Norland by irrigation with saline water (Tables 6.1 and 6.2). Salt stress significantly reduced yields of both cvs. However, it increased the number of small sized tubers and minitubers and therefore the total number of tubers and minitubers in cv. Norland in agreement with previous reports from field lysimeters (Zhang and Donnelly,

Table 6.2 Minituber yield, number (No.) and fresh weight (FW) in grams per plant, from pre-treated (saline or non-saline) microtuber-derived potato plants irrigated with 0 or 60 mM NaCl in a greenhouse pot trial.

Cultivar	Pre-Treatment	NaCl (mM)	Minituber size distribution (cm)					
			0.5 - 4.0		4.1 - 8.0		Total	
			No.	FW	No.	FW	No.	FW
Norland	Saline	0	4b[a]	35a	3a	220a	7b	254a
		60	49a	40a	1b	15b	49a	55b
	Non-saline	0	4b	72a	5a	276a	10b	348a
		60	71a	83a	1b	16b	71a	99b
Russet Burbank	Saline	0	7a	48a	1a	47a	8a	93a
		60	1b	1b	0b	0b	1b	1b
	Non-saline	0	6a	33a	0	26a	7a	59a
		60	1b	1b	0	0b	1b	1b

[a]Values with the same letter in each column are not significantly different at LSD $p < 0.05$.

1997). For seed production purposes, the number of tubers in the optimum size range may be more important than absolute yield (Nadler and Heuer, 1995). The ideal size for handling is Grade B; 40 g, 3 - 4.5 cm.

Potato is known for its plasticity with respect to tuberization, which can occur at any growing point, including lateral buds, stolons, even flowers (Lazin, 1980). Under salt stress, aerial tubers formed in the leaf-axils at the base of cv. Norland plants. The causes of aerial tuber formation and their relationship to salinity are not entirely known. These may be caused by inhibition of normal tuberization process, due to assimilate deprivation (Reviewed by Ewing and Struik, 1992) or diversion of assimilate transportation to alternate sinks (Dwelle, 1990).

The relative salinity tolerance of these two cvs. when salinity was applied after an establishment phase of 1 mo, contrasted with previous results from field lysimeter experiments, where saline treatment was applied from planting time onwards (Zhang and Donnelly, 1997). Potato plant response to salinity differed when salt treatment was applied at different plant developmental stages (Levy, 1992) and caused changes in the yield rankings of some cultivars. This was also true for several other crop plants (Shannon, 1984).

Growing conditions with soils or water having moderate salinity levels may be suitable for seed tuber production of more salinity tolerant cvs., without major yield loss or detectable salinity carry-over effect. Our results

suggest that potato seed tuber production could be achieved locally in countries where semi-arid conditions and saline soils prevail. However, other factors should also be considered, such as locating potato crops in areas remote from commercial production and disease pressure.

PREFACE TO CHAPTER 7

Chapter 7 reports the investigation of NaCl and mannitol effects on potato microtuber yield. The purpose was to understand which of the two-phase effects under saline (NaCl) stress, osmotic or specific-ion effects, was responsible for potato microtuber yield increase observed in some cultivars in the microtuberization bioassay (Chapter 4).

CHAPTER 7 DISTINGUISHING OSMOTIC AND SPECIFIC ION EFFECTS OF NaCl ON POTATO MICROTUBER YIELD

Abstract

As in the field, the yield of microtubers in culture are usually depressed by substrate salinity (NaCl), but are sometimes stimulated at low salinity levels. The objective of this experiment was to determine whether osmotic or specific ion stresses could be distinguished during microtuberization. Cvs. Norland and Russet Burbank potato (*Solanum tuberosum* L.) were grown on media containing isoosmotic levels of NaCl (0, 40, 80 mM) or mannitol (0, 72, 144 mM). Screening differentially for NaCl (osmotic stress and ion stress) and mannitol (osmotic stress) tolerance during in vitro microtuberization appeared possible. Cv. Russet Burbank was more salt tolerant than cv. Norland, but cv. Norland was more osmotic stress tolerant compared with cv. Russet Burbank. Microtubers of different size classes reacted differently to salt and osmotic stress, and may be useful in discrimination of osmotic stress or ion excess. Low concentration of NaCl increased microtuber FW in the larger microtuber size class in cv. Russet Burbank but isoosmotic mannitol concentration did not. Therefore, specific ion effect of Na^+ rather than osmotic effect, is likely the cause of yield stimulation but the mechanism(s) remain unknown.

7.1 Introduction

It has been hypothesized that plant growth inhibition and yield reduction under saline (NaCl) stress results from a two-phase growth response to salinity (Munns, 1993; Munns et al., 1995). In Phase 1 the osmotic strength of the salt solution reduces plant growth. In Phase 2 ion excess can cause relative growth reduction in genotypes sensitive to salt.

Curiously, anomalous growth or yield stimulation occurs in some genotypes exposed to relatively low salinity levels. When layered plantlets of potato (*Solanum tuberosum* L.) were exposed to medium containing 80 mM NaCl, relative increases of 141 % in cv. Kennebec and 203 % in cv. Russet Burbank occurred in microtuber fresh weight (FW) per container (Zhang and Donnelly, 1997). In three other cultivars, (Atlantic, Norland, and Spunta) microtuber FW was depressed at this salinity level. Microtuber yields were decreased in all cultivars when 160 mM NaCl was included in the medium. Similarly, relative shoot dry weights (DW) of 2 out of 6 micropropagated cultivars of low-land potato increased 10-16 % in culture medium containing 0.2 % crude sea salts (Potluri and Prasad, 1993). At salinity levels > 0.4 %, shoot height and DW were reduced in all cultivars. Similar observations were made when 4 potato cvs. (Cardinal, Multa, Patrones, and Red Bed) were grown in pots irrigated with mixed salt solutions from 0 to 1.0 %, at 0.2 % increments (Ahmad and Abdullah, 1979). As saline stress increased from 0.2 to 0.8 %, the relative tuber FW per plant in cvs. Cardinal and Patrones

increased 244 and 172 %, respectively at 0.2 %, and 141 and 121 % respectively, at 0.8 %, but progressively decreased in the other two cultivars. At 1.0 % salinity, relative tuber FW per plant was decreased in all cultivars. Among the 10 potato cultivars grown in moderately saline/sodic soil (0.268 % total salts with 0.167 % NaCl), tuber FW per plant in 6 cultivars were increased from 125 (3 cvs.), 175 (2 cvs.) to 213 % (1 cv.) relative to plants grown in normal production land (0.168 % total salts with 0.06 % NaCl) (Kang et al., 1995). An explanation is lacking for why plant growth, and yield of microtubers or tubers of some cultivars were increased under relatively low saline stress conditions.

Under different field situations, osmotically tolerant or salinity tolerant genotypes may be preferred (Ashraf, 1994). Bioassays involving differential screening and selection of potato genotypes during the microtuberization stage using NaCl and other osmotic agents such as mannitol would expedite evaluation and so accelerate the breeding process.

The objective of this experiment was to determine whether osmotic and specific ion stresses can be distinguished during microtuberization, and whether the increased microtuber yield observed in some cultivars when NaCl was incorporated into the medium was related to osmotic stress or to specific ion (Na^+) effect.

7.2 Materials and methods

Cvs. Norland and Russet Burbank were micropropagated using single-node cuttings on a modified Murashige and Skoog (MS, 1962) basal salt medium as previously described (Zhang and Donnelly, 1997).

