Salt Stress Tolerance in Potato Genotypes

A Thesis Submitted to the College of
Graduate Studies and Research
In Partial Fulfillment of the Requirements
For the Degree of Doctor of Philosophy
In the Department of Plant Sciences
University of Saskatchewan
Saskatoon

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Abstract

Soil salinity affects over 20% of the world's irrigated land. Potato (*Solanum tuberosum* L.), the most important vegetable crop worldwide, is relatively salt sensitive. However, relatively little work has been done on salt tolerance of the potato plant. This thesis investigated the methodology of treatment application and scion/rootstock effects on subsequent salt stress responses of four contrasting potato genotypes: 'Norland', 9506, 9120-05 [ABA-deficient mutant], and 9120-18 [ABA-normal sibling] grown hydroponically in sand. The effect of incremental salt stress were studied, using NaCl, $CaCl_2$ and combined NaCl + $CaCl_2$ pre-treatments as well as varying methods of ABA application with a specific focus on the role of rootstock and scion. Physiological responses of various potato genotypes to salt stress differed depending on how the salt stress was applied. An incremental salt stress regime was able to more effectively differentiate genotypes based on salt stress resistance and greater salt tolerance compared to a sudden salt shock. Generally, the ability to produce ABA was positively related to the degree of salt stress resistance, with higher ABA levels induced under incremental salt stress treatments compared to salt shock. The method of ABA application also had a marked effect on potato responses to salt stress. Slowly increasing concentrations of exogenous ABA maintained growth rates, enhanced root water content and induced more lateral shoot growth compared to a single ABA dose. The degree of salt tolerance induced by the grafted rootstock was primarily modulated by salt acclimation and was manifested in the scion as increased water content, stem diameter, dry matter accumulation, stomatal conductivity, and osmotic potential and was associated with reduced leaf necrosis. Using the salt-resistant 9506 line as a scion also significantly increased root fresh and dry weight and stem diameter as well as root water content of salt-sensitive ABA-deficient mutant rootstocks. Exogenous ABA appeared to enhance plant water status via the roots under salt stress beyond that of grafting alone. This was verified by more positive stomatal conductivity and greater upward water flow in ABA treated grafted and non-grafted plants as compared to the absence of upward water flow in non-treated grafted plants as measured via micro NMR imaging. NaCl pre-treatment produced greater salt stress resistance compared to pre-treatment with $CaCl_2$ and was associated with a specific Na^+ ion effect rather than a non-specific EC-dependent response. However, the presence of both ABA and $CaCl_2$ appears to be necessary in order to enhance Na^+ exclusion from the shoot and increases the K^+/Na^+ ratio.

I dedicate this work to the memory of mother,
(Bibijan Ahmadimanesh),
who supported and encouraged me throughout all my life.

*

She would wish me to expand this dedication
to include all who live as she lived:

To all who put others before oneself,
To all who don't eat until others are fed,
To all who don't sleep until others are rested,
To all who can realize pain, that others might not,
To all who can't read but make sure others can write,
To all who don't stop encouraging, until all goals are met,
To all who are determined to teach others how to be patient,
To all who go through hardships so others can climb mountains,
To all who know what it's like to be children of great mothers.

Acknowledgments

Finding adequate words is difficult for expressing my appreciation to my supervisor, Dr. K. K. Tanino, for her support, guidance, patience, gentleness and smile. Without her, I would not have been able to complete this research. I greatly value the help of Dr. D. R. Waterer, who provided tremendous assistance with editing my thesis, information on statistical analysis and, on the occasions that I have needed his assistance his door has always been open. I would also like to express my thanks to my advisory committee, comprising Drs. V. K. Sawhney, Y. Bai, P. J. Hucl, L.V. Gusta and B. Coulman, for their guidance and advice throughout the entire project. Appreciation is also extended to Dr. D. Ehret for his critical reading of the manuscript and making constructive suggestions. Thanks also go to Dr. H. De Jong of Agriculture and Agri-Food Canada in Fredericton, New Brunswick, for supplying plant materials. I extend special gratitude to Dr. S. Abrams and other staff in the Mass and Hormone Profiling Lab in the Plant Biotechnology Institute at the Saskatchewan Research Centre for their help in the hormone profiles analyses. I am also grateful to Dr. Ed Kendal for providing NMR facilities in RUH. For informative consultations, I thank Drs. B. Baker, S. Jana and M. Bandara. And for all their assistance and collaboration while I was working in the greenhouse, I wish to thank T. Ward and J. Peters.

I am grateful for the financial support provided for this research by the Agricultural Research Education and Extension Organization of Iran and by the University of Saskatchewan. Further, I wish to thank my friends and fellow graduate students in Saskatoon for their friendship and support during my time here. For their encouragement given me throughout this project, I offer sincere appreciation to my husband and our two daughters. Finally, my most heartfelt gratitude goes to my mother, for her tireless support of me all the way through my life.

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List of Abbreviations

Ψ_S	Unit of osmotic potential
ABA	Abscisic acid
ABA-	ABA-deficient mutant (9120-05)
ABA+	ABA-Normal sibling (9120-18)
9506	Salt Stress Resistant
9506/ ABA-	Resistant scion grafted onto the ABA-Deficient mutant rootstock
9506/ABA+	Resistant scion grafted onto the ABA-Normal sibling rootstock
ABA-/9506	ABA-Deficient mutant scion grafted onto the Resistant rootstock
ABA+/9506	ABA-Normal sibling scion grafted onto the Resistant rootstock
CIP	International Potato Center
DW	Dry weight
EC	Electrical conductivity
FAO	Food and Agriculture Organization
Flc	*Flacca*
Fr	*Flacca* rootstock
Fs	*Flacca* shoot
FW	Fresh weight
GA1-deficient	Gibberellic acid–1, Deficient mutant
GC-MS	Gas chromatography-mass spectrometry
GLM of SAS	General Linear Models of Statistical Analysis System
K^+/Na^+	Potassium to sodium ratio
LD_{50}	Median lethal dose
Not	Notabilis
NMR	Nuclear magnetic resonance
PAR	Photosynthetically active radiation
phu	Original *droopy (Solanum phoreja*)
PNRSV	*Prunus* Necrotic Ringspot Virus
PP_iase	Pyrophosphatase
S	Oppositional-allele of *Solanum tuberosum*
Sit	*Sitiens*
wil	*Wilty* mutant of pea
Wr	Wild type rootstock
Ws	Wild-type shoot
WT	Wild-type

1.0 Introduction

The progressive natural and anthropogenic salinization of arable lands at the rate of three hectares per minute worldwide (FAO, 2006) is a major concern for agricultural crop production (Parida and Das, 2005). The sustainability of agriculture production in many areas of the world including North and South America, Asia, Europe and Australia is at risk due to soil salinization (Schoups *et al*., 2005; Chaves *et al*., 2006; Rengasamy, 2006). In the Great Plains of North America, soil salinization threatens the productivity of agricultural land (Mckee *et al*., 2004) and agricultural sustainability (Waisel, 2001). Soil of the Canadian Prairie provinces generally contains high concentrations of $MgSO_4$, Na_2SO_4 and $CaSO_4$ salts (Eilers *et al*., 1995). These salts are the result of chemical action on the minerals in the upper layers of the glacial till that underlies the soils in these regions (Eilers *et al*., 1995). About 1.6 million ha of cultivated land in Saskatchewan are negatively affected by salinity (Crosson, 1976).

Salt tolerance is the ability of plants to grow and complete their life cycles on a substrate that contains high concentrations of soluble salt. Based on this definition, plant species are divided into two groups: halophytes and glycophytes. Halophytes can survive on high concentrations of salt in the rhizosphere: they can grow well and complete their life cycles in saline environments. Glycophytes, which include all major crops, cannot grow well and complete their life cycles and/or suffer a loss in productivity or yield under saline conditions. At high salt concentrations, glycophytic plants cannot set seed and often die (Borsani *et al*., 2003; Parida and Das, 2005). The plant salinity threshold is defined as the maximum soil electrical conductivity (EC) a plant can tolerate, after which yield decreases as salinity increases (Maas, 1986). On the basis of their tolerance to salt, plant species can be subdivided into four groups, namely (1) salt tolerant, (2) moderately salt tolerant, (3) moderately salt sensitive and (4) salt sensitive (Maas and Hoffman, 1977; Katerji *et al*., 2003). Among herbaceous crops, the most salt sensitive is Jerusalem artichoke (*Helianthus tuberosus* L., EC=0.4 dS m^{-1}) while canola (*Brassica napus* L., EC=11.0 dS m^{-1}) is the most salt tolerant. The salinity thresholds for barley (*Hordeum vulgare* L.), kenef *(Hibiscus cannabinus* L.), cotton (*Gossypium hirsutum*), wheat tall grass (*Agropyron elongatum* L.), sugar beet (*Beta vulgaris* L.) and Bermuda grass (*Cynodon dactylon* L.) range from 6.9 to 8.1 dS m^{-1}. These crops are known to tolerate high concentrations of salt. Potato is the fourth most important food crop globally (CIP, 2007). However, most cultivated potato genotypes are relatively salt sensitive (Katerji *et al*., 2003).

Salinity levels as low as 2.3 dS m^{-1} reduce both growth and tuber yield (Katerji *et al*., 2003). The responses of potato cultivars and wild species to elevated levels of NaCl and Na_2SO_4 are variable (Li *et al*., 2006). However, despite the economic importance and apparent salt sensitivity of potato, relatively little work has been reported on salinity resistance mechanisms in potato. Approximately 4,047 ha of Saskatchewan land are used for the production of 111,100 tons of potato, representing this province's most economically important vegetable crop (ASFS, 2008). The application of mineral nutrients is required for maximum potato production; however, the application of excess fertilizer has contributed to increasing levels of soil salinity. Potato is a heavily irrigated crop which also contributes to salinization of potato fields.

Salinity stress is generally caused by osmotic stress and ionic imbalance (Zhu, 2002; Rodriguez *et al*., 2005). The enzymes in living cells have evolved to function at very low ionic concentrations, mainly 100-200 mM K^+ and less than 50 mM Na^+ and Cl^-. When salinity results from the excess of NaCl, which is by far the most common type of salt stress, the increased intercellular concentration of Na^+ and Cl^- is deleterious to cellular systems (Serrano *et al*., 1999). In addition, the homeostasis of not only Na^+ and Cl^- but also of essential cations such as K^+ and Ca^{2+} is disturbed (Serrano *et al*., 1999; Hasegawa *et al*., 2000; Tattini *et al*., 2002).

Plant survival and growth under salt stress depend on adaptations that re-establish ionic homeostasis, thereby reducing the cellular exposure to ionic imbalances. High concentrations of salt impose a hyperosmotic shock by decreasing the chemical activity of water, thereby causing loss of turgor. This negative effect in the plant cell is thought to be similar to the effects caused by drought (Borsani *et al*., 2003). Therefore, salt-induced water stress reduction of chloroplast stoma volume and regeneration of reactive oxygen species is thought to play an important role in the inhibition of photosynthesis seen in salt-stressed plants (Price and Hendry, 1991; Allen, 1995).

The effective regulation of water is central to resistance of many stresses, including salt stress. A common indicator of salt stress is the reduction of growth due to inadequate water uptake (Munns, 2002; Borsani *et al*., 2003). Regulation of water can occur at the whole plant, the tissue or the cellular levels, and has been shown to be modified by several factors, particularly abscisic acid (Miao *et al*., 2006) and calcium (Alleva *et al*., 2006).

Plants respond to salt stress at the cellular, tissue and whole plant levels. Cell-based mechanisms of ion homeostasis and synthesis of osmoprotectants are essential determinants of

salt stress tolerance. Integration and coordination of the responses of tissue and organs are also required for proper tolerance of salt stress and are useful in selection of salt- tolerant crops. For example, the *Arabidopsis* AtHKT1 mutant facilitates Na^+ homeostasis and modulates K^+ status of seedlings grown *in vitro* and plants grown in controlled environmental conditions through higher accumulation of Na^+ content in the shoot and lower Na^+ content in the root, thereby suppressing NaCl sensitivity (Rus *et al*., 2004). Inhibition of shoot growth accompanied by continued root growth has been considered a whole plant adaptation to salt stress or water stress (Meloni *et al*., 2001; Akhtar *et al*., 2003; Mulholland *et al*., 2003; Qaderi *et al*., 2006).

Given all the factors determining the pleiotropic deleterious effects of salt stress, it is not surprising that adaptation to salinity may involve the interplay or interaction of a large number of responses. Upon exposure to a sublethal level of an environmental stress such as salt stress, many plants acclimate to the stress. Pre-exposure to mild stress can result in a significant increase in tolerance to that stress. In the case of *Sorghum,* the adapted plant can develop and produce seeds under stress conditions that would otherwise be lethal to a non-adapted plant (Amzallag *et al*., 1990b).

Calcium, a well-known protectant against sodium toxicity, increases the selectivity of root K^+/Na^+ uptake (Cramer *et al*., 1987; Bolat *et al*., 2006). Calcium is mainly found in the bound form in the plant cell and has an apoplastic role in promoting structural cell wall development, reducing membrane damage and leakage of K^+ (Piyasiri *et al*., 1986; Renault, 2005; Bolat *et al*., 2006). Maintenance of adequate K^+ levels in the cytoplasm is essential for normal growth of the cell (Gorham, 1993; Khatun and Flowers, 1995; Pilot *et al*., 2003; Chen *et al*., 2005). Calcium appears to play an important role in water transport of plants under salt stress conditions and the restoration of hydraulic conductivity can also be related to Ca^{2+} concentration in the nutrient solution (Azaizeh and Steudle, 1991; Cabanero *et al*., 2006). Calcium ameliorates the negative effects of salts on water transport by regulating aquaporin activity (Carvajal *et al*., 2000; Cabanero *et al*., 2006). Calcium is also a secondary messenger of abscisic acid (ABA) and is involved in environmental signalling in response to stresses such as salinity (Rengel, 1992; Poovaiah and Reddy, 1993; Lee *et al*., 2004).

Abscisic acid mediates the primary responses of plants to environmental stresses such as drought and salinity (Giraudat *et al*., 1994; Yan *et al*., 2006). ABA induces physiological changes which predispose plants to tolerate salt and water stress (Davies and Mansfield, 1983;

Van Steveninck and Van Steveninck, 1983; Lee *et al*., 2003). Exposure to desiccation and salt stress is generally accompanied by endogenous elevation of ABA (Zeevaart and Creelman, 1988; Davies and Jones, 1991; Aswath *et al*., 2005). This increase in ABA levels contributes to the induction of several water and salt-stress-induced genes (Chandler and Robertson, 1994; Barrero *et al*., 2006; Umezawa *et al*., 2006). ABA affects the water status of plants by reducing transpiration via regulation of stomata and increasing water flux into the roots (Van Steveninck and Van Steveninck, 1983; Sansberro *et al*., 2004). Hydraulic conductivity, solute flow and osmotic adjustment are important factors in increasing water flux into the root (Van Steveninck and Van Steveninck, 1983; An *et al*., 2002). ABA-deficient and insensitive mutants have been employed to understand the role of ABA in the transduction pathway (Jensen *et al*., 1996; Kof *et al*., 2006) and the cellular and molecular mechanisms of drought tolerance in plants (Vartanian, 1996; Lin *et al*., 2007). A better understanding of these mechanisms has implications for crop production (Quarrie, 1991; Deng *et al*., 2005).

The well-characterized *wilty* mutant of potato, *droopy* (Quarrie, 1982), results from reduced levels of endogenous ABA due to a blockage in the conversion of ABA-aldehyde to ABA (Duckham *et al*., 1989). This mutant is one of many deleterious recessive mutants that has been characterized in the cultivated diploid (2n=24) potato (Simmonds, 1965). Droopy potato (*Solanum tuberosum* L. group *phureja*) is a recessive mutant occurring in a progeny of a cross between two clones (C.P.C. 2862 X 2863 of *Solanum tuberosum* L. group *phureja* [Simmonds, 1965]). Droopy lines tend to wilt due to excessive stomatal opening (Waggoner and Simmonds, 1966). Wilt prone mutants were also identified in other species including corn (Postlethwaite and Nelson, 1957; Rock and Ng, 1999), tomato (Alldridge, 1964; Taylor *et al*., 2000), sunflower (Giordani *et al*., 1999), bean (*Phaseolus vulgaris* L.) (Yang and Zeevaart, 2006), pepper (*Capsicum annum* L.) (Tal *et al*., 1976; Murthy and Tester, 2006), barley (Chen *et al*., 2004), *Arabidopsis* (*Arabidopsis thaliana*) (Koiwai *et al*., 2004), tobacco (*Nicotiana tabacum* L.) (Wigger *et al*., 2002), rice (*Oryza sativa*) (Agrawal *et al*., 2001) and pea (*Pisum sativum* L.) (Dodd, 2003). The wilt prone tomato mutants *flacca*, *sitiens* and *notabilis* were unable to control their stomatal apertures, largely because of a deficiency in ABA production (Tal and Nevo, 1973; Thompson *et al*., 2000). Treatment with ABA reverts the wilty tomato to the normal phenotype (Mulholland *et al*., 2003). Another diploid potato similar to the earlier *droopy* mutant described by Simmonds (1965) was rediscovered by De Jong (2001) in New

Brunswick (Agriculture and Agri-Food Canada Potato Research Centre, Fredericton). This mutant had a longer tuber dormancy than the original *droopy,* was also deficient in abscisic acid, and was unable to regulate water loss from its leaves.

Grafting is a useful tool in horticultural crop production. In typical grafts, rootstocks with desirable traits such as resistance to soil-borne disease and/or above stress factors are grafted on to scions with superior horticulural characteristics, i.e., yield and quality. The ability to use grafting to combine superior rootstock and scion characteristics has resulted in increasing commercial cultivation of grafted vegetable crops like melons and tomatoes over the past few years (Lee and Oda, 2003). Grafting has also been used to study the responsive role of the root and shoot in the control of ion accumulation under salt stress conditions in soybean (Grattan and Maas, 1985), chickpea (Dua, 1997), tomato (Santa-Cruz *et al*., 2001; 2002; Fernández-García *et al*., 2002; 2004b; Estañ *et al*., 2005), melon and watermelon (Romero *et al*., 1997; Edelstein *et al*., 2005; Colla *et al*., 2006a; 2006b) and tobacco (Ruiz *et al*., 2005).

In summary, potato is an important crop that is salt-sensitive. Salinization of agricultural land is increasing due to over-fertilization and the use of poor water quality and/or inappropriate irrigation practices. Studies on salt tolerance mechanisms in potato are rare. Some wild species and only a few genotypes have been studied. However, in order to fully understand the mechanisms of salt stress resistance, it is important to appropriately apply the treatments. Therefore, this thesis initially developed an NaCl salt stress and ABA application regime for salt stress evaluation. Four contrasting genotypes were used in this study: a salt sensitive ABA-deficient mutant, its normal sibling, a salt stress resistant genotype (9506) and a widely utilized commercial cultivar of potato 'Norland'. To further study the role of shoots and roots the NaCl salt pre-treatment, reciprocally grafted genotypes were exposed to salt stress. The separate and combined effects of NaCl salt pre-treatment and calcium in alleviating the adverse effects of salt stress on growth parameters and ion accumulation were then evaluated.

Hypotheses

1- Potato can salt-acclimate and this response is genotype-dependent.

2- Salt tolerance is associated with ABA in the potato genotypes.

3- Salt tolerance can be transferred from rootstock to scion and from scion to rootstock.

4- Calcium plays an important role in salt stress tolerance of potato genotypes.

Overall questions and objectives

1- Is potato able to salt acclimate and does this ability vary among potato genotypes?

To investigate this capability, four contrasting potato genotypes were subjected to two levels of salt acclimation for three weeks.

2- Is ABA involved in salt stress tolerance of potato genotypes?

To study the role of ABA in salt tolerance, four different levels of exogenous ABA with different methodology of application were applied on four potato genotypes. In addition, endogenous ABA was measured during salt acclimation. Finally, responses to exogenous ABA were compared between an ABA-deficient mutant and its normal sibling.

3- Can salt tolerance be transferred from rootstock to scion and from scion to rootstock?

To answer this question, reciprocal grafting between salt tolerant and salt sensitive genotypes was performed.

4- What is the effect of calcium in salt stress tolerance of potato genotypes?

To address this point, combinations of NaCl and $CaCl_2$ were supplied to grafted and non-grafted contrasting potato genotypes of differing salt tolerance, including the ABA-deficient mutant and its normal sibling, with subsequent salt stress application. In addition, endogenous levels of calcium, sodium and potassium were measured and localization assessed in the roots and shoots in salt stress experiments. Finally, whether the response was related to an ion-specific or EC effect was assessed.

2.0 Literature review

Potato (*Solanum tuberosum L.*) is one of the most important food crops globally (Spooner and Salas, 2006). The suitability of the potato to a wide range of growing conditions coupled with its ability to produce large quantities of nutritious food has driven a rapid increase in potato production (Horton and Sawyer, 1985). Currently, potatoes are grown in more than 150 countries on an estimated 180,000 sq km of farmland globally, with production of more than 323 million tonnes per year (FAO, 2006; CIP, 2007). Potatoes are grown in different climatic zones including temperate regions, subtropics and tropics under very different agro-economic conditions; in lowland as well as highland regions and from high-input agriculture by large scale corporate farms to small holders (Struik and Wiersema, 1999). Potatoes are best adapted to the cool temperature zones of the high altitudes in the Andes (2000-3500 m), at sea level in temperate regions of North America, Europe, South Chile and Argentina and at appropriate altitudes in intermediate latitudes (Hawkes, 1992b).

Abiotic stress, especially salinity and drought are considered to be the most serious growth-limiting factors for crop plants (Boyer, 1982; Vinocur and Altman, 2005). Recent investigation suggested that 67% of agricultural area has potential for transient salinity, a type of ground water-associated salinity (Rengasamy, 2006). The total area of salt-affected soils including saline and sodic is 831 million hectares (Martinez-Beltran and Manzur, 2005), extending over all the continents including Africa, Asia, Austrlia and the Americas (Schoups *et al*., 2005; Chaves *et al*., 2006; Rengasamy, 2006), Potato is relatively salt-sensitive; however, little research has been performed on salt stress resistance mechanisms in this crop.

Potato is used mainly for food production as a source of carbohydrates. However, in some developed countries such as Europe, potato is also used as a food source for animals. Potato is also used for starch and alcohol production (Horton and Sawyer, 1985). Approximately 60% of potato is consumed by humans, 25% is used as stock feed and in industry (starch and alcohol production), 10% is stored for seed and 5% is wasted (Horton and Sawyer, 1985).

In terms of yield of edible energy and protein per hectare, the potato is near the top of the list of major world crops (Spooner and Salas, 2006) and is well balanced with regard to the ratio of protein to calories. Potato tubers contain 75-80% water, 16-20% carbohydrates, 2.5-

3.2% protein, 0.8-2% mineral (low sodium, high potassium), 0.6% fibre and 0.1-0.2% fat (Bajaj, 1987). The potato tuber contains high-quality protein and substantial amounts of essential vitamins, minerals and trace elements (Horton and Sawyer, 1985; Bajaj, 1987; Spooner and Salas, 2006). Potatoes are a good source of ascorbic acid (vitamin C) and B vitamins, especially niacin, thiamine, vitamin B_6 and riboflavin (Horton and Sawyer, 1985; Bajaj, 1987). The appreciable quantity of lysine in potato makes it a valuable supplement to cereals, which are limited in lysine. Potato is also a good source of phosphorous and magnesium, a moderate source of iron and rich in potassium (Horton and Sawyer, 1985).

2.1 Origin and evolutionary relationship of potato species

The potato originated in the mountains of South America, specifically in the Andes of Peru and Bolivia where wild prototypes still exist. Archaeological studies clearly show the potato was already domesticated in South America for centuries before the Spaniards arrived. The gene pool of potato is extremely large, providing a valuable source of genetic diversity to breeders. Its ancestor could have been the wild species of *Solanum leptophyes*. The name *potato* is derived from the native name *batata* (Hawkes, 1992a).

The genus *Solanum,* to which the cultivated potato belongs, is an extremely large one, containing about 1,000 species. In addition to the widely cultivated *S. tuberosum*, subsp. *tuberosum* and *andigenum,* seven other related species are cultivated, namely *S. ajanhuriri*, *S. chaucha*, *S. curtilobum*, *S. goniocalyx*, *S. juzepcukii*, *S. phureja* and *S. stenotomum*. Over 230 wild species of potato are generally recognized (Hawkes, 1992a; Struik and Wiersema, 1999). The cultivated potato has probably more related wild species than any other crop species (Hawkes, 1994). The wild species of potato occur as diploids, triploids, tetraploids, pentaploids and hexaploids with n=12. Most species (73%) are diploid, 4% are triploid, 15% are tetraploid, 2% are pentaploid and 6% are hexaploid. Cultivated potatoes (tetraploid) originally were derived from the primitive diploid *S. stenotomum*, either by mutation, selection or hybridization (Hawkes, 1992a).

The evolution of cultivated potato began from S. *stenotomum* (Hawkes, 1992a). The diploid S. *stenotomum* grown in Peru and Bolivia is quite primitive and is probably derived from the diploid wild species S. *leptophyes* or possibly S. *canasense*, both still occurring in the

distribution area (Hawkes, 1994). Hybridization of S. *stenotomum* with S. *sparsipilum*, the weedy species and the subsequent chromosome doubling produced the tetraploid S. *tuberosum* subs. andigena in the central Andes (Gribb and Hawkes, 1986). However, others consider that tetraploid Andean potatoes are derived from simple chromosome doubling of S. *stenotomum* (Hawkes, 1994). A series of crosses between S. *stenotomum* and subsp. *andigena* gave rise to S. *chaucha*, the triploid hybrid (Jackson *et al*., 1977; 1978)

2.2 Growth habit

Potato is an herbaceous C_3 annual plant, with wide genotypic differences in number of branching and pattern of branch development (Toosey, 1963; Bajaj, 1987). This results in differences in the number of stolons per plant, tubers per plant or tubers per stolon (Wurr, 1974). Stolon formation may start before or after plant emergence and stolon number/stem declines with increasing stem numbers. However, the quantity of assimilates available for growth also affects this relation (Struik and Wiersema, 1999). Early-maturing types tend to produce more nodes and subtending stolons than do late maturing types, but the ratio can be affected by environmental factors (Ewing, 1987).

Generally it is common to distinguish between determinate types and indeterminate types of potato. Determinate types have a tendency to be early maturing, remain short, do not produce many successive orders within one main stem and produce short-cycle crops. Indeterminate types require a longer growing season (late maturing) to fully mature and have a higher yield potential if provided with an adequate duration growing season (Struik and Wiersema, 1999).