Microtuberization was carried out using the two-step procedure of Leclerc et al. (1994). Step-1 involved layering 3 plantlets of 5 nodes each, with root and apex severed, in 50 ml of liquid potato micropropagation medium containing (mg l^{-1}): 6-benzylaminopurine (BAP; 0.5), gibberellic acid (GA_3; 0.4), and reduced sucrose (2 %) in a 400 ml plastic container (Better Plastics, Kissamee, FL, USA). After 4 wk incubation under conditions of 25 ± 2 °C, 16/8 h D/N cycle, and 80 μmol $m^{-2}s^{-1}$ photosynthetic photon flux (PPF) (cool white fluorescent lights), residual medium was drained off and replaced with 50 ml of step-2 liquid growth regulator-free medium with sucrose increased to 8 % and isoosmotic levels of NaCl (0, 40, or 80 mM) or mannitol (0, 72 and 144 mM) (Salisbury and Ross, 1992). For step-2 the cultures were incubated for 4 wk more under 15 ± 1 °C with 8/16 h D/N cycle and PPF of 50 μmol $m^{-2}s^{-1}$.

There were 15 containers at each NaCl or mannitol treatment level for cv. Norland and 10 for cv. Russet Burbank. Data were collected on microtuber yield (number (No.) and fresh weight (FW) in grams) and size distribution per container. Data was analysed using the general linear model (GLM) (SAS, 1989). The difference among treatments was tested with least square

difference (LSD) comparison at the 5 % level of significance.

7.3 Results and discussion

In cv. Norland, microtuber No. and FW in the small size class (<0.5 cm) were not affected by either NaCl or mannitol concentrations (Table 7.1). The greatest concentrations of NaCl (80 mM) and mannitol (144 mM) reduced microtuber FW but not No. in the large size class (>0.5 cm) and this caused the total microtuber FW reduction.

In cv. Russet Burbank, NaCl at 40 mM did not affect microtuber yield in the small size class yield and increased microtuber FW but not No. in the large size class. This resulted in unaffected total No. and a slight, but not significant, increase in total microtuber FW. It is interesting that an isoosmotic concentration of mannitol (72 mM) had no effect on yield in the large size class, but reduced yield in the small size class, causing a reduction in total microtuber yield. At 80 mM NaCl and 144 mM mannitol, microtuber No. and FW in the small size class was reduced, and this caused total microtuber No. and FW reduction. This experiment underlines the possibility of screening differentially for salt and osmotic tolerance during in vitro microtuberization, using NaCl (osmotic plus specific ion effects) and isoosmotic concentrations of agents such as mannitol (osmotic effects). Averaged over 40 and 80 mM NaCl levels, the relative mean microtuber FW were 70.51 % and 85.05 % for cvs. Norland and Russet Burbank,

Table 7.1 Microtuber yield, including number (No.), fresh weight (FW) in grams, and size distribution per culture container, at 0, 40, and 80 mM NaCl or 0, 72 and 144 mM mannitol.

		Microtuber size distribution (cm)					
		<0.5		>0.5		Total	
Cultivar	Treatment (mM)	No.	FW	No.	FW	No.	FW
Norland	Control	4.1a[a]	0.28a	2.1a	0.50a	6.2a	0.78a
	NaCl 40	5.8a	0.36a	1.5a	0.41a	7.3a	0.77a
	Man. 72	3.3a	0.28a	1.7a	0.45a	5.0a	0.73a
	NaCl 80	4.1a	0.24a	0.5a	0.08b	4.6a	0.33b
	Man.144	4.1a	0.24a	1.0a	0.17b	5.1a	0.41b
Russet	Control	15.1a	0.67a	1.9a	0.40b	17.0a	1.07a
Burbank	NaCl 40	13.1a	0.55a	2.8a	0.81a	15.8a	1.36a
	Man. 72	6.2b	0.37b	1.0a	0.23b	7.2b	0.60b
	NaCl 80	3.1b	0.15b	1.7a	0.31b	4.8b	0.46b
	Man.144	6.0b	0.32b	0.5a	0.11b	6.5b	0.42b

[a]Values in each column with the same letter are not significantly different at LSD $p<0.05$.

respectively. We conclude that cv. Russet Burbank were more tolerant than cv. Norland under NaCl stress, in agreement with Zhang and Donnelly (1997). Averaged over 72 and 144 mM mannitol levels, the relative means of microtuber FW were 73.08 % and 47.66 % for cvs. Norland and Russet Burbank, respectively. Mean No. of microtubers was unaffected by mannitol stress in cv. Norland but significantly decreased in cv. Russet Burbank. We conclude that cv. Norland was more tolerant to osmotic stress in vitro than cv. Russet Burbank.

It may also be possible to distinguish the two-phase osmotic and specific ion effects of NaCl stress by evaluating the impact of NaCl on microtubers of different sizes, and developmental stages at the time of harvest. In cv. Norland, the larger microtuber size class was more affected than the smaller one, implying that the larger microtubers responded to both osmotic and specific ion effects while the smaller microtubers responded primarily to osmotic effects. In cv. Russet Burbank, the larger microtuber size class was less affected than the smaller one, implying that the older tubers were more tolerant to Na^+ ion excess.

Low concentrations of NaCl stimulated microtuber FW in the larger microtuber size class in cv. Russet Burbank but isoosmotic mannitol concentration did not have the same result. Therefore, specific ion effect of Na^+, not osmotic effect, is likely the cause of yield stimulation but the mechanism(s) remain unknown. Growth and yield stimulation in the presence

of NaCl is genotype dependent and may only occur in the relatively more NaCl tolerant genotypes.

Microtuberization bioassays for osmotic and salinity tolerance could potentially be linked to in vitro screening procedures for determination of other abiotic stress resistance, such as heat tolerance (Nowak and Colborne, 1989), or seasonality and yield factors such as earliness (Lentini et al., 1988). In vitro evaluations pose many advantages compared to the more costly, labour-intensive and sometimes more problematic field-based assessments.

CHAPTER 8 GENERAL SUMMARY AND CONCLUSIONS

In vitro bioassays were investigated for salinity tolerance screening and selection as a means of providing rapid and reliable results. These methods are less labour intensive than field trials. In vitro evaluations of salinity tolerance in wild potato species and cultivars were reported using single-node cuttings (Arslan et al., 1987), five-node stem cuttings (Morpurgo, 1991; Morpurgo and Rodriguez, 1987), microtuberization (Kim et al., 1995) and root tip segments cultures (Naik and Widholm, 1993). The latter two reports came out while this work was in progress. These reports showed the possibility of using in vitro bioassays to screen and select salt tolerant potato genotypes.

Chapter 3 describes experiments where potato genotypes were evaluated using different in vitro bioassays under saline stress. NaCl stress significantly reduced single-node cutting growth in potato cultivars. Among the cvs. tested, Russet Burbank and Spunta were the least and Norland was the most affected by NaCl stress (Fig. 3.1 A-C). NaCl stress decreased all of the growth parameters: SL, SDW, RL, and RDW in the six hybrids tested in the single-node cutting bioassay (Fig. 3.2 A-D). Hybrids 9787-07 and 9506-04, derived from *S. chacoense* and crosses between *S. chacoense, S. microdontum,* and *S. tuberosum* were more tolerant than other hybrid genotypes under NaCl stress. The seed germination and early seedling

growth bioassay was used to evaluated salinity tolerance in wild potato species. *S. chacoense* outperformed *S. gourlayi* and *S. microdontum* in true seed germination and early seedling growth (Fig. 3.3 A-D).

Even though direct comparison of salinity tolerance among these groups was not possible from these experiments because they were performed at different times, the variations of salinity tolerance within each potato genotype group were detected and could be observed with different in vitro bioassays. This was in agreement with previous reports (Arslan et al., 1987; Morpurgo, 1991; Morpurgo and Silva-Rodriguez, 1987; Naik and Widholm, 1993). Salinity tolerance evaluation of potato hybrids will be important since salt tolerant hybrid genotypes can be used in future breeding programs.

Chapter 4 describes the evaluation of potato salinity tolerance using three in vitro bioassays; single-node cutting, root tip segment, and microtuberization. In the single-node cutting bioassay, NaCl at 80 and 120 mM reduced plantlet growth (Table 4.1). Since the six growth parameters were only partially correlated across the range of NaCl levels tested (Tables 4.2 and 4.3), potato genotype salinity tolerance at higher salinity levels could not be predicted according to their growth in control medium (vigour) or relative growth data at 40 or 80 mM NaCl. Also no recommendation could be made in choosing any one of the six growth parameters over any other for salinity tolerance ranking. These conclusions underline the complexity in ranking genotype salinity tolerance. Multivariate cluster analysis was used to

group cultivars. The potato genotypes tested were divisible into two groups (Table 4.4). The hybrid and cvs. Kennebec and Russet Burbank were consistently found in one group at every NaCl treatment level and were considered relatively salt tolerant compared with the other group (Tables 4.4 and 4.5). These results suggest that, if many growth parameters are involved in the ranking of genotype salinity tolerance, it is very important to chose a proper statistical procedure for correct data interpretation. Cluster analysis can better enable evaluation of potato salinity tolerance than the methods used in earlier reports (Arslan et al., 1987; Kim et al., 1995; Naik and Widholm, 1993). In these reports, cultivar salinity tolerance was simply evaluated based on the relative vegetative growth under saline stress by using the general linear model (GLM). Since different growth parameters respond differently under saline stress (Tal, 1994), GLM can give inconsistent and ambiguous rankings if more than one growth parameter or salinity level was involved in the evaluation process.

In the root tip segment bioassay, 80 and 120 mM NaCl inhibited root tip segment extension growth on both solid and liquid media in all the genotypes tested (Table 4.6 A,B). Cvs. Kennebec and Russet Burbank were more NaCl tolerant than cv. Norland (Table 4.6 A,B) when ranked based on the average relative root extension growth over three NaCl treatment levels (Naik and Widholm, 1993). Although the root tip segment bioassay saved significant time and labour compared with Naik and Widholm 's (1993)

method, and was more rapid and utilized fewer growth parameters than the single-node bioassay, it required twice the operational time and more delicate in vitro handling.

In the microtuberization bioassay, cvs. Kennebec and Russet Burbank were more salt tolerant than cv. Norland based on their yield under NaCl stress. Total microtuber FW per container in cvs. Kennebec and Russet Burbank were increased at 80 mM due to the increase in the $\geq$0.5 cm size class (Table 4.7). This might be useful for a microtuber production system, but needs further research for practical application. Physiologically, the microtuberization bioassay best mimics potato tuber yield response under field saline stress. Although it took 1 mo longer than other in vitro bioassays and excluded certain genotypes where induction parameters were unknown, it may help to distinguish abiotic, including NaCl stress on yield.

The three in vitro bioassays utilized different organs. Although the genotype rankings of salinity tolerance were similar in these bioassays, the single-node cutting bioassay was recommended for in vitro screening of salinity tolerance since it was simpler to perform and adaptable to more genotypes.

Since different genes are apparently expressed at different saline stress levels in vitro in vivo (Tal, 1994), it is useful to test a range of salinity levels in the evaluation. Evaluation could be performed at 0, 80, and 120 mM NaCl, followed by a proper statistical analysis method, e.g., use six growth

parameter measurements with cluster analysis in the single-node cutting bioassay, to partition the genotypes into groups with different salinity tolerance.

The ranking results of three in vitro bioassays were verified to some extent with one field lysimeter trial (Chapter 5). Cvs. Kennebec, Russet Burbank, and Norland were tested under saline stress. Based on the tuber yield, cvs. Kennebec and Russet Burbank were ranked more salt tolerant than cv. Norland (Table 5.2). This was similar to the rankings in the three bioassays; single-node cutting (Tables 4.4 and 4.5), root tip segment (Table 4.6 A,B), and microtuberization (Table 4.7). Field lysimeter results confirmed the possibility that in vitro bioassays could be used as a substitute for salinity tolerance screening and selection.

In the correlation of in vitro and saline field experiments (Morpurgo, 1991), the saline conditions in the field were varied from EC levels of 4.0 to 7.0 dSm^{-1}. In our field lysimeter trials (Chapter 5), the EC levels in the lysimeters were varied from 1.19 to 1.7 dSm^{-1} in the control and from 3.15 to 3.4 dSm^{-1} in the saline water irrigation treatments throughout 11 weeks observation in the growth season (Appendix 6). Lysimeters provide more standardized environmental conditions which make experimental results more repeatable and reliable. This lysimeter experiment strongly supported two hypotheses: a) in vitro bioassays can be effective and valid for potato salinity tolerance screening and selection; b) the lysimeter is a very useful

system in future saline stress research to assist with interpretation and validation of the in vitro bioassay results.

Plant response under salinity stress is complex and varied with field locations and environmental interactions. The limited number of lysimeters available in this research permitted few cultivars to be tested. Therefore caution must be exercised before concluding that our method can be generalized to any potato genotype. It is strongly recommended that a wider range of genotypes should be tested under various levels of saline stress in lysimeters as well as in vitro.

In Chapter 6, we tested whether there was a salinity carry-over effect on the yield of potato plants derived from microtubers (mTD) and seed tubers (STD), some of which were exposed to salt stress conditions during their growth. Whether NaCl stress was present or not, the tuber source (saline or non-saline) did not affect total minituber or tuber yields nor their size distributions in either cultivar (Tables 6.1 and 6.2). Moderately salinized fields may be used to produce seed tubers of more salt tolerant potato cultivars. Since cvs. Norland and Russet Burbank performed differently in the field lysimeter (Zhang and Donnelly, 1997) and the greenhouse (Zhang et al., 1998b) experiments, it is suggested that potato cultivars may express different salinity tolerance with reversed ranking positions if the saline stress is applied at different plant growth stages.

In Chapter 7 potato plantlets were exposed to saline (NaCl) and osmotic

(mannitol) stress to determine whether osmotic or specific ion effects stimulated microtuber yield under NaCl stress. Cv. Russet Burbank microtuber FW was stimulated under low NaCl level (40 mM) but not under isoosmotic mannitol (72 mM) in the larger microtuber size class (>0.5 cm). It is likely that specific ion (Na^+) effect rather than osmotic effects caused microtuber yield increase. This might help to explain why the tuber yields of some potato cultivars increased in the moderately salinized field (EC level of 2.0 - 3.0 dSm^{-1}). This preliminary information also reemphasized the importance of in vitro salt tolerant potato screening and selection because it appears that only relatively salt tolerant cultivars can grow in moderately salinized soil with no major yield loss.

This microtuberization experiment was consistent with the previous report (Zhang and Donnelly, 1997) confirming cultivar response under NaCl stress, microtuber yield increase in the larger size class (> 0.5 cm), and microtuber yield (number and fresh weight) in different microtuber size distributions, except that the two experiments were conducted under different NaCl stress levels. We can recommend the microtuberization bioassay to distinguish NaCl from purely osmotic tolerance and possibly for other abiotic stress tolerance screening and selection.