Potato is the most widely cultivated vegetatively propagated crop in the world. Tubers are the organ harvested for consumption. Tuber formation requires a sequence of physiological events, which are internally regulated by plant hormones, triggered by photoperiod and modified by genetic and other environmental factors (Ewing and Struik, 1992). Short days and moderate temperature enhance tuber formation while long photoperiods enhance vegetative growth (Moreno, 1985).

Tuber formation takes place over a relatively short period of time, with the duration of this period depending on the maturity class of the potato (Jackson, 1999; Struik *et al*., 1999).

Tuber development involves several stages, starting from induction, initiation, rapid growth and branching, cessation of longitudinal growth and swelling (Struik and Wiersema, 1999).

2.3 Salt stress

Crops are rarely grown in ideal environments and they rarely attain their full yield potentials because of limitations on physiological processes imposed by environmental and edaphic stresses. Salinity represents a stress of increasing importance in agriculture. Increasing salinity of irrigation water has contributed to progressive salinization of agricultural soils inhibiting agricultural productivity in many semi-arid and arid regions of the world (Flowers *et al*., 1977; Qadir *et al*., 2000). In the Great Plains of North America, salinization threatens the productivity of agricultural land. Saline and saline sodic soils in the Canadian provinces generally contain high concentrations of $MgSO_4$ and Na_2SO_4 salts (Redmann and Fedec, 1987; Eilers *et al*., 1995). About 1.6 million hectares of cultivated land in Saskatchewan are affected by salinity (Crosson, 1976). Most of the salts in soils are sodium and magnesium sulphate (Mermut and Arshad, 1987).

Saline soils contain a high percentage of soluble salts, with one or more of these salt components being present in excess. Sodium chloride (NaCl) is the most commonly encountered source of salinity (Henry *et al*., 1987; Li *et al*., 2006). However, sulphate and bicarbonate anions and calcium and magnesium cations may contribute to salinity problems. Alkali soils are low in soluble salts but have a high sodium content and high pH (Henry *et al*., 1987). Electrical conductivity of the moderate saline soils is between 4 to 8 dS m^{-1} (Henry *et al*., 1987).

Salt stress arises from excessive uptake of salts by plants (Luttge and Smith, 1984). There are at least three components of salt stress in plants, including osmotic effects, nutritional effects and toxic effects (Leopold and Willing, 1984). The degree to which each one of these components of salinity stress influences growth is dependent upon many factors e.g., plant species, ionic composition of the saline water, humidity and stage of plant development.

Levitt (1980) has drawn a line between ion stress and salt stress. He defined salt stress as a condition in which salt concentration is high enough to make the water potential in the plant excessively negative. Conversely, in ion stress conditions, salt concentration is not high

enough to lower plant water potential. Ion imbalance specifically results from disturbed ionic ratios in the cells after an accumulation of ions present in the salt (Cheeseman, 1988).

Exposure of plants to extreme conditions such as high salinity causes a diverse set of physiological, morphological and developmental changes (Jensen *et al*., 1996). Much of the strain in salinity stress is related to water stress arising from excessive uptake of salts by the plants and the resulting reduction in water potential. This stress reduces leaf expansion due to reduction in leaf turgor, and can severely restrict crop yield (Addicott, 1983). Maintenance of cell and tissue turgor pressure is important for many metabolic processes and growth (Morgan, 1984). For example, Gandar and Tanner (1976) found that leaf growth stopped at leaf water potentials between -0.4 and -0.5 MPa in greenhouse-grown potato plants.

The effects of salinity are generally summarized as water stress, salt stress and stress due to ionic imbalance (Greenway and Munns, 1980). Therefore at least one part of salt stress is associated with water stress, which is a general condition, and it can be expected that plant adaptation to salinity may show features similar to those characteristic of adaptation to water stress.

2.3.1 Salt stress resistance

Salt resistance is a complex characteristic controlled by a number of genes or groups of genes and involving a number of component traits that are quantitative (Flowers and Yeo, 1995). The complexity of polygenic control for salt tolerance makes selection of salt-tolerant species difficult (Greenway and Munns, 1980).

Salt stress tolerance varies among various plant crop species. Vegetation of saline soils is designated as “halophyte,” while that of non-saline soils is referred to as “glycophytic” (Luttge and Smith, 1984). The salt content of soil can be estimated by measuring its electrical conductivity (EC), which is expressed in deci-Siemens per metre (Henry *et al*., 1987). The plant salinity threshold is also expressed in electrical conductivity (EC,) which is defined as the level of tolerance of the plant. There is negative correlation between crop yield and increasing salinity (Maas, 1986). Generally speaking, and on the basis of tolerance to salt, plant species are grouped into four categories, namely salt tolerant, moderately tolerant, moderately sensitive and salt sensitive (Maas and Hoffman, 1977; Katerji *et al*., 2003). Crops that can tolerate high concentrations of salt include barley (*Hordeum vulgare* L.), kenef *(Hibiscus cannabinus* L.),

cotton (*Gossypium hirsutum*), tall wheat grass (*Agropyron elongatum* L.), sugar beet (*Beta vulgaris* L.) and bermuda grass (*Cynodon dactylon* L.). The salt stress threshold for these crops ranges from 6.9 to 8.1 dS m^{-1}. Sorghum (*Sorghum bicolor* L.), wheat (*Triticum aestivum* L.), soybean (*Glycine max* L.), cowpea (*vigna unguiculata* L.) and sunflower (*Helianthus annuus* L.) are rated as medium salt- tolerant crop species with salt stress thresholds ranging from 4.8 to 6.8 dS m^{-1}. Tomato (L*ycopersicon esculentum* L.), alfalfa (*Medicago sativa*), corn (*Zea mays* L.), sugarcane (*Saccarum officiarum* L.), clover berseem (*Trifolium alexandrinum* L.), potato (*Solanum tuberosum* L.) and broad bean (*Vicia faba* L.) have threshold salinity levels from 1.6 to 2.5 dS m^{-1} so are classified as moderately salt-sensitive. Onion (*Allium cepa*), strawberry (*Fragaria x ananassa* Duch.), carrot (*Daucus carota*), bean (*Phaseolus vulgaris* L.) and fruit trees such as apricot (*Prunus armeniaca* L.), peach (*Prunus persica* L.) and orange (*Citrus sinensis* L.) are classified as salt-sensitive plants that tolerate only low concentrations of salt, with damage thresholds ranging from 1.0 to 1.8 dS m^{-1}.

To transfer genes from salt-tolerant species (halotolerance genes) to crop plants, several classic breeding techniques have been attempted with some success (Epstein *et al*., 1980; Tal, 1985; Wyn Jones and Gorham, 1986; Epstein and Rains, 1987). To recover useful agronomic features of these crosses, several backcrosses are also required. However, this strategy is not applicable if salt-resistant wild species do not exist (Serrano and Gaxiola, 1994). Genetic engineering offers new techniques for application to this issue. This technology is now available for transferring genes to plants (Schell, 1987; Potrykus, 1991; Klein *et al*., 1992; Delk *et al*., 2005). However, the isolation of relevant halotolerance genes is still a major problem. Selection or screening of existing germplasm with different sources of resistance to salt might be an important method for potato cultivation in soil with accumulating salt. An alternate strategy for salt tolerance improvement is regeneration of salt- tolerant cells with heritable traits (Winicov, 1991; 1996; Teixeira *et al*., 2006).

2.3.2 Mechanisms of salt stress resistance

Saline stress occurs when concentrations of certain ions increase in the soil above the range to which a species has become adapted for optimum growth. The degree to which a plant can maintain growth and normal metabolism in such diverse conditions is described as salinity "resistance" (Yeo, 1983). The available options observed in plants in response to saline

environments are separable into avoidance mechanisms (the means by which a plant can avoid the salt stress of its environment) and tolerance mechanisms, which enables a plant to survive high concentrations of electrolytes in its environment (Levitt, 1980).

2.3.2.1 Avoidance mechanisms

Stress avoidance is stress resistance by avoiding thermodynamics equilibrium with the stress. The plant with avoidance is able to exclude the stress, either partially or completely, either by means of a physical barrier which insulates its living cells from the stress, or by a steady state exclusion of stress. By avoiding the stress, it also avoids the starain (Levitt, 1980). In avoidance mechanisms, the orgaism responds by somehow reducing the impact of salt stress. The majority of the research on Na^+ metabolism in plants has been concerned with the initial uptake across the root cell plasmalemma. At the cellular level, the steady state must be maintained either by very effective exclusion of Na^+ or by the extrusion or turnover of internal pools of Na^+ (Cheeseman, 1988).

a) Salt extrusion

Salt extrusion occurs via either (1) salt glands or (2) membrane channels and pumps (which involves internal translocation of ions and can help prevent increased deleterious concentration [McKersie and Leshem, 1994]). While salt extrusion from glands on leaves or petioles is a common mechanism in many wild plants, it is less common in cultivated species (McKersie and Leshem, 1994). Salt extrusion via membrane channels is encountered in many cultivated species, especially in salt-resistant cultivars, and it is basically associated with transport regulation, with the production of compatible solutes or in combination with both processes (McKersie and Leshem, 1994).

The three membrane barriers to salt are (1) cell membrane of the root cortical and epidermal cell, (2) the tonoplast of the vacuole and (3) the plasmalemma of the xylem-parenchyma cells. With respect to these membrane barriers, three types of transport systems have been considered: (1) ion, Na^+ or Cl^- conducting channels, (2) Na^+/H^+ transporter (antiporter) and (3) the ATPase/H^+ pump (McKersie and Leshem, 1994). Located in membranes are selective protein pores called ion channels. Transportation of ions through these

channels occurs in response to the electrochemical gradients of those ions across the membrane. In other words, passive transport is involved. It is believed that under saline conditions, Na^+ can enter the cell through these channels passively. However, this is not true for Cl^-, which is believed to be taken up via an active transport system. Until now, no specific Na^+ pathway entry has been detected.

In some plants there must exist an active system which, at an expense of energy, transports Na^+ from the cytoplasm to the external medium (Maathius and Prins, 1990). Removal of surplus Na^+ and also K^+ from the cytosol into the vacuole in halophyte plants has been attributed to this system (Hassidim *et al*., 1990). The final form of transportation is the ATPase/H^+ pump, which transports protons across the membrane against their electrochemical gradients and is coupled to the splitting of ATP. It is reported that in *Atriplex*, salt resistance is associated with this system (Braun *et al*., 1986). The plasma membrane-localized Na^+/H^+ antiporter functions in the extrusion of toxic Na^+ from cells and is essential for plant salt tolerance of SOS1 mutant plants (Katiyar-Agarwal *et al*., 2006). Modulation of proton extrusion and ATP-dependent H^+ transport through the plasma membrane in response to osmotic shock was studied in tomato (*Lycopersicon esculentum*) (Kerkeb *et al*., 2002).

b) Salt exclusion

Another mechanism of salt avoidance is exclusion of Na^+ or Cl^- entry by a root membrane-associated mechanism (McKersie and Leshem, 1994). This involves regulating net ion transport both qualitatively and quantitatively, and may be increased by reducing membrane passive permeability (Yeo, 1983). The salt resistance of such plants depends on their impermeability to salt in the presence of high external concentrations. It has been known that differential permeability at the cellular level depends on a balance between monovalent (K^+, Na^+) and divalent (Ca^{2+}) cations (Levitt, 1972). According to Flowers *et al*. (1977), most glycophytes respond to salinity by ion exclusion. In the majority of these species such exclusion occurs in their leaves, so they may accumulate high levels of Na^+ in their roots and stems.

Plant cell structure is well established for sequestration of ions because of the presence of large membrane-bound vacuoles. In salt-resistant plants, through the Na^+/H^+ antiport, compartmentation of Na^+ into vacuoles provides an efficient mechanism both to prevent the deleterious effects of Na^+ in the cytoplasm and also to maintain osmotic balance by using Na^+

and Cl^- accumulated in the vacuole to drive uptake of water into the cells (Glenn *et al*., 1999). This Na^+/H^+ transport of Na^+ into the vacuoles utilizes the electrochemical proton gradient generated by the vacuolar enzymes H^+-adenosine triphosphatase (ATPase) and H^+-inorganic pyrophosphase (PP_iase) (Blumwald, 1987; Rea and Sanders, 1987; Blumwald *et al*., 2000). Although avoiding the stress at the cellular level, these plants are termed "salt tolerant" since transgenic salt-tolerant plants (expressing a vacuolar Na^+/H^+ antiport) grew and completed their life cycles in the presence of 200 mM sodium chloride (Zhang and Blumwald, 2001). The capability of vacuolar compartmentation seems to be correlated with salt tolerance (Serrano and Gaxiola, 1994; Chinnusamy *et al*., 2005). Transgenic plants which over-express the vacuolar Na^+/H^+ antiport have potential for use in agriculture because while salt (Na^+ and Cl^-) accumulates in the leaves, such high accumulation are not found in fruits. Rather, K^+ content of the fruits from transgenic plants grown in salt was higher than the K^+ content of the leaves.

An important organ-specific factor that regulates adaptation of plant cells to salinity is xylem-loading at the root central cylinder. Resorption of Na^+ from the xylem sap and accumulation of Na^+ by xylem parenchyma in the root and stem base are the main mechanisms of this phenomena (Läuchli, 1984). Alteration of the structure of the cell is another aspect of adaptation of plant cells to salinity (Zhong and Läuchli, 1988; Iraki *et al*., 1989). A decrease in the cellulose framework caused structure-weakening in salt-adapted cells and consequently facilitated turgor-driven growth under osmotic stress.

2.3.2.2 Tolerance mechanisms

Salt tolerance may be defined generally as sustained growth of plants in an environment containing substantial amounts of salt (Tal, 1984). Several mechanisms have been attributed to salt tolerance (Gorham, 1995). Compartmentation of ions in vacuoles (Yeo, 1998) and accumulation of compatible solutes in the cytoplasm (osmoprotectants) (Hare *et al*., 1998; Yeo, 1998) are common proposed mechanisms. Compatible osmotica such as proline and glycine betaine can function as osmoprotectants for proteins (Gorham, 1995; Bohnert and Jensen, 1996). In addition to osmoregulation, possible roles for proline are protection of cellular membranes and enzymes, and conversion of energy and amino groups for post-stress growth (Mansfield and McAinsh, 1995). Osmolytes have been proposed to generally prevent the

deleterious effects of water loss on cellular membranes (Crowe *et al*., 1988; Ashraf and Foolad, 2007).

Tolerance to osmotic stress may operate either through dehydration tolerance, which permits the cell to survive without growing when the turgor decreases, or by avoidance of dehydration through osmoregulation (Tal, 1984). The roots of salt-tolerant species exhibit a higher rate of potassium uptake than do salt-sensitive varieties (Rush and Epstein, 1981; Watad *et al*., 1991; Wang *et el*., 2002). Increased osmoprotectant synthesis has been produced in transgenic plants by overexpression of enzymes leading to increased mannitol synthesis (Tarczynski *et al*., 1993; Thomas *et el*., 1995; Hu *et el*., 2005), ononitol production (Sheveleva *et al*., 1997), fructan synthesis (Pilon-Smits *et al*., 1995), trehalose synthesis (Holmstrom *et al*., 1996; Almeida *et al*., 2007) and the amino acid proline (Kishor *et al*., 1995; Xiang *et al*., 2007). In the case of mannitol, this solute may provide protection by reducing oxidative damage in the choloroplast (Shen *et al*., 1997).

In the case of halophytes, salt tolerance is definitely the main factor since the salt content of the cells approaches that of the external medium (Levitt, 1980). Therefore we must first consider whether salt accumulation can quantitatively explain osmotic adjustment by making the water potential more negative (Flowers *et al*., 1977). Halophytes are unique in their ability to accumulate and concentrate salts to levels equalling or exceeding those of seawater in their leaves without damage; thus, accumulation has a positive function. The combined effect of this osmoregulation with an exclusion of salts by the endodermal layer helps, for example, the mangrove plant to maintain the osmotic potential of the xylem sap to nearly zero (Scholander *et al*., 1965). Such species must normally maintain a high cell water content in the presence of the negative external water potential brought about by the high salinity. In a number of herbaceous halophytes such as *Atriplex*, this osmotic adjustment is mainly due to Na^+ absorption (Tal, 1984). Halophytic plants can tolerate high concentrations of Na^+ and Cl^- by sequestering the toxic ions away from important metabolic processes (Flowers *et al.*, 1977) even though energy is required for the accumulation and compartmentalization of the ions (Yeo, 1983).

Osmoregulation is one mechanism by which plants under salinity may undergo regulation of osmotic potential (MacKersie and Leshem, 1994). Potato plants respond to water stress or salt stress with an increased synthesis of osmoprotectants including proline and soluble carbohydrates (Sasiakala and Prasad, 1993; Teixeira and Pereira, 2007) and proteins (Pruvot *et*

al., 1996b; Hmida-Sayari *et al*., 2005b). Sasiakala and Prasad (1993) determined levels of proline increased in leaves and shoots of potato in response to salt stress. It is well documented that abscisic acid (ABA) may initiate physiological reactions in response to environmental stresses in potato (Sukumaran *et al*., 1975; Stephen *et al*., 1995; Liu *et al*., 2006). According to Palmer (1985), the addition of ABA to potato during growth increased the levels of proline which then served as an osmoprotectant.

2.3.3 NaCl induction of salt stress tolerance

2.3.3.1 Salt pre-exposure and acclimation

Pre-exposure to mild stress for a period from a few days up to 3 weeks before exposure to a more severe stress can result in a significant increase in tolerance of the severe stress event. In the case of *Sorghum,* the acclimated plant developed and produced seeds under stress conditions that were lethal for non-acclimated plants (Amzallag *et al*., 1990b).

Acclimation can be induced rapidly if the stress is applied progressively. However, for acclimation to occur, the cell, organ or organism must be competent to undergo the change. For example, in *Sorghum* the plant was competent to undergo adaptation to salinity only during a short period of time (between days 5 and 10) following germination (Amzallag *et al*., 1993). When this developmental window was open, plant acclimation was a function of stress intensity and duration (Seligmann *et al*., 1993) and environmental stability (Amzallag *et al*., 1993). The potential for adaptation also varies considerably among species (Baker *et al*., 1986) and among genotypes within species (Amzallag *et al*., 1993). Not all genotypes have the capacity to acclimate (Amzallag and Lerner, 1995).

Acclimation can be observed only with several narrow experimental requirements: a) the developmental window must be open; b) environmental conditions must be constant until the adaptation process is completed; c) the plant must be vigorous and healthy, with optimum light intensity, humidity and temperature conditions; and d) prolonged growth studies are essential to recognize the phenomena. These requirements may considerably limit the possibility of acclimation under field conditions.

2.3.3.2 The effect of calcium on salt stress resistance

Calcium/total cation ratio is known to influence dry matter production and salt tolerance of several grass species (Suhayda *et al*., 1992). NaCl reduced the Ca^{2+} content of barley foliage and, when applied in nutrient solution, inhibited the root-to-shoot transport of Ca^{2+}. Salinity can interfere with K^+ and Ca^{2+} nutrition (Rengel, 1992; Cramer and Jones, 1996; Chen *et al*., 2005), causing nutrient deficiency.

Recent evidence suggests a role of Ca^{2+} in salinity resistance (Bilski *et al*., 1988a; Katsuhara and Kawasaki, 1996; Suhayda *et al*., 1997; Anil *et al*., 2005). Salinity injuries resulted in separation of the cell wall from the cell membrane, due to dehydration of the cytoplasm. Evidence also suggests Ca^{2+} to be an important factor in the maintenance of membrane integrity and membrane transport functions (Poovaiah and Leopold, 1976; Hanson, 1984; Cramer *et al*., 1985; Renault, 2005; Gilliham *et al*., 2006). Thus, the impact of environmental stress on plants mediated by cytosolic Ca^{2+} is expected to play an important role in stresses, particularly in salinity stress.

Several reports have suggested the general protective effect of Ca^{2+} on many cellular processes during stress (Epstein, 1961; Bilski *et al*., 1988a; Arora and Palta, 1989; Palta, 1996; Reddy and Reddy, 2004). Some plants have the capability to maintain high tissue Ca^{2+} levels and to exclude Na^+ from their shoots as an essential feature in adaptation to salt stress (Murillo-Amador *et al*., 2002; Karrenberg *et al*., 2006). Several pathological and physiological potato tuber defects have been correlated with low calcium levels in the potato tuber peel (Bamberg *et al*., 1993). Some greenhouse-grown wild species of potato significantly exceeded cultivated potatoes in accumulating calcium in tubers under both low and high levels of calcium. Species with high calcium-accumulating capacity may provide a resource for breeding programs.

According to Cramer *et al*. (1989), Na^+ can disrupt membrane integrity and inhibit transport of K^+ and Ca^{2+} ions into the root and up to the shoots. It is essential to have Ca^{2+} in the extracellular solution to ensure the maintenance of selective permeability (membrane integrity). It is also well known that Ca^{2+} is an integral part of the cell wall, where it provides stable, but reversible, intramolecular linkage between pectic molecules, resulting in cell wall rigidity. In addition, Ca^{2+} may stabilize cell membranes by binding phosphate and carboxyl groups of phospholipids at the membrane surface (Legge *et al*., 1982).

In addition to its role in the cell wall membrane, Ca^{2+} is now regarded as an important intracellular secondary messenger. Several studies have provided strong evidence implicating the regulation of various cell functions by cytosolic Ca^{2+}. These studies suggest Ca^{2+} to be a messenger in transducing external stimuli in plants. These signals often involve plasma membrane-associated protein-kinases (Poovaiah and Reddy, 1987; Reddy and Reddy, 2004).

Supplemental Ca^{2+} generally decreases Na^{+} and increases K^{+} and Ca^{2+} uptake, which are usually associated with an increase in plant growth. Supplementing the medium with calcium alleviates growth inhibition imposed by salt in glycophytic plants (Yan *et al*., 1992; Kinraide, 1998; Anil *et al*., 2005). Tissue calcium concentration is also related to salt tolerance and may play a regulatory role in response to saline conditions (He and Cramer, 1992; Karrenberg *et al*., 2006). In salt-tolerant wild species of barley (Suhayda *et al*., 1992) root calcium levels were twice that of salt-sensitive cultivated plants. Salt-tolerant species may have a more efficient Ca^{2+} uptake and translocation mechanism (Suhayda *et al*., 1992; Karrenberg *et al.*, 2006).

Calcium is known to move exclusively in the xylem. This movement is due to mass flow of transpirational water to the point of lowest water potential (Bangerth, 1979; Busse and Palta, 2006). Wiersum (1966) demonstrated that nonsenescing leaves with high transpiration rates act as a strong sink for Ca^{2+} and restrict its movement to slowly transpiring organs such as fruits and tubers. Calcium appears to play an important role in the water transport of plants under salt stress conditions, and the restoration of hydraulic conductivity also can be related to its concentration in the nutrient solution (Azaizeh and Steudle, 1991; Cabanero *et al.*, 2006).

Root elongation in media containing high concentrations of NaCl was positively correlated to the computed amount of calcium bound to the plasma membrane (Yermiyahu *et al*., 1997). Addition of calcium to growing media treated with NaCl improved percentage germination of *Brassica* but had no effect on germination rate (Ashraf and Naqvi, 1992).

2.3.3.3 Role of ABA in plant stress resistance

ABA is a 15-carbon sesquiterpenoid synthesized partly in the chloroplast and other plastids by the mevalonic acid (MVA) pathway (Walton and Li, 1995). ABA is usually present in the plant in very low concentration. In most tissues, the levels range from 10 to 50 ng/g FW (4×10^{-8} M to 2×10^{-7} M); only in water-stressed leaves, developing and mature seeds and dormant buds is its level higher than 10^{-6} M (Walton and Li, 1995).

ABA can be considered a stress hormone because the amounts produced when the plants are subjected to various kinds of stresses are much higher than those produced when the plants are not under stress. The importance of ABA as a stress hormone was first suggested by Wright and Hiron (1969). The roles of ABA, sugars and heat-stable proteins in low-temperature stress tolerance were also studied by Gusta *et al*. (1996). In addition, there is evidence that ABA may be used as a potential surrogate for the leaf gas exchange regulation response and could become part of a drought tolerance selection strategy (Bauerle *et al.*, 2006). The positive effects of exogenous ABA on morphological and physiological responses of plants under saline stress conditions have been widely described (Gómez-Codenaz *et al*., 2003; Shaterian *et al*., 2005a; Fricke *et al*., 2006; Khadri *et al.*, 2006). The expression of genes in response to NaCl was also reported to be mediated by ABA (Zhu *et al*., 2005). ABA exogenously applied to the roots transiently induced ZmPIP2;4 as well as ZmPIP1;2. The ZmPIP2;4 which was inducted by NaCl may be mediated by ABA (Zhu *et al*., 2005).

A combination of synthesis, metabolism, import and export may determine the ABA levels in tissues (Walton and Li, 1995). The effect of osmotic constraints on endogenous ABA levels and synthesis of proteins in potato under high salinity was investigated by Pruvot *et al.*, (1996b). The endogenous ABA content increased upon salinity treatment in rice (Bostock and Quatrano, 1992; Fricke *et al*., 2004; 2006). A significant increase in leaf ABA content (at least 2.5-fold) was measured in plants exposed to high salinity (Jeschke *et al.*, 1997; Chen *et al*., 2002c; Fricke *et al*., 2004). The thylakoid proteins (CDSP 32 and CDSP 34) exhibited enhanced synthesis and substantial accumulation of proteins in response to high salinity. The contribution of ABA to the synthesis of these two proteins was also investigated by spraying well-watered plants with 100 mM ABA solution for 15 days. This treatment resulted in a 15-fold increase in the leaf ABA content. Whereas synthesis of the CDSP 32 protein was not affected by exogenous ABA, synthesis of CDSP 34 protein was enhanced. It was concluded ABA can mediate the synthesis of CDSP 34 upon high salinity and suggested another signal, related to high osmolarity, is involved in synthesis of CDSP 32 protein (Pruvot *et al.*, 1996a). The CDSP 32 protein is suggested to be involved in osmoregulation of the stroma. The CDSP 34 protein is also suggested to play a role in structural mechanisms involved in the tolerance of thylakoids to dehydration stress (Pruvot *et al*, 1996).

Downton and Loveys (1981) showed an increase in ABA up to 9-fold within 6 hours in the 50 and 150 mM NaCl-treated grapevine. The involvement of ABA in induction of newly synthesized protein associated with salt tolerance was also reported in barley (Ramagopal, 1987), rice (Moons *et al*., 1995) and tomato (Jin *et al*., 2000). When leaves of mesophytic plants were water stressed, ABA concentration rose 10 to 50-fold within 4 to 8 hours due to increased rates of ABA biosynthesis. When the plants were rewatered, the ABA levels dropped to pre-stress levels after 4 to 8 hours. This drop was believed to be due to a reduced biosynthetic rate (Walton and Li, 1995). The active concentration of ABA in plant tissue was determined by the rates of its biosynthesis and catabolism (Gillard and Walton, 1976).

a) ABA and osmotic adjustment

Accumulation of ABA can precede the accumulation of osmotically active solutes such as proline and reducing sugars, which supports the idea that ABA stimulates the cellular osmotic adjustment process. Solute accumulation or osmotic adjustment in response to water deficits is a key metabolic adaptation to water (Morgan, 1984) and salinity stress (Greenway and Munns, 1980).