In summary, salinity tolerance of various potato (*Solanum* spp.) genotypes, e.g., wild species, hybrids, and cultivars was evaluated by using different in vitro bioassays (single-node cutting, root tip segment, and

microtuberization) under NaCl stress and validated in a preliminary way with the field lysimeter trial. In vitro bioassays could substitute for the more labour intensive and time consuming conventional field trials for salinity tolerant potato genotype screening and selection. Since other abiotic stresses, such as heat stress, can be evaluated using microtuberization culture (Nowak and Colborne, 1991), combined in vitro screening for more than one stress agent may be feasible. It is hoped that this study will inspire and promote in vitro potato research into other abiotic stress tolerance screening and selection.

On a cautionary note, lysimeter studies should continue for more solid confirmation of the utility of in vitro ranking. Furthermore, field trials are essential, especially in the arid, saline zones, to justify further pursuit of this approach.

CHAPTER 9 CONTRIBUTIONS TO KNOWLEDGE

1. The single-node cutting bioassay was recommended for salt tolerance screening and selection because of its simplicity, economy of experimental materials, and adaptability to a wide range of potato genotypes. The multivariate cluster analysis was more effective than the general linear model (GLM) in grouping potato genotypes according to their salinity tolerance when more than one growth parameter was used in the single-node cutting bioassay. Compared with the original method of Naik and Widholm (1993), the root tip segment bioassay utilized was simplified and shortened. The microtuberization bioassay was promising for potato salinity tolerance evaluations although it took 1 mo more and was not suitable for all of the genotypes. The increased microtuber size in cvs. Kennebec and Russet Burbank at 80 mM NaCl are potentially important for saline stress research as well as microtuber production.

2. The salinity tolerance of some potato cultivars was greater than the wild species tested. This suggests that there is no need to go back to wild progenitors to obtain genes for salinity tolerance. One hybrid (*Solanum* sp.) genotype (9506-04), obtained from Dr. H. De Jong, consistently appeared in the salt tolerant genotype group in the single-node cutting bioassay. This is the first time an in vitro bioassay has been used to evaluate potato hybrid salt tolerance. It may be possible to routinely incorporate salinity tolerance

screening into potato breeding programs and may accelerate the release of salt tolerant cultivars.

3. This is the first report of potato cultivar salinity tolerance evaluation in field lysimeters. The relatively stable salinity levels achieved during the lysimeter trial eliminated many soil micro-environment effects which occur in conventional field-based salinity stress experiments, and underline the utility of lysimeters. However, the lysimeter trial should be repeated, and field trials done eventually, to confirm the validity of our approach.

4. The effect of tuber source (saline or non-saline environment) on plant growth and tuber yield in seed tuber- (STD) and microtuber-derived (mTD) plants proved to be minimal. Moderately salinized soil may not be the limiting factor for tuber or seed tuber production of salt tolerant cultivars in salinized areas.

5. This is the first attempt to distinguish osmotic and specific-ion (Na^+) effects under NaCl stress on potato tuber yield. However, the effect of Cl^- was not considered, and should be evaluated for potato. The identified specific-ion (Na^+) effect on microtuber yield, in the different microtuber size classes, will help to establish a method to understand mechanisms of saline stress on potato yield.

CHAPTER 10 SUGGESTIONS FOR FUTURE RESEARCH

Different in vitro bioassays were used to evaluate potato (*Solanum* spp.) salinity tolerance and their validity confirmed in the field lysimeter trial.

1. The single-node cutting and root tip segment bioassays require little improvement, except that more genotypes may be evaluated with NaCl or other salt agents (KCl, Na_2SO_4, $CaCl_2$, or mixed salts) and concentrations. The microtuberization bioassay is presently successful for potato cvs. (Kim et al., 1995; Zhang and Donnelly, 1997). It is possible to expand this technology to evaluate salinity tolerance on a wider range of genotypes, e.g., hybrids and wild species, with NaCl, other salt agents, and osmotic agents at different concentrations.

2. It would be ideal if future experiments could be carried out with greater numbers of lysimeters. Only three potato cvs. were tested due to the limitation of lysimeters in this research. More genotypes need to be tested at different salinity concentrations and plant developmental stages to verify many of the in vitro and greenhouse observations of potato growth under saline conditions. The work in lysimeters could be extended to evaluate other abiotic stresses, alone and in combination.

3, Field evaluations are essential, preferably in arid, saline soil areas of the Middle east or South America. Field rankings should be compared with in vitro bioassay results based on yield criteria, especially with the single-node

cutting and microtuberization bioassays.

4. The conditions that increased potato tuber or minituber number under saline stress in the greenhouse experiment need to be further explored to achieve the optimum production level for seed tuber production.

5. The comparisons of time factor on the effectiveness and efficiency of in vitro bioassays will be necessary and important. The single-node cutting and microtuberization bioassays could be extended, e.g., 4, 6, or 8 wk under NaCl stress and may reveal whether plant salt tolerance changes during exposure to saline stress. The optimum duration of these bioassays should be established.

6. Yield increase under low saline stress was related to specific-ion (Na^+) effect. It is necessary to understand the mechanism(s) and to rule out the involvement of Cl^{-1}. The microtuberization bioassay could be used to explore plantlet growth, microtuber development, effect of plant nutrition and plant growth regulators which are possibly involved in microtuberization and certainly affected under NaCl stress.

REFERENCES

Acevedo E. and E. Fereres, 1990. Resistance to Abiotic Stresses. In: Plant Breeding: Principles and Prospects. Hayward M.D., N.O. Bosewark, and I. Romagosa (eds.). Chapman and Hall, London. pp.406-418.

Ahmad R. and Z.U.N. Abdullah, 1979. Salinity induced changes in the growth and chemical composition of potato. Pak. J. Bot. 11:103-112.

Arslan N., G. Mix, and N.E. Bassam, 1987. Ermittlung der Salztoleranz in vitro bei Wildarten und Kultursorten der Kartoffel. Landbauforschung Völkenrode 37:128-131.

Ashraf M., 1994. Breeding for salinity tolerance in plants. Crit. Rev. Plant Sci. 13:17-42.

Ashraf M. and A. Waheed, 1993. Responses of some local/exotic accessions of lentil (*Lens culinaris* Medic.) to salt stress. J. Agron. Crop Sci. 170:103-112.

Austin S. and A. Cassells, 1983. Variation between plants regenerated from individual calli produced from separated potato stem callus cells. Plant Sci. Lett. 31:107-114.

Ayers A.D., J.W. Brown, and C.H. Wadleigh, 1952. Salt tolerance of barley and wheat in soil pots receiving several salinization regimes. Agron. J. 44:307-310.

Barker W.G., 1953. A method for the in vitro culturing of potato tubers. Sci. 118:384.

Beloualy N. and J. Bouharmont, 1993. Sodium chloride-tolerant plants of *Poncirus trifoliata* regenerated from tolerant cell lines. Theor. Appl. Genet. 83:509-514.

Bernstein L., 1974. Crop Growth and Salinity. In: Drainage for Agriculture. Van Schilfgaade J. (ed.). Am. Soc. Agron., Madison, Wisconsin. pp.39-54.

Bernstein L. and H.E. Hayward, 1958. Physiology of plant salt tolerance. Ann. Rev. Plant Physiol. 9:141-158.

Bilski J.J., D.C. Nelson, and R.L. Conlon, 1988a. The response of four potato cultivars to chloride salinity, sulphate salinity and calcium in

pot experiments. Am. Pot. J. 65:85-90.

Bilski J.J., D.C. Nelson, and R.L. Conlon, 1988b. Response of six wild potato species to chloride and sulphate salinity. Am. Pot. J. 65:605-612.

Bouaziz E., 1980. Tolerance à la salure de la pomme de terre. Physiolog. Veg. 18:11-17.

Bourque J.E., J.C. Miller, and W.D. Park, 1987. Use of in vitro tuberization system to study tuber protein gene expression. In Vitro Cell. Dev. Biol. 23:381-386.

Bressan R.A., N.K. Singh, A.K. Handa, A. Kononowicz, and P.M. Hasegawa, 1985. Stable and Unstable Tolerance to NaCl in Cultured Tobacco Cells. In: Plant Genetics. Proc. of the Third Annual ARCD Plant Cell Res. Institute-UCLA Symp. on Plant Biol., Freeling M. (ed.). New York. pp.755-769.

Bruns S. and R., Caesar, 1990. Sproßentwicklung und knollenerträge mehrerer kartoffelsorten bei hohen salzkonzentrationen zu verschiedenen entwicklungsstadien. Pot. Res. 33:23-32.

Bryan J.E., 1988. Implementation of rapid multiplication and tissue culture methods in third-world countries. Am. Pot. J. 66:199-207.

Burgutin A.B., A.G. Butenko, B.A. Kaurov, and N. Iddagoda, 1996. In vitro selection of potato for tolerance to sodium chloride. Russ. J. Plant Sci. 43:520-531.

Chapman H.W., 1955. Potato tissue cultures. Am. Pot. J. 32:207-210.

Christiansen M.B., 1982. World Environment Limitations to Food and Fibre Culture. In: Breeding Plants for Less Favourable Environments. Christianson M.B. and C.F. Lewis (eds.). John Wily and Sons, New York. pp.1-12.

Dwelle, R.B., 1990. Source/sink relationships during tuber growth. Am. Pot. J. 67:829-833.

Ekanayake I.J. and J.H. Dodds, 1993. In vitro testing for the effects of salt stress on growth and survival of sweet potato. Scient. Hort. 55:239-248.

Epstein E., 1976. Genetic Potentials for Solving Problems of Soil Mineral Stress: Adaptation of Crops to Salinity. In: Plant Adaptation to Mineral Stress in Problem Soils. Wright M.J. (ed.). Ethaca, New York. pp.73-82.

Epstein E. and D.W. Rains, 1987. Advances in salt tolerance. Plant Soil 99:17-29.

Espinosa N.O., R. Estrada, D.S. Rodriguez, P. Tovar, R. Lizarraga, and J.H. Dodds, 1986. The potato: A model crop plant for tissue culture. Outl. Agric. 15:21-26.

Estrada R., P. Tovar, and J.H. Dodds, 1986. Induction of in vitro tubers in a broad range of potato genotypes. Plant Cell, Tis., Org. Cult. 7:3-10.

Ewing, E.E. and P.C. Struik, 1992. Tuber formation in potato: Induction, initiation, and growth. Hort. Rev. 89-198.

FAO, 1990. Food and Agriculture Organization of the United Nations. 1989 FAO Production Yearbook. Vol.43. FAO, Rome.

FAO, 1995. Potato in the 1990's: Situation and Prospects of the World Potato Economy. Int'l Potato Centre and Food and Agric. Org. of the United Nations, Rome. pp.1-39.

Flowers T.J., P.F. Troke, and A.R. Yeo, 1977. The mechanism of salt tolerance in halophytes. Ann. Rev. Plant Physiol. 28:89-121.

Flowers T.J. and A.R. Yeo, 1989. Effect of Salinity on Plant Growth and Crop Yield. In: Biochemical and Physiological Mechanisms Associated with Environmental Stress Tolerance in Plants. Cherry J. (ed.). Springer-Verlag, Berlin. pp.151-165.

Freytag A.H., J.A. Wrather, and A.W. Erichsen, 1990. Salt tolerant sugar beet progeny from tissue cultures challenged with multiple salts. Plant Cell Rep. 8:647-650.

Garcia-Torrez L. and C. Gomez-Campo, 1973. In vitro tuberization of potato sprouts as affected by ethel and gibberellic acid. Pot. Res. 16:73-79.

Greenway H., 1965. Plant response to saline substrates. VII. Growth and ion uptake throughout plant development in two varieties of *Hordeum vulgare*. Aust. J. Biol. Sci. 18:763-769.

Gregory L.E., 1956. Some factors for tuberization in the potato. Ann. Bot. 43:281-288.

Hamdy A., S. Abdel-Dayem, and M. Abu-Zeid, 1993. Saline water management for optimum crop production. Agri. Water Mgt. 24:189-203.

Hamid A. and O. Talibuddin, 1976. Effect of sodium on the growth of and ion uptake by barley, sugar beet and broad beans. J. Agric. Sci. 86:49-56.

Hawkes J.G., 1992. History of the Potato. In: The Potato Crop: The Scientific Basis for Improvement. Harris M. (ed.). Chapman and Hall, London. pp.1-11.

Heuer B. and A. Nadler, 1995. Growth and development of potatoes under salinity and water deficit. Aust. J. Agric. Res. 46:1477-1486.

Heyser J.W. and M.W. Nabors, 1982. Long term plant regeneration, somatic embryogenesis and green spot formation in secondary oat (*Avena sativa*) callus. Z. Pflanzenphysiol. 107:153-160.

Huang P., P.R. Bohac, and J.C. Miller, 1988. Differential response of in vitro cultured potato cultivars in a temperature gradient system. HortSci. 23:754.

Hussey G. and N.J. Stacey, 1984. Factors affecting the formation of in vitro tubers of potato (*S. tuberosum*). Ann. Bot. 53:565-578.

Ishida B.K., G.W. Snyder, and W.R. Belknap, 1989. The use of *in vitro* grown microtuber disks in *Agrobacterium*-mediated transformation of *Russet Burbank* and *Lemhi Russet* potatoes. Plant Cell Rep. 8:325-328.

Jones E.D., 1988. A Current assessment of in vitro culture and other rapid multiplication methods in North America and Europe. Am. Pot. J. 65:209-220.

Jones R.A., 1986. The development of salt-tolerant tomatoes: Breeding strategies. Acta Hort. 190:101-114.

Kang Y., L. Xiu, D. Wang, and W. Pang, 1995. Selection of potato varieties/strains adapted in saline/sodic soils. Acta Hort. 256:249-252.

Khan M.A. and I.A. Ungar, 1984. The effect of salinity and temperature on the germination of polymorphic seeds and growth of *Atriples triangularis* Wild. Am. J. Bot. 71:481-489.

Khrais T., 1996. Evaluation of Salt-Tolerance in Potato (*Solanum* spp.). McGill University, M.Sc. Thesis.

Kim H.S., J.H. Jeon, Y.H. Jeung, and H. Joung, 1995. In vitro selection of salt-resistant *Solanum tuberosum* L. varieties. J. Korean Soc. Hort. Sci.

36:172-178.

Kirki P.B., S. Hadi, P.A. Kumar and V.L. Chopra, 1991. Production of sodium-chloride-tolerant *Brassica juncea* L. somaclones and their parent cv. Prakash. Plant Cell Rep. 9:684-687.

Kumar D., 1985. Emergence, establishment and seed yield of chickpea as affected by sodicity. Ann. Arid Zone. 24:334-340.