The synthesis of organic osmolytes is due to the synthesis of the necessary enzymes in response to salt stress (McCue and Hanson, 1992). Several reports (eg., Pesci, 1989, 1992; Misra and Gupta, 2005; Verslues and Bray, 2006) showed the accumulation of proline due to both salt and ABA treatment.

Exogenous ABA accelerates adaptation of isolated cells to osmotic stress elicited by salts (La Rosa *et al.*, 1985). Exogenous ABA can affect accumulation of sugars (Saftner and Wyse, 1984; Arenas-Huertero *et al*., 2000), amino acids (especially proline) and ions (Pesci and Beffagna, 1985; Khadri *et al*., 2007). Exogenous ABA can also activate transcription of several genes induced by salt/drought stress, while other salt/drought-inducible genes are not activated by ABA, suggesting both ABA-dependent and ABA-independent signalling pathways (Bray, 1997; Shinozaki and Yamaguchi-Shinozaki, 1997). Also, under stress conditions, specific genes are expressed that can be induced in non-stressed tissues by exogenous ABA (Mundy and Chua, 1988; Ma *et al*., 2006; Srivastava *et al*., 2006).

Cachorro *et al*. (1995) measured ABA accumulation in control and salt-stressed *Phaseolus vulgaris* plants and related ABA levels to the accumulation of solutes and other

physiological conditions. The addition of exogenous ABA to control plants caused a retardation in growth. The amount of ABA applied to the growth medium caused ABA concentrations to become close to those of salinized plants. Addition of ABA to plants under control conditions resulted in proline and total sugar accumulation very similar to that observed in plants under salt stress. These authors also discussed the possibility that ABA stimulates the cellular processes of osmotic adjustment.

b) ABA and adaptation

ABA not only assists the plant in the short term, but also plays a role in the enhancement of plant tolerance to a stress (Amzallag and Lerner, 1995). When plants respond to stress, response is tied to a pre-existing program that enables survival while maintaining (more or less) the original developmental program (Amzallag and Lerner, 1995). Resistance reflects the capacity of the plant to express its original developmental program under stress conditions. Generally, expression of a pre-existing program occurs relatively rapidly, after 48 h of stress exposure. The reaction is not specific to a particular stress but there is usually an ABA increase in the organ exposed to the stress. Growth decreases and then rapidly stabilizes at a new (lower) level. The magnitude of this decrease in growth is proportional to stress intensity and inversely proportional to inherent tolerance. At the beginning of the acclimation process, there is considerable decrease in growth. When the plant is pre-acclimated, growth after stress increases and can reach levels similar to the average relative growth rate of control plants of the same age (Amzallag *et al*., 1990b). Growth rate increased at the end of the acclimation process, indicating that the environment was no longer stressful for the plant (Ingestand and Lund, 1979; Amzallag *et al*., 1990b; Wignarajah, 1990).

Exogenous ABA enhanced growth and accelerated the process of salt stress adaptation in *Sorghum* briefly exposed to salinity stress. However, if the plant was stressed continuously by increasing salinity, exogenous ABA no longer enhanced growth but rather acted as a growth inhibitor (Amzallag *et al*., 1990b). This effect of ABA on plants during the first exposure to salinity indicated ABA played an active role in the adaptation process. Resistance or pre-adaptation to stress appeared linked to the potential of a plant to maintain the hormonal balance close to that of pre-stress conditions (Amzallag and Lerner, 1995). In contrast, adaptation involves the establishment of a new hormonal balance necessary for integrated plant

development under stress, which probably includes several genetic changes. The new hormonal balance was not similar to the previous level (Amzallag and Lerner, 1995).

An indication of the non-specificity of the response to stress produced by ABA is cross adaptation, by which a given stress increases resistance to other unrelated stresses. For example, the increase in ABA induced by environmental stress such as drought or salinity also increased tolerance of cucumber to cold (Rikin *et al*., 1976).

c) ABA and calcium

Calcium is known to inhibit stomatal opening in some plants (De Silva, 1985; Mansfield and Atkinson, 1990; Cousson, 2007). It was proposed that Ca^{2+} might act as a secondary messenger during ABA-stimulated stomatal closure (Mansfield and McAinsh, 1995; Cousson, 2007). Use of ^{45}Ca has clearly demonstrated influx of Ca^{2+} into isolated stomatal guard cells (McRobbie, 1989). McAinsh *et al.* (1992) reported that 10^{-7} M ABA stimulated an increase in Ca^{2+} content of the guard cell and preceded stomatal closure by approximately 5 min. ABA-stimulated increase in Ca^{2+} may be the result of either influx of apoplastic Ca^{2+} or release of Ca^{2+} from intercellular spaces.

2.3.4 Importance of ABA-deficient mutants in stress research

Monogenic mutants are important tools for investigation of plant physiology under stress. Generally, phytohormone mutants are split into two groups: (1) synthesis mutants, which influence hormone levels and (2) sensitivity mutants, which influence hormone response (Reid, 1990). The most common types of synthesis mutants such as GA1-deficient dwarfs in peas and maize (Phinney 1984; Ingram *et al*., 1986; Reid, 1986; Ross *et al*., 1999) block synthesis of the active hormone. Overproducing mutants have also been reported for cytokinin in moss and *Physcomitrella patens* (Wang *et al*., 1981; Schulz *et al*., 2001). Mutants, which have low levels of hormone due to increased conjugation or catabolism of the active hormone, also belong to the synthesis mutant category (Reid, 1990). ABA-deficient mutants have been found in a number of plant species. These mutants have been used successfully to understand the role of ABA in stresses (Tal and Nevo, 1973; Koornneef *et al*., 1982; Quarrie, 1982; Mulholland *et al*., 2003).

Many wilty mutants of higher plants have been isolated, and several causes of wiltiness have been described: abnormal xylem-vessel development (Postlethwaite and Nelson, 1957; Alldridge, 1964), alteration in the ionic content of guard cells (Tal *et al*., 1976), low osmotic pressure in the cell sap (Landrige, 1958) and deficiencies in, or insensitivity to, ABA (Quarrie, 1987; De Jong *et al*., 2001).

In most species, ABA synthesis is stimulated by wilting, while application of ABA to turgid leaves and epidermal tissues induces stomatal closure. Therefore, it has been proposed that the primary effect of these mutations is to lower endogenous levels of ABA, with wilting symptoms arising as a consequence of this effect (Tal and Nevo, 1973; Tal, *et al*., 1979; De Jong *et al*., 2001). These results with different genotypes provide good evidence that one of the primary roles of ABA is regulation of stomatal homeostasis (Neill and Horgan, 1985; Mishra *et al*., 2006).

The best-characterized wilty, ABA-deficient mutants are the *notabilis* (*not*), *flacca* (*flc*) and *sitiens* (*sit*) mutants of tomato (Tal, 1966; Tal and Nevo, 1973; Neill and Horgan, 1985; Borsani *et al*., 2002). Other ABA-deficient mutants include potato (*droopy*, Quarrie, 1982; De Jong *et al*., 2001), pea (*wilty*, Wang *et al*., 1984; Kof *et al*., 2006), *Arabidopsis thaliana* displaying reduced seed dormancy (*aba*, Koorneef *et al*., 1982; Umezawa *et al*., 2006), *Nicotiana pilumbaginifolia* (*1217*, Bitoun *et al*., 1990; *CKR1*, Blonstein *et al*., 1991; *Esg 152*, Rousselin *et al*., 1992); *Hordeum vulgarie, Helianthus annuus* L. (*nd*-1; *w*-1) (Fambrini *et al*., 1993; 1995; 2004), viviparous corn mutants (Smith, *et al*., 1978; Robichaud *et al*., 1980; Suzuki *et al*., 2006) and wheat (Holappa *et al*., 2005). These mutants provide excellent background for understanding the role of ABA in stresses (Tal and Neo, 1973; Koornneef *et al*., 1982; Quarrie, 1982; Mäkelä *et al*., 2003; Mulholland, *et al*., 2003; Verslues and Bray, 2006).

Research with ABA-synthesis mutants shows that several genes and enzymes are necessary to synthesize ABA (Walker-Simmons *et al.,* 1989; Skriver and Mundy, 1990; Taylor *et al*., 2005). The block to ABA synthesis in an ABA-deficient barley mutant is the inability to convert ABA-aldehyde to ABA. In tomato, three recessive wilty mutants have been isolated: *flacca* (*flc*), *sitiens* (*sit*) and *notabilis* (*not*) (Tal and Nevo, 1973). The mutants also possess much higher rates of transpiration because their stomata open wider and resist closure in dark or wilted leaves. If treated with ABA, these mutant plants behave like normal varieties

(Mulholland *et al*., 2003). The endogenous level of ABA was low in all these mutants when measured by gas chromatography-mass spectroscopy (GC-MS) (Neill and Horgan, 1985).

In one tomato mutant (*notabilis* or *not*), conversion of a carotenoid to a 15-carbon intermediate (perhaps xanthoxin) was blocked while in the two others (*flacca* or *flc* and *sitiens* or *sit*) and in the *droopy* mutant of potato, the conversion of ABA-aldehyde to ABA was blocked (Duckham *et al.,* 1989). Both mutants (*flacca* and *sitiens*) rapidly reduced and isomerised the exogenous ABA-aldehyde to form 2, *trans* ABA-alcohol (Taylor *et al*., 1988). These results explain the earlier observation that *flacca* and *sitiens* accumulate 2, *trans* ABA-alcohol instead of ABA when subjected to water stress (Lindforth *et al*., 1987).

The mutant *droopy* (*dr*) is one of many deleterious recessive mutants known in cultivated diploid potatoes (*Solanum tuberosum*) of the Andes (Simmonds, 1965). All such diploids (with 2n=24) have an oppositional-allele (*S*) incompatibility system. Geographically, they are distributed down the Andes from Venezuela to Bolivia. *Droopy* potato is a recessive mutant occurring in progeny of a cross between two clones (C.P.C. 2862 X 2863) of *Solanum tuberosum* L. group *Phureja* (Simmonds, 1965). Simmonds (1966) also showed that seeds of *droopy* progeny were less dormant than normal seeds. Premature germination in the berry of plants from two backcross families was always associated with the *droopy* phenotype and Simmonds (1966) suggested the association was a pleiotropic effect of the *dr* (*droopy*) genes on dormancy. The ABA-deficient potato mutant also provides a suitable model for analysis of the signal transduction pathway in wound-induced gene activity (Peña-Cortés *et al*., 1995).

Because of the inhibitory effects of ABA on both stomatal opening and seed germination (Milborrow, 1974), the excessive stomatal opening and premature seed germination of *droopy* suggest that it may be deficient in ABA (De Jong *et al*., 2001). Progeny of the cross between potato clones C.P.C. 4461 and C.P.C. 4463 showed characteristics similar to those of the original *droopy* potato (Quarrie, 1982). These plants wilted at high vapour pressure deficit and their stomatal conductance in the light and dark were higher than those of normal plants. Conductances were reduced by applied ABA, but stomata remained partially open even when guard cells were plasmolysed. Leaves of *droopy* plants accumulated very little ABA when water-stressed.

Waggoner and Simmonds (1966) compared *droopy* mutant of potato with a normal sibling. Leaves of the mutant lost fresh weight more rapidly and had more open stomata than

leaves of the normal sibling. When the stomata of the abnormal mutant were closed by a chemical spray (phenylmercuric acetate), its excised leaves lost water no more rapidly than did the normal leaves. Thus, the wilting of mutant appeared to be caused by inability to close stomata. The wilting of abnormal leaves and the small dry weight of the plants indicated the advantage of the stomatal hydrostatic pressure in normal plants.

The *wilty* pea mutant is virtually indistinguishable from non-mutant controls when grown under the low light levels typically encountered in greenhouse (Wang *et al*., 1984; Kof *et al*., 2006). However, it is inclined to wilt when suddenly exposed to bright sunlight as in the field. This mutant is defective in its ability to accumulate ABA rapidly following the onset of water stress (Wang *et al*., 1984). The *wilty* phenotype of the pea is due to a single recessive gene pair (Marx, 1976). Leaves of *wilty* have a lower-percent water content, a lower water potential and a lower diffusive resistance, even in well-watered plants. In addition, the dimensions of the stomatal cells of the *wilty* mutant are larger than those of non-wilty lines.

Three ABA-deficient maize mutants had blocks in carotenoid biosynthesis. They were albinos and lacked protection against photo oxidation of chlorophyll. These mutants also had a reduced ability to accumulate ABA in their leaves and roots (Moore and Smith, 1985). *Wilty* mutant of pea (*wil*) and *Arabidopsis* (*aba*) had low levels of ABA. The ABA biosynthetic pathway can be readily determined by feeding labelled ABA-aldehyde to mutants and monitoring its incorporation into ABA. Duckham *et al*. (1989) determined the final step of ABA synthesis (oxidation of ABA-aldehyde to ABA) by feeding ABA-aldehyde to three ABA-deficient mutants of potato, pea and *Arabidopsis thaliana*. When Duckham *et al*. (1989) applied ABA-aldehyde to ABA-deficient mutants of potato (*droopy*), pea (*wilty*) and *Arabidopsis thaliana* (*aba*) both the *wilty* and *aba* mutants readily oxidized the ABA-aldehyde to ABA. In contrast, the *droopy* mutants reduced and isomerized ABA-aldehyde to a mixture of 2, *cis* and 2, *trans* ABA-alcohol.

The *wilty* (*w*-1) mutant of sunflower occurred due to a spontaneous mutation, which resisted stomata closure in darkness. When treated with foliar ABA spray at 10^{-4} and 10^{-5} M the mutant plants expressed phenotypic reversion to a normal type (Pugliesi *et al*., 1994). Several similarities existed between the *flacca* and *sitiens* mutants of tomato and *droopy* in potato. Two of these were (a) inability to accumulate ABA in response to drought, resulting in the failure of stomatal closure, and (b) higher shoot:root ratio in the mutants than in the wild types (Quarrie,

1987). Further similarities between *sitiens* and *droopy* were vivipary under some special conditions (Simmonds, 1966; Groot and Karssen, 1992) and genetic linkage to S-locus (Simmonds, 1966; Duckham, *et al*., 1989). Because of these similarities, *droopy* and *sitiens* may be equivalent to each other and may possibly code for the same gene product (Duckham *et al*., 1989).

Although the original *droopy* line of potato (*Solanum phoreja, phu*) (Simmonds, 1965) is cultivated in South American countries including Bolivia, Colombia and Peru, it is poorly adapted to grow in the field under the relatively long days of North America and Northern Europe. If this unadapted *phu* is grown under field conditions on the two aforementioned continents, it either does not produce tubers at all or produces very small and immature tubers. This mutant is known for its lack of tuber dormancy (Suttle and Hultstrand, 1994; De Jong *et al*., 2001). Unlike viviparous albino mutants, wilty genotypes are green and viable, because they contain normal carotenoid levels and are only impaired in the last step of ABA biosynthesis (Taylor, 1991; Reid, 1993). Some other mutants wilt because of abnormal stomatal functioning. In *Scabrous diminutive*, a mutant of *Capsicum annum*, excessive stomatal opening is due mainly to an increased concentration of ions in guard cells (Tal *et al*., 1976).

Grafts between normal and mutant plants with different ABA levels could be helpful in establishing the role of roots and shoots in water stress perception and adaptation. The effect of reciprocal grafts on the phenotype of the wilty (*w-l*) mutant and normal sunflower and the relative importance of the root and the shoot in controlling stomatal conductance, water potential and ABA levels under well-watered and drought condition has been reported (Fambrini *et al*., 1995). Reciprocal grafting between ABA-deficient mutant of tomato (*flacca*) and wild type was also performed to study the role of root and shoot in regulation of plant growth under salinity stress (Chen *et al*., 2002a; Chen *et al*., 2003a).

2.3.5 Grafting responses and salt stress tolerance

The physiological and morphological properties of rootstock are important factors in the interaction between rootstock and scion. In vegetable crops, grafting is often performed to provide vigor (Lee and Oda, 2003) by uptake and translocation of ions, photosynthates and plant hormones from vigorous root systems of rootstock to the scions (Ruiz *et al*., 1997; Lee and Oda, 2003). One of the striking effects of rootstock is the enhanced absorption by grafted

plants of low-mobile nutrients such as phosphorous (Ruiz *et al*., 1996), and other ions like nitrogen (Pulgar *et al*., 2000) and iron (Rivero *et al*., 2004). Grafting has been widely used in the production of standard trees on rootstocks (Webster, 1996). Grafting is also used to quantify ultrastructural changes associated with wound recovery (Moore, 1982).

Modern agriculture has made extensive use of specific rootstocks and grafting to improve fruit crop production, enhance disease resistance and increase stress tolerance. One of the most visually dramatic effects of rootstock grafting is reduced tree size on dwarfing rootstocks. This dwarfing effect facilitates harvest, reduces the need for pruning and allows planting at high density all leading to increased yield per area (Autio and Southwick, 1986; Jensen *et al*., 2003). Fruiting at an earlier age, improvement of fruit quality and increased disease resistance are also desirable characteristics conferred to fruit trees by grafting onto suitable rootstock (Autio, 1991; Lee, 1994; Ogasanovic and Papic, 1995; Colla *et al*., 2006a; 2006b).

One of the most widely reported effects of grafting is its influence on disease control. Grafting of disease sensitive plants on resistant rootstocks is an alternative way of controlling soil-borne diseases (Bletsos *et al*. 2003; Vuong and Hartman, 2003). Grafting of watermelon on gourd rootstocks has been used for protection from soil-borne *Fusarium* wilt since the 1900s, and subsequently this method has been expanded to other vegetables including melon, cucumber, tomato, soybean and eggplant (Lee, 1994; Oda, 1995; Ferrari, 1998; Vuong and Hartman, 2003). Since all eggplant cultivars had been susceptible to *Verticillium* wilt, grafting eggplant scions onto resistant tomato rootstocks was found to be an efficient control method (Yamakawa, 1983 referred by Oda, 1995; Ginaux and Douple, 1985; Alconero *et al*., 1988; Sakata *et al*., 1989; Oda, 1995). Recently, wild species of eggplants that were resistant to *Verticillium* wilt were used as rootstocks in a grafting programme (Bletsos *et al*., 2003). Micrografting *in vitro* of peach was used to investigate the effect of heterografting and autografting on *Prunus* Necrotic Ringspot Virus (PNRSV) (Heuss-LaRosa *et al*., 1995; Heuss *et al*., 1999).

The responses of grafted scions and rootstocks are varied under salinity stress. When scions of salt-sensitive cultivars of soybean were grafted onto the rootstocks of salt-tolerant cultivars, enhanced salt tolerance in terms of continuing growth and nitrogen fixation was observed. Conversely, when scions of salt-tolerant soybean cultivars were grafted onto the

rootstocks of salt-sensitive cultivars, greater salt-sensitivity in terms of growth, nitrogen fixation and ionic concentration was noted. Therefore, it is evident that in soybean, root genotype is dominant in growth regulation and nitrogen fixation under salinity stress (Velagaleti *et al*., 1990). However, other researchers (Dua, 1997; Schmutz and Lüdders, 1999), using salt-sensitive and salt-tolerant genotypes of mango and gram in grafting experiments, showed that scions play a critical role in salt tolerance of grafted plants. In tomato, scion genotype and its ABA level also played the most important role in the growth of grafted plants, regardless of the rootstock genotypes and salinity of growth media (Chen *et al*., 2003a). When grafted plants had ABA-normal shoots (Ws), they produced more biomass than those having the ABA-deficient mutant shoots (*Flacc).* Growth of *Flacca* when it was grafted on the ABA-normal rootstock was superior to that of ABA-mutant grafted on its own ABA-mutant rootstock. The phytohormone ABA has a role in coordinating the growth of roots and shoots of plants (Sharpe and LeNoble, 2002) and regulating tolerance responses to a number of stresses including water and salt. Chen *et al*. (2002a) showed that *Flacca* scions grafted onto wild type rootstock exhibited higher ABA levels, lower transpiration rate and higher water content than those of a control graft of *Flacca* scion on *Flacca* rootstock. *Flacca* rootstock grafted to wild type scion showed a higher ABA level, xylem exudation rate, ABA xylem-loading rate, dry weight biomass and length than grafts to *Flacca* scion.

Grafting has been applied to various crops to increase salt stress resistance. *Cucurbitaceae* (*Cucurbita* spp., *Lagenaria siceraria*, cucumber, watermelon and melon) as rootstocks provided salt stress tolerance in cucumber scions (Matsubara, 1989). Reciprocal grafts of mango were assessed under saline conditions (Schmutz and Lüdders, 1999). While leaf Cl^- content was significantly affected by both the scion and rootstock, leaf Na^+ content was influenced only by the scion. Choice of scion governed the K^+/Na^+ ratio in the leaves, but the rootstock had no effect on this balance. Fernández-García *et al*. (2002) found that concentrations of Cl^- and Na^+ were lower in the xylem sap and leaves of grafted tomato plants (Fanny and Goldmar/AR-9704) as compared to ungrafted plants. This suggests that salinity tolerance of these plants could be related to ion exclusion. Estañ *et al*. (2005) also showed that changes in salt tolerance of tomato shoot induced by rootstock were related to ionic balance rather than to osmotic stress caused by salinity. Fruit yield responses of different graft combinations were mainly related to the relative abilities of rootstocks to regulate the transport

of saline (Na^+ and Cl^-) ions. Watermelon rootstock also showed significantly restricted absorption of saline ions. Grafting reduced the concentration of Na^+ but not the concentration of Cl^- in the leaves (Colla *et al*., 2006a; 2006b). Grafting also increased total fruit yield relative to non-grafted plants. Similarly, grafted melon plants showed reduced leaf Na^+ levels but not Cl^- concentrations (Edelstein *et al*., 2005; Colla *et al*., 2006b). However, marketable fruit yields were higher in grafted than in non-grafted plants (Colla *et al*., 2006b). Edelstein *et al*. (2005) showed that grafted melon accumulated less boron at both low and high salinity levels than did non-grafted plants.

To assess the relative roles of roots and shoots in imparting salt tolerance in chickpea (*Cicer arietinum* L.), reciprocal grafting was attempted between scions and rootstocks of salt-sensitive and salt-tolerant genotypes (Dua, 1997). Fifty percent of the grafts were successful. The scion of the sensitive genotype grafted on the tolerant rootstock was salt affected and died after 11 days of salinization, while the tolerant scion grafted on the sensitive rootstock remained tolerant to salinity. On the basis of dry weight, the tolerant scion had equivalent or greater concentration of ions except Na^+, but on a fresh-weight basis the concentration of all ions was almost halved, indicating the potential for higher water retention by the salt-tolerant scion. These results emphasized the importance of shoot tolerance in chickpea. In contrast, the rootstock of the sensitive genotype absorbed more water from the saline solution and retained higher concentrations of ions compared to the salt-tolerant type. Therefore, crossing salt-tolerant and salt-sensitive genotypes can be a good indicator of the possibility of obtaining better recombinants for salt stress tolerance. The evidence of differences in root response of two sensitive and resistant genotypes comes from observations of the scion of the sensitive genotype, which died 11 days after salinization, compared with non-grafted plants of the sensitive genotype, which survived for 18 days. The upper young shoots of the sensitive genotype had greater concentrations of all ions except K^+ than the tolerant type, but the tolerant genotype had mature leaves with higher concentrations of salt than had those of the sensitive plant.

The responses of graft combinations of two mango cultivars (Turpentine and ‘13-1’) to NaCl salinity was assessed (Schmutz and Lüdders, 1999). Turpentine is salt-sensitive, while ‘13-1’ is salt-resistant. The strongest reduction in CO_2 assimilation and transpiration due to salt stress was observed in the combination of Turpentine scion grafted on ‘13-1’ rootstocks. The

highest Cl^- content in leaves and the greatest reduction in shoot growth was also in this graft combination, indicating that '13-1' as a rootstock was not able to protect the salt-sensitive Turpentine scion. '13-1' as a scion was able to maintain higher K^+/Na^+ ratio in the leaves, but as a rootstock had no corresponding protective effect on the scion.

Scion and rootstock efficiencies in water utilization can have marked importance in determining physiologic and agronomic performances under semi-arid conditions. Two scions and three rootstocks of winegrape with different vigours and resistances to drought stress were investigated to test their water-use efficiencies, yields and water potentials under drought stress conditions (Novello and de Palma, 1997). The characteristics of root and shoot that determine shoot response to low water availability can be separated. Root genotypes can be more important than shoot genotypes in some species during drought stress (White and Castillo, 1989).

A wide range of morphologic and physiologic characteristics are affected by rootstocks, scions and their interactions (Lockard and Schnieder, 1981; Syvertsen, 1985; Fernández-García *et al*., 2002; Massai *et al*., 2004; Solari and De Jong, 2006). Some of these characteristics have the potential for improvement of plant water relations, growth and development during water stress and salt stress. For example, rootstocks influence transpiration rates and water use efficency (Natali *et al*., 1983; Satisha *et al*., 2006), leaf conductance (Giulivo *et al*., 1985; Fernández-García *et al*., 2002; Satisha *et al*., 2006), leaf osmotic potential at full turgor and hydraulic conductivity (Olien and Lakso, 1986; Fernández-García *et al*., 2002; Massai *et al*., 2004), root distribution (Castle and Krezdorn, 1975; 1977) and mid-day leaf water potentials (Natali *et al*., 1983; Giulivo *et al*., 1985; Olien and Lakso, 1986).

In potato grafting can be used to assess the transmissibility of factors from foliage to tubers. Gregory (1956) demonstrated that the tuber initiation signal could be transmitted from a grafted scion into the non-induced rootstocks. Source/sink relationships of autografts and cross heterografts of (1) a low-specific-gravity, high sugar content, non-chipping cultivar and (2) a high-specific-gravity, low sugar content, chipping potato were assessed (Tai *et al*., 1988). Heterografting of non-chipping scions onto chipping rootstocks reduced specific gravity of tuber components to levels similar to their autografts. Grafting of chipping potato scions onto non-chipping rootstocks did not significantly increase the tuber specific gravity compared to that of non-grafted plants. Heterografting of non-chipping scions onto chipping rootstocks

resulted in significantly darker chip colour, but this was not related to differences in reducing sugar content. By contrast, potatoes from heterografts of chipping scions grafted onto non-chipping rootstocks showed a slight improvement in chip colour and significantly lower reducing sugars than did non-chipping autografts.