Lazin, M.B., 1980. Screening for Heat Tolerance and Critical Photoperiod in the potato (*Solanum tuberosum* L.). Ph.D. Thesis. Cornell Univ. Ethaca, New York.

Leclerc Y., 1993. The Production and Utilization of Potato Microtuberization. McGill University, Ph.D thesis.

Leclerc Y., D.J. Donnelly, and J.E.A. Seabrook, 1994. Microtuberization of layered shoots and nodal cuttings of potato: The influence of growth regulators and incubation periods. Plant Cell, Tis., Org. Cult. 37:113-120.

Lentini Z., R.L. Plaisted, and E.D. Earle, 1988. Use of in vitro tuberization as a screening system for potato earliness. Am. Pot J. 65:488.

Levitt J., 1977. Salt and Ion Stress. In: Responses of Plant to Environmental Stresses. Kuzlowsky T.T. (ed.). Acad. Press, New York. pp.506-524.

Levy D., 1992. The response of potatoes (*Solanum tuberosum* L.) to salinity: Plant growth and tuber yields in the arid desert of Israel. Ann. Appl. Biol. 120:547-555.

Levy D., E. Fogelman, and Y. Itzhak, 1988. The effect of water salinity on potatoes (*Solanum tuberosum* L.): Physiological indices and yielding capacity. Pot. Res. 31:601-610.

Lewis L.N., 1984. A vital resources in danger. Calif. Agric. 38:33-34.

Maas E.V., 1984. Crop tolerance. Calif. Agric. 34:20-21.

Maas E.V., 1987. Salt Tolerance of Plants. In: CRC Handbook of Plant Science in Agriculture. Christie, B.R. (ed.). CRC Press, Inc. Boca Raton, Florida. Vol.II, pp.57-75.

Maas E.V. and G.J. Hoffman, 1977. Crop salt tolerance-current assessment.

J. Irrig. Drainage 103:115-134.

Maas E.V. and Nieman R.H., 1978. Physiology of Plant Tolerance to Salinity. In: Crop Tolerance to Suboptimal Land Conditions. ASS, CSA, SSSA, Madison, WI: a Special Publ. No. 32, 277.

Maddock S.E., V.A. Lancaster, R. Rissiott, and J. Franklin, 1983. Plant regeneration from cultured immature embryos and inflorescence of 25 cultures of wheat (*Triticum aestivum*). J. Exp. Bot. 34:915-926.

Massoud F.I., 1974. Salinity and Alkalinity as Soil Degradation Hazards. FAO/UNEP Expert Consultation on Soil Degradation, FAO, Roam.

Meuleman M., J. Dumont, C.S. Anceau, and G. Fouarge, 1986. In vitro tuberization of potato. Med. Fac. Landbouww. Rijksuniv. Gent. 51/2a:527-531.

Milbocker D.C., 1988. Salt tolerance of Azalea cultivars. J. Amer. Soc. Hort. Sci. 113:79-84.

Miller D.R., R.M. Waskom, M.A. Brick, and P.L. Chapman, 1991. Transferring in vitro technology to the field. Biotech. 9:143-146.

Morpurgo R., 1991. Correlation between potato clones grown *in vivo* and *in vitro* under sodium chloride stress conditions. Plant Breed. 107:80-82.

Morpurgo R. and D.S. Rodriguez, 1987. In vitro differential response of the potato (*Solanum tuberosum* L.) under sodium chloride stress conditions. Riv. Di Agric. Subtrop. e Trop. 81:73-77.

Munns R., 1993. Physiological process limiting plant growth in saline soil: some dogmas and hypothesis. Plant Cell Environ. 16:15-24.

Munns R., D.P. Schachtman, and A.G. Condon, 1995. The significance of a two-stage growth response to salinity in wheat and barley. Aust. J. Plant Physiol. 22:561-569.

Murashige T. and F. Skoog. 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol. Plant. 15:473-479.

Nabors M.W., 1990. Environmental Stress Resistance. In: Plant Cell Line Selection: Procedures and Applications. Dix P.J. (ed.). Weinheim, New York. pp.167-186.

Nabors M.W., S.E. Gibbs, C.S. Bernstein, and M.E. Meis, 1980.NaCl tolerant tobacco plants from cultured cells. Z. Pflanzenphysiol. 97:13-18.

Nadler A. and B. Heuer, 1995. Effect of saline irrigation and water deficit on tuber quality. Pot. Res. 38:119-123.

Naik P.S. and J.M. Widholm, 1993. Comparison of tissue culture and whole plant responses to salinity in potato. Plant Cell, Tis., Org. Cult. 33:273-280.

Nieman R.H. and M.C. Shannon, 1976. Screening Plants for Salinity Tolerance. In: Plant Adaptation to Mineral Stress in Problem Soils. Proc. of a Workshop at National Agricul. Lib., Wright M.J. (ed.). Ethaca, New York. pp.359-372.

Norlyn J.D., 1980. Breeding Salt Tolerant Plants. In: Genetic Engineering of Osmoregulation: Impact on Plant Productivity for Food, Chemicals and Energy. Rains D.W., R.C. Valetine, and Alexander Hollaender (eds.). Plenum Press, New York. pp.293-310.

Nowak J. and D. Colborne, 1989. In vitro tuberization and tuber proteins as indicators of heat stress tolerance in potato. Am Pot. J. 66:35-45.

Paliwal K.V. and B.R. Yadav, 1980. Effect of saline irrigation water on the yield of potato. Indian J. Agric. Sci. 50:31-33.

Palmer C.E. and O.E. Smith, 1970. Effect of cytokinin on tuber formation of isolated stolons of *Solanum tuberosum* cultured in vitro. Plant Cell Physiol. 11:303-314.

Picchioni G.A. and S. Miyamoto, 1990. Salt effects on growth and ion uptake of *Pistachio* rootstock seedlings. J. Amer. Soc. Hort. Sci. 115:647-653.

Picchioni G.A. and S. Miyamoto, 1991. Rapid testing of salinity effects on *Pistachio* seedling rootstocks. J. Amer. Soc. Hort. Sci. 116:555-559.

Ponnamperuma F.N., 1977. Screening rice for tolerance to mineral stresses. IRRI Res., Philippine, Paper Ser. p.6.

Ponnamperuma F.N., 1984. Role of Cultivar Tolerance in Increasing Rice Production on Saline Lands. In: Salinity Tolerance in Plants. Staples R.C. and G.H. Toenniessen (eds.). Wiley-Interscience Pub., John Wiley and Sons, New York. pp.125-150.

Potluri S.D.P. and D.P.V. Prasad, 1993. Influence of salinity on axillary bud cultures of six lowland tropical varieties of potato (*Solanum tuberosum*). Plant Cell, Tis., Org. Cult. 32:185-191.

Rajashekar G., D. Palmquist, and C.A. Ledbetter, 1995. In vitro screening procedure for osmotic tolerance in Prunus. Plant Cell, Tis., Org. Cult. 41:159-164.

Ram N.V.R. and M.W. Nabors. 1985. Salinity Tolerance. In: Biotechnology: Applications and Research. Cheremisinoff P.N. and R.P. Ouelette (eds.). Technomic, Lancaster, Basel. Chapter 46, pp.623-642.

Ramage R.T., 1980. Genetic Methods to Breed Salt Tolerance in Plants. In: Genetic Engineering of Osmoregulation. Rains D.W., R.C. Valentine (eds.). Plenum Press, New York. pp.311-318.

Ramagopal S. and J.B. Carr, 1991. Sugarcane proteins and messenger RNAs by salt in suspension cells. Plant Cell and Environ. 14:47-56.