Jensen *et al*. (2003) have been reported that rootstocks affect gene expression in the scion. In a study of two rootstocks, one rootstock reduced the susceptibility of the scion to fire blight (*Erwinia amylovora*), while another rootstock did not alter fire blight susceptibility of the scion. Scions grafted onto dwarfing rootstocks showed higher expression of a number of photosynthesis, cell division-related genes at the transcription/translation level, while scions grafted onto semi-dwarfing rootstocks exhibited increased stress-related gene expression.

Disadvantages of grafting are the required time, space, materials and high labour demand, as well as the possible resultant incompatibility between scion and rootstock which sometimes reduces growth, yield and quality (Oda, 1995; Bletsos *et al*., 2003). Robotic grafting methods have been established for eggplant, tomato and pepper grafting (Kurata, 1994; Oda, 1995). Grafting via robots has been reported to be 10 times faster than the conventional hand-grafting method. In eggplant, fruit yield from plants grafted by robots was similar to that from plants grafted by the conventional hand method (Oda *et al*., 1997). In recent years cultivation of grafted *Solanaceae* has been increasing (Fernández-García *et al*., 2002; Santa-Cruz *et al*., 2002; Chen *et al*., 2003a).

2.3.6 Nuclear magnetic resonance

Microimaging based on Nuclear Magnetic Resonance (NMR) is an experimental technique that can provide a unique view of a variety of plant physiological processes (Van As *et al*., 1985; Köckenberger, 2001; Pietrzak *et al*., 2002; Köckenberger *et al*., 2004). Investigations of water movement and the transport and accumulations of labelled molecules are popular applications of this technique (Köckenberger, 2001). Measurement of water flow rate and velocity pattern in the xylem vessels of intact maize (Kuchenbrod *et al*., 1996), water flow rates in leaves of cereals (Bhattacharya and Tiwari, 1998), phloem and xylem flow in cucumber (Scheenen *et al*., 2002) and castor bean (Köckenberger *et al*., 1997; Peuke *et al*., 2001), plant water status in response to salt stress in tomato (Rajasekaran *et al*., 2001), developmental formation and structure of vacuoles in *Brassica napus* siliques (Köckenberger *et*

al., 2004) and anisotropic diffusion of water in chive (*Allium schoenoprasum*) (Qiao *et al*., 2005) are recent applications of NMR imaging in agriculture.

Proton (^{1}H) NMR imaging has been used to non-destructively probe water distribution and transport in plant root systems grown in natural and artificial soil media under normal and stress conditions (Bottomley *et al*., 1986; Brown *et al*., 1986; Rogers and Bottomley, 1987) and water in stem and other plant tissue (Connelly *et al*., 1987; Johnson *et al*., 1987; Jenner *et al*., 1988; Tamiya *et al*., 1988). Elevated atmospheric CO_2 produced significant increases in water imaged in upper roots, hypogeal cotyledons and lower stems of *Vicia faba* in response to a short term drying cycle (Bottomley *et al*., 1993).

Nuclear magnetic resonance imaging is also capable of measuring flow parameters in xylem vessels. Under some circumstances, the spatial resolution of NMR imaging was recorded down to approximately 10 μM (Brandl *et al*., 1995). In addition, flow sensitivity of the ^{1}H-NMR signals permits measurement of net water flow and velocity (Caprihan and Fukushima, 1990). Flow-sensitive NMR microscopy determined the water flow rate and velocity pattern at the xylem vessels level in single vascular bundles of maize plants (Kuchenbrod *et al*., 1996).

The resolution of nuclear magnetic resonance microimaging technique allowed discrimination between xylem and phloem water flow in castor bean. Both the xylem and the phloem average flow velocities in the intact seedling were quantified. The total conductive cross-sectional area of the xylem vessel and the phloem sieve elements were determined by a non-invasive and non-destructive NMR microimaging technique (Köckenberger *et al*., 1997).

NMR has also been applied to study various phenomena in plant systems, such as quantification of proline in barley (Jones *et al*., 1986), heat injury in grape (Abass and Rajashekar, 1991), chilling injury in zucchini squash (*Cucurbita pepo*) (Wang and Wang, 1992) and cold acclimation and freezing effects on winter wheat (Millard *et al*., 1995). NMR was also applied to investigate the uptake of Mn^{2+} by maize roots (*Zea maize* L.) (Connelly *et al*., 1987; Quiquampoix *et al*., 1993) and the proportion of Eu (III) in the roots of water hyacinth (*Eichhornia crassipes*) (Kelley *et al*., 2000).

Tracing of water in plant seeds for germination and processing purposes is another application of NMR (Pietrzak *et al*., 2002). The applicability of NMR to the morphological studies of seed in maize (*Zea maize* L.), bean (*Phaseolus coccineus* L.) (Connelly *et al*., 1987) and tall fescue (*Festuca arundinacea*) (Anderson *et al*., 1997) is documented. NMR technology

has been applied to monitor water dispersion in seeds of barley, wheat and dry beans (Yin *et al.*, 1996; Zeng *et al*., 1996; McEntyre *et al.*, 1998) and pea (Wojtyla *et al*., 2006).

3.0 Impact of salt stress regime on salinity stress responses in salt sensitive and tolerant potato genotypes

3.1 Abstract

In most salt stress research, the salt stress is applied as a transient but intense salt shock. The resulting responses may not reflect responses in nature, where salt stress typically increases slowly over the growing season. Relative salt tolerance and physiological mechanisms of resistance to salt stress may also differ depending upon how the stress is applied. Hydroponically grown salt-stress-resistant and -sensitive potato seedlings (*Solanum tuberosum* L.) were compared in their responses to a salt stress applied either gradually or as a salt shock treatment. Exposure of plants to incremental salt stress reduced leaf necrosis during subsequent exposure to higher concentrations of salt. The relative ranking of the salt tolerance of the genotypes tested also differed, depending upon the nature of the stress application. Incremental salt stress treatment differentiated among the cultivars more effectively than did salt shock. Plants exposed to salt shock had lower endogenous ABA levels compared to those that underwent the acclimation treatment. Salt tolerance seemed to be associated with higher endogenous levels of ABA. The acclimation treatment slowed growth but generally maintained higher leaf osmotic potential relative to salt shock. Breeding and research programs should carefully consider the nature of the salt stress regime applied when screening for stress resistance, since differing regimes will cause selection of different genotypes.

3.2 Introduction

Salinity is one of the most important factors limiting agricultural crop production (Munns, 2002) and agricultural sustainability (Waisel, 2001). Potatoes are the fourth most important food crop globally (CIP, 2007). Although potato is adapted to different climatic zones and agro-economic conditions (Struik and Wiersema, 1999), most cultivated potato genotypes are relatively salt sensitive (Katerji *et al*., 2003). Soil salinity levels as low as 2.3 dS m^{-1} reduce both growth and tuber yield of potato (Katerji *et al*., 2003). Despite the apparent salt sensitivity of this important crop, little research has addressed salt stress resistance in potato.

Adapted plants have an inherent level of salinity resistance and that level of resistance can significantly increase following salt acclimation (Amzallag and Lerner, 1995). Salt

acclimation results from pre-exposure to low, non-lethal levels of salt stress (Matthews and Boyer, 1984; Conroy *et al*., 1988; Guy, 1990). Acclimated plants will grow at salt concentrations that are lethal to non-acclimated plants (Amzallag *et al*., 1990b). For acclimation to occur, the cell, organ and organism must be in a proper physiological state (Amzallag and Lerner, 1995). The capacity for acclimation varies considerably among plant species (Baker *et al*., 1986) and also varies among genotypes of the same species (Durrant, 1981; Amzallag *et al*., 1993; Azevedo Neto *et al*., 2004). Virtually all plant species can acclimate to salt stress to some degree if the stress is imposed gradually (Amzallag *et al*., 1990b; Hasegawa *et al*., 1994).

In nature, plants are typically subjected to a gradual buildup of salt due to: (1) application of the fertilizers required for crop growth and (2) increase in salt concentration in the soil solution as soil water is depleted (Eilers *et al*., 1995). This gradual increase in salt concentration is ideal for salt acclimation that might occur in the soil. While salt shock is uncommon in nature (Maas and Grattan, 1999), most research on salt stress responses involves short-term exposure to relatively high concentration of salt on non-acclimated plants. Correspondingly, there are fewer studies on acclimation to salt stress compared to the extensive literature using salt shock. Strognov (1964) showed that salt tolerance of plants species was increased by pre-treatment with low concentrations of salt. Amzallag *et al*. (1990b) also reported that *Sorghum* pre-treated with 150 mM NaCl at the seedling stage for 20 days could then survive and produce seeds in the presence of salt concentrations that were lethal to non-acclimated plants. Enhanced salt tolerance following pre-treatment with low concentrations of NaCl was observed in bell pepper (*Capsicum annuum* L.) (Bethke and Drew, 1992), jojoba (*Simmondsia chinensis*) (Ben Raïs *et al*., 1993), maize seedlings (*Zea mays*) (Rodríguez *et al*., 1997), rice (*Oryza sativa*) (Hassanein, 2000; Djanaguiraman *et al*., 2006), soybean (*Glycine max*) (Umezawa *et al*., 2000) and cowpea (*Vigna unguiculata*) (Silveira *et al*., 1999; 2001).

To our knowledge, there are no reports comparing the effects of pre-treatment with incremental salt stress versus salt shock (non-acclimation) on the salt stress response of potato. The hypothesis that a sudden imposition of salt stress (salt shock) would induce a different response from a more gradual increase in salinity (salt pre-treatment) was tested in salt-sensitive and -tolerant potato genotypes. Specifically, our objectives were (a) to compare the effects of incremental NaCl salt stress (salt acclimation) and salt shock on potato genotypes differing in salt stress sensitivity and (b) to examine ABA levels under different salt stress regimes.

3.3 Materials and Methods

3.3.1 Potato genotypes tested

Four potato genotypes of varying salt tolerance were evaluated in this experiment: Three diploid potato lines were provided by Dr. H. De Jong of Agriculture and Agri Food Canada, Fredericton. Line 9506 is a hybrid cross between *S. chacoense*, *S. microdontum,* S. *Phureja* and *S. tuberosum* and is resistant to salt both *in vitro* (Zhang and Donnelly, 1997) and *in vivo* (Shaterian *et al*., 2005b). Line 9120-05 is an ABA-deficient mutant. ABA-deficient mutants were first described by Simmonds (1965) as a single gene (*dr*) mutant, *droopy*, in an unadapted accession of *Solanum tuberosum* L. group *Phureja* (*phu*). A similar mutation was discovered in line 9120-05 (De Jong *et al*., 2001). The sensitivities of this line to drought and to salt stress were shown by De Jong *et al*. (2001) and Shaterian *et al*. (2005a). Line 9120-18 is an ABA-normal sibling of 9120-05. It has an intermediate level of salt tolerance (Shaterian *et al*., 2005a). A moderately salt-tolerant tetraploid commercial cultivar, 'Norland' (Shaterian *et al*., 2005b), was included as a control.

3.3.2 Greenhouse conditions

To eliminate mother tuber effects, the potato germplasm was propagated using stem cuttings. The cuttings were rooted in Ottawa sand (75.5% 1-2 mm diameter, very coarse sand; 24.4% 0.5-1 mm, course sand; and less than 0.1%, less than 0.5 mm sand). Four-week-old rooted cuttings were transferred to 1.5-L pots containing Ottawa sand. Ottawa sand has minimal ion-binding potential. This prevents interference in salinity testing by ion absorption to the medium. Natural light in the greenhouse was supplemented with high pressure sodium halogen lamps to a total of 600 $\mu M\ s^{-1}\ m^{-2}$ PAR with a 15-hour photoperiod. Temperatures were 24/17^{o}C day/night with about 60% relative humidity.

One pot for each line was placed in a tray (20 x 40 x 60 cm) that was connected to a tank containing a nutrient solution amended with different concentrations of NaCl. The trays were flooded 3-5 times per day with the nutrient solution, depending on the growth stages and greenhouse conditions. The trays were flooded for 5 minutes with the appropriate salt solution treatment and then drained. All nutrition solutions contained 1.27 g L^{-1} fertilizer solution (20-20-20 N-P-K, including micro-nutrients, Plant Products Co. Ltd., Brampton, Ontario). The

percentage of micro- and macro-nutrients of 20-20-20 N-P-K fertilizer are discussed in detail in the appendix I. The EC and pH of solution were monitored weekly. The EC ranged from 1.22 for 0 mM NaCl, to 22.16 dS m^{-1} for 180 mM NaCl. The pH ranged between 6.52 to 6.96 for 0-180 mM NaCl (Appendix II).

3.3.3 Salt pre-treatment regime

The salt regime was started one week after plant establishment, which coincided with the emergence of the fourth leaf. Due to differences in salt tolerance of the four potato genotypes observed in the preliminary experiment, the NaCl treatment concentrations used in the pre-treatments were adjusted. The ABA-deficient line and its sibling were exposed to 25 mM NaCl in the first week, 50 mM in the second week and 75 mM in the third week of the acclimation treatment (incremental salt stress of 75 mM). The more salt-tolerant 'Norland' and 9506 genotypes were exposed to 33.3 mM NaCl in the first week, 66.6 mM in the second week and 100 mM in the third week (incremental salt stress of 100 mM). Non-acclimated plants were grown in nutrient solution (as above) for three weeks. Both acclimated (incremental stress) and non-acclimated (salt shock) plants were subsequently exposed to two weeks of salt stress. The salt stress treatment involved two weeks exposure to 150 mM for the salt-sensitive genotypes (ABA-deficient line and its sibling) and 180 mM for the salt-tolerant ('Norland' and 9506) genotypes.