Rowland G.G., A. McHughen, and C. McOnie, 1989. Field performance at saline-affected sites of a somaclonal variant of *McGregor flax* selected for salt tolerance in vitro. Can. J. Plant Sci. 69:49-60.

Rowley G., 1993. Multinational and national competition for water in the Middle East: Towards the deepening crisis. J. Environ. Mgt. 39:187-197.

Salisbury F.B. and C.W. Ross, 1992. Osmosis. In: Plant Physiology. Salisbury F.B. and C.W. Ross (eds.). Wadsworth Pub. Co., Belmont, CA. pp.49-50.

SAS Institute Inc, 1989. SAS/STAT User's Guide, Version 6, 4th ed. Volume 1, SAS Institute Inc. Cary, NC.

Satti S.M.E., M. Lopez, and F.A. Al-Said, 1994. Salinity induced changes in the vegetative and reproductive growth in tomato. Commun. Soil Sci. Plant Anal. 25:501-510.

Saxena N.P., M.C. Saxena, P. Ruckenbauer, R.S. Rana, M.M. El-Fouly, and R. Shabana, 1994. Screening techniques and sources of tolerance to salinity and mineral nutrient imbalances in cool season food legumes. Euphytica 73:85-93.

Scott S.J., R.A. Jones, and W.A. Williams. 1984. Review of data analysis of methods for seed germination. Crop Sci. 248:1192-1199.

Serrano R. and R. Gazelle, 1994. Microbial models and salt stress tolerance in plants. CRC Crit. Rev. Plant Sci. 13:121-138.

Shah G.H., S.J. Wainwright, and M.J. Merrett, 1993. Cation tolerance in callus cultures of *Medicago sativa* L. tolerant to sodium chloride. Plant Sci. 89:81-84.

Shannon M.C., 1979. In quest of rapid screening techniques for plant salt tolerance. HortSci. 14:587-589.

Shannon M.C., 1984. Breeding, Selection, and the Genetics of Salt Tolerance. In: Salinity Tolerance in Plants: Strategies for Crop Improvement. Staples R.C. and G.H. Toenniessen (eds.). Wiley-Interscience Pub., John Wiley and Sons, New York. pp.125-150.

Shannon M.C., 1996. New Insights in plant breeding for improved salt tolerance. HortTech. 6:96-99.

Shannon M.C., M.C. Grieve, and L.E. Francois, 1994. Whole plant Response to Salinity. In: Plant-Environment Interactions. Wilkinson R.E. (ed.). Maicel Decker Inc., New York. pp.199-245.

Sparague J.B., 1973. The ABC's of Pollution Bioassay Using of Fish. In: Biological Methods for the Assessment of Water Quality. Cairns J. Jr, and K.L. Dickson (eds.). Am. Soc. for Testing and Materials, Philadelphia. pp.6-60.

Stallknecht F.G. and S. Farnsworth, 1982a. General characteristics of coumarin-induced tuberization of axillary shoots of *Solanum tuberosum* cultured *in vitro*. Am. Pot J. 59:17-32.

Stallknecht F.G. and S. Farnsworth, 1982b. The effect of the inhibitors of protein and nucleic acid synthesis on the coumarin-induced tuberization and growth of excised axillary shoots of potato sprouts *Solanum tuberosum* L.) Cultured *in vitro*. Am Pot. J. 59:69-75.

Sweby D.L., B.I. Huckett, and M.P. Watt, 1994. Effects of nitrogen nutrition on salt-stressed *Nicotina tabacun* var. Samsun in vitro plantlets. J. Exp. Bot. 45:995-1008.

Tal M., 1984. Selection for Stress Tolerance. In: Hand Book of Plant Cell Culture. Evans D.C., W.R. Sharp, P.V. Ammirato, and Y. Yamada (eds.). MacMillam, New York. Vol.1, pp.481-488.

Tal M., 1990. Somaclonal Variation for Salt Tolerance. In: Biotechnology in Agriculture and Forestry. Vol. 11: Somaclonal Variation in Crop Improvement. Bajaj Y.P.S. (ed.). Springer-Verlag, Berlin, Heidelberg. pp.236-257.

Tal M., 1993. In vitro methodology for increasing salt tolerance in crop plants. Acta Hort. 336:69-78.

Tal M., 1994. In vitro selection for salt tolerance in crop plants: Theoretical and practical considerations. In Vitro Cell Dev. Biol. 30:175-180.

Vayda M.E., 1990. Environmental Stress and Its Impact on Potato Yield. In: The Molecular and Cellular Biology of the Potato. Vayda M.E. and W.D. Park (eds). CAB Int'l., Wallingford. pp.239-250.

Wang P.J., 1977. Regeneration of Virus-Free Potato From Tissue Culture. In: Plant Tissue Culture and It's Biotechnological Application. Barz W., E. Reinhard, and M.H. Zenk (eds.). Proc. Int'l. Congr. Med. Plant Res. pp.386-391.

Wang P.J. and C.Y. Hu, 1982. In vitro mass tuberization and virus-free potato production in Taiwan. Am. Pot. J. 59:33-39.

Wang P.J. and C.Y. Hu, 1985. Potato tissue culture and its application. In: Potato Physiology. Li P.H. (ed.). Acad. Press, Orlando. pp.503-577.

Wattimena G., B. McCown, and G. Weis, 1983. Comparative field performance of potatoes from micro-culture. Am. Pot. J. 60:27-33.

Wenzel G. and B. Foroughi-Wehr, 1990. In Vitro Selection. In: Plant Breeding: Principles and Prospects. Hayward M.D., N.O. Bosemark, and I. Romagosa (eds.). Chapman and Hall, London. pp.352-370.

Wiersema S.G., R. Cabello, P. Tovar, and J.H. Dodds, 1987. Rapid seed multiplication by planting into beds microtubers and in vitro plants. Pot. Res. 30:117-120.

Winicov I., 1991. Characterization of salt tolerant alfalfa (*Medicago sativa* L.) plants regenerated from salt tolerant cell lines. Plant Cell Rep. 10:561-564.

Woolfe J.A., 1986. The Potato in the Human Diet. Cambridge University press. Cambridge.

Yeo A.R., M.E. Yeo, S.A. Flowers, and T.J. Flowers, 1990. Screening of rice

(*Oryza sativa* L.) genotypes for physiological characters contributing to salinity resistance and their relationship to overall performance. Theor. Appl. Genet. 79:377-384.

Zekri M., 1993. Osmotic and toxic ion effects on seedling emergence and nutrition of citrus rootstocks. J. Plant Nutr. 16: 2013-2028.

Zeroni M., 1988. Plant tolerance of salinity in greenhouse-physiological and practical considerations. Acta Hort. 229: 55-72.

Zhang, Y., J.E. Abdulnour, and D.J. Donnelly, 1998. Effects of NaCl stress on yield of potato plants derived from previously saline conditions. (HortSci., in submission).

Zhang Y., M. Brault, V. Chalavi, and D. Donnelly, 1993. In Vitro Screening for Salinity Tolerant Potato. In: Biometeorology. Proc. of the 13th Int'l Cong. of Biometeor.. Calgary, AB, Canada. Part 2, pp.491-498.

Zhang Y. and D.J. Donnelly, 1995. A modified nodal cutting bioassay for assessing salinity tolerance in potato (*Solanum* spp.). HortSci. 30:752.

Zhang Y. and D.J. Donnelly, 1997. In vitro screening for salinity tolerant screening of potato. Pot. Res. 40:285-295.

Zhang, Y., D.J. Donnelly, and J.E. Abdulnour, 1998. Distinguishing osmotic and specific-ion effects of NaCl on potato microtuber yield (HortSci., in submission).

Zurayk R., M. Nimah, and M. Hamze, 1993. The salt tolerance potential of local cultivars of eggplant (*Solanum melongena* L.). Biolog. Agric. & Hort. 9:317-324.

Appendix 1 List and description of the cultivars, hybrids, and wild species used in the in vitro bioassays.

A: Cultivars[a]	Description	
Atlantic	Midseason maturity	(120 d)
Kennebec	Midseason maturity	(120 d)
Norland	Early season maturity	(90 d)
Russet Burbank	Very late season maturity	(140 d)
Shepody	Midseason maturity	(120 d)
Spunta	Midseason maturity	(120 d)
Superior	Early-midseason maturity	(100 d)

B: Hybrid clones[b]	Origin	Pedigree
9787-07	Poland	*S. chacoense* X *S. tuberosum*
9506-04	Argentina	*S. chacoense* X *S. microdontum* X *S. tuberosum*
10908-06	Clones 10908-06 to 10911-04 were backcross hybrids between Agentinian materials and *S. tuberosum*	
10910-08		
10911-02		
10911-04		

C: Wild species[c]	PI# of accessions	
S. chacoense:	458309	473403
S. microdontum:	218223	458353
S. gourlayi:	473029	473073

[a]Performance evaluations of potato clones and varieties in the northeastern states. Maine Agricultural Experiment Station, 1985.
[b]Dr. H. De Jong, pers.comm.. Agriculture and Agri-Food Canada, Fredericton NB, Canada.
[c]Dr. J.B. Bamberg, Inter-Regional Potato Introduction Station, Sturgeon Bay, WI,USA.

Appendix 2 Media used for potato propagation and bioassays. All were Murashige-Skoog (1962) basal salt medium with the following additions.

	<1> Single-node cutting	<2> Seed germination	<3> Microtu-berization	<4> Root tip segment (agar)	<5> Root tip segment (liquid)
(mg l^{-1})					
myo-inositol	100	100	100	100	100
GA_3			0.4*		
BAP			0.5*		
sucrose (g l^{-1})	30	30	20*/80**	30	30
agar (g l^{-1})	7	7		7	
pH	5.7	5.7	5.7	5.7	5.7

MS (1962) media components	(mg l^{-1})
NH_4NO_3	1,650
KNO_3	1,900
$CaCl_2.2H_4O$	440
$MgSO_4.7H_2O$	370
KH_2PO_4	170
$MnSO_4.4H_2O$	22.3
$ZnSO_4.7H_2O$	8.6
H_3BO_3	6.2
KI	0.83
$Na_2MoO_4.H_2O$	0.25
$CoCl_2.6H_2O$	0.025
$CuSO_4.5H_2O$	0.025
NaEDTA.$2H_2O$	37.5
$FeSO_4.7H_2O$	27.8
Ca-Pantothenate	2.0
Glycine	2.0
Thiamine·HCl	1.0
Niacin	0.5
Pyridoxine·HCl	0.5

*Step-1; **Step-2.

Appendix 3 Data for Fig. 3.1; effect of NaCl (0, 80, and 120 mM) on single-node cuttings of seven potato cultivars.

Parameters (LSD 5%)	NaCl (mM)	Cultivars						
		Atlantic	Kennebec	Norland	R.Burbank	Shepody	Spunta	Superior
Shoot length (mm) (LSD 5% = 2.63)	0	44.9	59.1	60.2	48.9	43.5	43.1	52.1
	80	15.5	24.2	11.4	15.1	16.2	15.9	20.5
	120	5.9	6.4	3.8	13.9	8.4	14.5	4.1
Root length (mm) (LSD 5% = 6.02)	0	56.2	58.3	82.1	76.4	79.5	59.2	51.3
	80	35.0	28.9	31.1	50.3	21.4	37.2	30.1
	120	17.3	28.6	13.5	42.9	14.6	28.7	18.5
Shoot dry weight (mg) (LSD 5% = 0.07)	0	2.6	3.9	5.1	2.9	4.7	2.8	5.3
	80	1.7	2.0	2.8	2.2	2.1	1.9	2.3
	120	0.9	0.9	1.1	1.0	1.4	1.1	1.2

Appendix 4 Data for Fig. 3.2; effect of NaCl (0, 80, and 120 mM) on single-node cuttings of potato hybrids.

Parameters (LSD 5%)	NaCl (mM)	Hybrids					
		9708-07	9506-04	10908-06	10910-08	10911-02	10911-04
Shoot length (mm) (LSD 5% = 2.48)	0	20.5	25.8	57.9	28.2	65.1	62.8
	80	11.7	8.4	22.0	0.4	7.0	3.9
	120	4.3	6.1	1.5	0.0	1.7	1.8
Root length (mm) (LSD 5% = 2.67)	0	19.8	52.2	38.1	20.1	28.9	24.7
	80	4.9	1.1	0.0	0.0	2.6	0.0
	120	0.0	3.5	0.0	0.0	0.0	0.0
Shoot dry weight (mg) (LSD 5% = 0.59)	0	5.8	9.1	9.8	3.9	5.2	9.3
	80	3.3	4.7	0.7	0.5	1.6	0.3
	120	0.9	2.8	0.4	0.0	0.4	0.3
Root dry weight (mg) (LSD 5% = 0.04)	0	0.8	1.8	1.6	0.6	0.5	2.0
	80	0.2	0.1	0.0	0.0	0.0	0.0
	120	0.0	0.0	0.0	0.0	0.0	0.0

Appendix 5 Data for Fig. 3.3; effect of NaCl (0, 80, and 120 mM) on three potato species seed germination and early seedling growth in vitro.

Parameters (LSD 5%)	NaCl (mM)	Wild Species		
		S. chacoense	*S. gourlayi*	*S. microdontum*
Germination rate (LSD 5% = 10.7)	0	92.5	40.0	68.1
	80	78.9	13.2	27.0
	120	49.6	2.8	8.6
Shoot length (mm) (LSD 5% = 1.99)	0	32.6	25.1	31.3
	80	20.0	7.8	15.1
	120	14.1	0.6	7.3
Root length (mm) (LSD 5% = 4.56)	0	60.1	50.9	55.8
	80	48.5	15.4	24.2
	120	39.1	0.5	10.1
Root dry weight (mg) (LSD 5% = 0.08)	0	0.5	0.3	0.3
	80	0.3	0.2	0.2
	120	0.2	0.01	0.1

Appendix 6 Lysimeter salinity level EC (dSm^{-1}) variations examined at weekly intervals (0 - 10) during the 1995/field trial (from Mr. S. Patel, pers. comm., 1997).

Salinity level (EC dSm^{-1})		0	1	2	3	4	5	6	7	8	9	10	Average
Calib-ration	Subirri-gation												
1.0	1.0	1.19	0.84	0.97	1.04	1.19	1.26	1.24	1.32	1.52	1.52	1.7	1.26
	9.0	1.24	0.77	0.9	0.97	1.12	1.33	1.76	2.13	2.73	3.03	3.3	1.75
2.0	1.0	3.15	2.44	2.84	3.02	3.3	3.38	3.15	3.09	3.31	3.37	3.4	3.13
	9.0	3.08	2.51	2.93	3.08	3.46	3.78	3.97	4.34	4.97	5.2	5.5	3.89