The experiment was conducted as a randomized complete block design in a factorial experiment (4 genotypes x 2 salinity treatments) with 8 replicates and three plants per replicate, with one plant per pot. The GLM program of SAS (SAS Institute, 2002-2003) was used to analyze the data. The ANOVA tables for all parameters are presented in Appendix III. Mean comparisons were performed by LSD tests ($P = 0.05$).

After two weeks' exposure to the salt stress, plant injury, change in plant height, leaf water content and leaf osmotic potential, shoot fresh and dry weight and shoot water content were evaluated. Leaf necrosis was scored on a 1-5 scale: 1 - (0% leaf area necrosis), 2 - (1-25% leaf area necrosis), 3 - (26-50% leaf area necrosis), 4 - (51-75% leaf area necrosis) and 5 - (76-100% leaf area necrosis) (Shaterian *et al*., 2005b). The lowest non-wilted leaves and the youngest fully expanded leaves were used for this assessment. Plant height was measured from

the plant collar to the uppermost tip of the main stem. Heights were monitored weekly to calculate change in plant height:

$$\text{Change in plant height} = \frac{\text{height at T2} - \text{height at T1}}{\text{T2 - T1}}$$

T1= before stress

T2= after stress

After two weeks of salt stress, the water content and osmotic potential in the fourth and fifth fully expanded leaves were measured based on tissue samples taken between 10 a.m. and 12 p.m. Water content was calculated as: (FW- DW) / FW x 100. Osmolality was quantified by a Wescor vapour pressure osmometer (Wescor Model 5500, U.S.A) from samples taken after two-week salt stress, obtained by crushing frozen leaf tissue with a hand press. Osmotic potentials were calculated by the formula $\Psi s = - CiRT$. Ψs = osmotic potential, C = concentration of the solution (moles of solute per kg H_2O), i = a constant that accounts for the ionization of a solute, R = the universal gas constant (0.00831 kg. MPa $mole^{-1}$ K^{-1}), T = absolute temperature (K) = degree C + 273. Shoot fresh and dry weights and shoot water contents were determined at the termination of the experiment. The sample numbers per treatment for all parameters measured in this experiment were 24.

3.3.4 ABA measurements

For ABA measurements (Schmitz *et al*., 2000), 15-20 g (fresh weight) samples from the youngest fully expanded leaves were collected between 10 a.m. - 12 p.m. at the completion of the salt stress treatments. Leaf samples were immediately frozen in liquid N_2, stored at -20^{o}C and lyophilised prior to extraction. Approximately 100 mg of samples were ground with 3 mL of aqueous 80% acetone containing 1% acetic acid (v/v). The internal standard D3-ABA was added to each sample at 10 µL (2 ng mL^{-1}). The supernatant was dried by stream on a nitrogen evaporator. An Oasis HLB 1 cc cartridge (Waters, U.S.A.) was pre-conditioned with methanol and equilibrated with water under vacuum. The extract was dissolved in 200 µL 99% methanol (v/v) containing 1% acetic acid and mixed with 800 µL 1% acetic acid (v/v). The supernatant was loaded and washed with 1 mL water under vacuum. ABA was eluted from the column with

1 mL of 80% methanol containing 1% acetic acid and dried by speed vacuum (Eppendorf Vacufuge ™, Brinkmann Instruments, Inc., Canada). High-performance liquid chromatography (HPLC) and mass spectrometry (MS) were used to quantify the abscisic acid (ABA) (Ross *et al*., 2004). ABA measurements are described in more detail in Appendix IV.

3.4 Results

The extent of leaf necrosis varied depending on the type of salt stress applied and the genotype (Figure 3.1). In the salt shock treatment, leaf necrosis ratings for 'Norland', the ABA-deficient mutant and the ABA-normal sibling were similar. The salt-resistant 9506 line showed less leaf necrosis than the ABA-deficient mutant. The incremental salt stress treatment significantly reduced leaf necrosis compared to the salt shock regime (Figure 3.1). 'Norland' and the 9506 genotypes had the greatest reductions in leaf necrosis associated with the incremental salt stress treatment. In the incremental salt stress treatment, leaf necrosis of the ABA-deficient mutant was more severe than in all other genotypes.

There were no significant differences between the growth rates (changes in plant height) of any of the incrementally salt-stressed and -shocked plants after three weeks of salt stress (Table 3.1). The incremental stress slowed growth of all plants during the subsequent salt stress event. The salt pre-treatment reduced shoot fresh weight of all genotypes relative to the salt shock, but this effect was only statistically significant in line 9506 (Table 3.1). The incremental stress treatment also reduced shoot dry weight of 'Norland', 9506 and the ABA-normal sibling compared to the salt-shocked plants.

After 3 weeks of salt stress, the shoot water content of the 9506 genotype and the ABA-deficient mutant under the incremental stress treatment was higher than in the corresponding salt shock treatments (Table 3.1). The more salt-resistant genotypes ('Norland' and 9506) had higher shoot water contents compared to those of other genotypes under both salt stress regimes. Since leaf water content was generally not different between the two salt stress regimes (Table 3.1), the observed differences in shoot water content were probably due to differences in stem water content. Leaf osmotic potential was less negative for plants exposed to the incremental stress treatment compared to plants exposed to salt shock, except for the salt-tolerant 9506 genotype, which had a consistently high osmotic potential, irrespective of the salt stress regime.

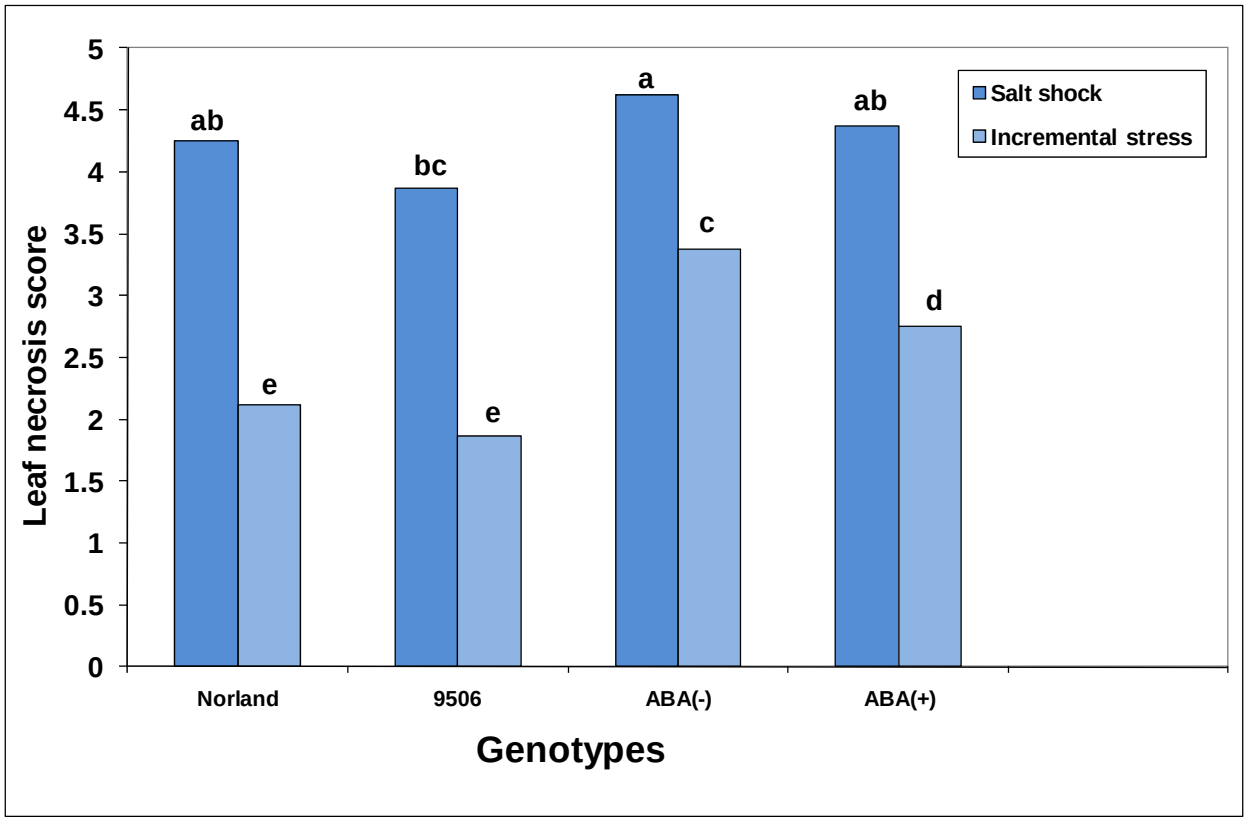

Figure 3.1 The effects of incremental salt stress pre-treatments (75-100 mM NaCl) on leaf necrosis scores of four potato genotypes measured after two weeks of salt stress (150-180 mM NaCl). Leaf necrosis score, 1-5: 1- (0% leaf area necrosis), 2- (1-25% leaf area necrosis), 3- (26-50% leaf area necrosis), 4- (51-75% leaf area necrosis), 5- (76-100% leaf area necrosis). Means with the same letters are not significantly different (LSD *P = 0.05*).

Table 3.1. Change in plant height (growth rate), shoot fresh and shoot dry weight, shoot water content, leaf water content and leaf osmotic potential of four potato genotypes measured after 3 weeks of salt stress (150-180 mM NaCl) applied to either salt shocked (0 mM NaCl) or incrementally salt stressed (75-100 mM NaCl) pre-treated plants. Means for each parameter with the same letters are not significantly different (LSD *P = 0.05*).

Responses	**Pre-treatments**	**'Norland'**	**9506**	**ABA-Mutant**	**ABA-Normal**	**Mean**
Growth rate	Salt shock	0.35 ab	0.61 a	0.55 a	0.57 a	0.52 a
cm d^{-1}	Incremental stress	0.18 b	0.39 ab	0.26 ab	0.43 ab	0.31 ab
Shoot fresh weight	Salt shock	127.24 a	123.55 a	10.86 d	29.94 c	72.89 a
(g)	Incremental stress	113.53 ab	104.97 b	7.23 d	26.46 c	63.04 b
Shoot dry weight	Salt shock	29.25 b	37.55 a	5.61 e	9.38 d	20 44 a
(g)	Incremental stress	20.43 c	20.97 c	2.18 e	4.30 e	11. 97 b
Shoot water content	Salt shock	73.68 abc	68.63 cd	33.73 f	61.77 de	59.45 a
(%)	Incremental stress	80.61 a	78.94 ab	53.30 e	69.64 bcd	70.62 b
Leaf water content	Salt shock	83.45 a	79.79 abc	57.40 d	72.76 c	73.35 a
(%)	Incremental stress	83.19 b	79.36 abc	40.14 e	73.52 bc	69.06 a
Leaf osmotic potential	Salt shock	-1.74 b	-1.22 ef	-1.99 a	-1.88 ab	-1.70 a
(Ψs=MPa)	Incremental stress	-1.26 def	-1.21 efg	-1.74 b	-1.43 cd	-1.41 d

Concentrations of ABA in the incrementally salt stressed plants were 33% (ABA mutant) to 44% (9506 genotype) higher than the ABA levels in salt-shocked plants at the end of the salt stress treatment (Figure 3.2). The ABA mutant had the lowest ABA concentration but was not completely devoid of ABA. Its ABA-normal sibling plants also had lower ABA concentrations compared to those of the more salt-tolerant 'Norland' and the 9506 genotype. Salt-shocked plants of 'Norland' and the 9506 genotype had comparable leaf ABA concentrations but under incremental salt stress, the 9506 genotype had higher levels of ABA than did 'Norland'.

3.5 Discussion

Exposing potato plants to 3 weeks of incremental salt stress treatment prior to imposition of salt stress suppressed growth during the stress period but reduced salt stress damage to the leaves and improved plant water relations compared to salt-shocked plants. This is consistent with previous observations on *Sorghum* (Amzallag *et al*., 1990b), where *Sorghum* pre-treated with 150 mM NaCl for 20 days prior to exposure to 300 mM NaCl survived the salt stress treatment, while control plants that either were not pre-treated or were pre-treated for less time died when exposed to high concentrations of salt (Amzallag *et al*., 1990b). The observed acclimation of *Sorghum* to salinity was proposed to result from a modulation of gene expression triggered by exposure to non-lethal NaCl concentrations. Amzallag *et al*. (1993) indicated that *Sorghum* plants coping with salinity through pre-existing resistance mechanisms (tolerance mechanisms existing prior to salt pre-treatment) lost their leaves when exposed to salinity, while plants that had been pre-acclimated (adaptation accompanied by morphological modification) to salt stress retained their leaves. In our study, pre-treatment with incremental salt stress treatment also decreased leaf necrosis during subsequent exposure to high concentrations of salt. Leaf necrosis was suggested as a sensitive indicator of salt tolerance in potato (Shaterian *et al*., 2005b) and other crops (Wahome *et al*., 2000; Chen *et al*., 2001; 2002). A reduction in the growth occurred under both incremental salt stress treatment and salt shock in our study. Umezawa *et al.* (2000) found no effect of salt pre-treatment on growth rates of soybean subsequently exposed to high concentrations of salt. By contrast, Silveria *et al*. (2001) found a decrease in the shoot growth of non-acclimated cowpea plants compared to pre-acclimated plants when the plants were subsequently exposed to salt stress.

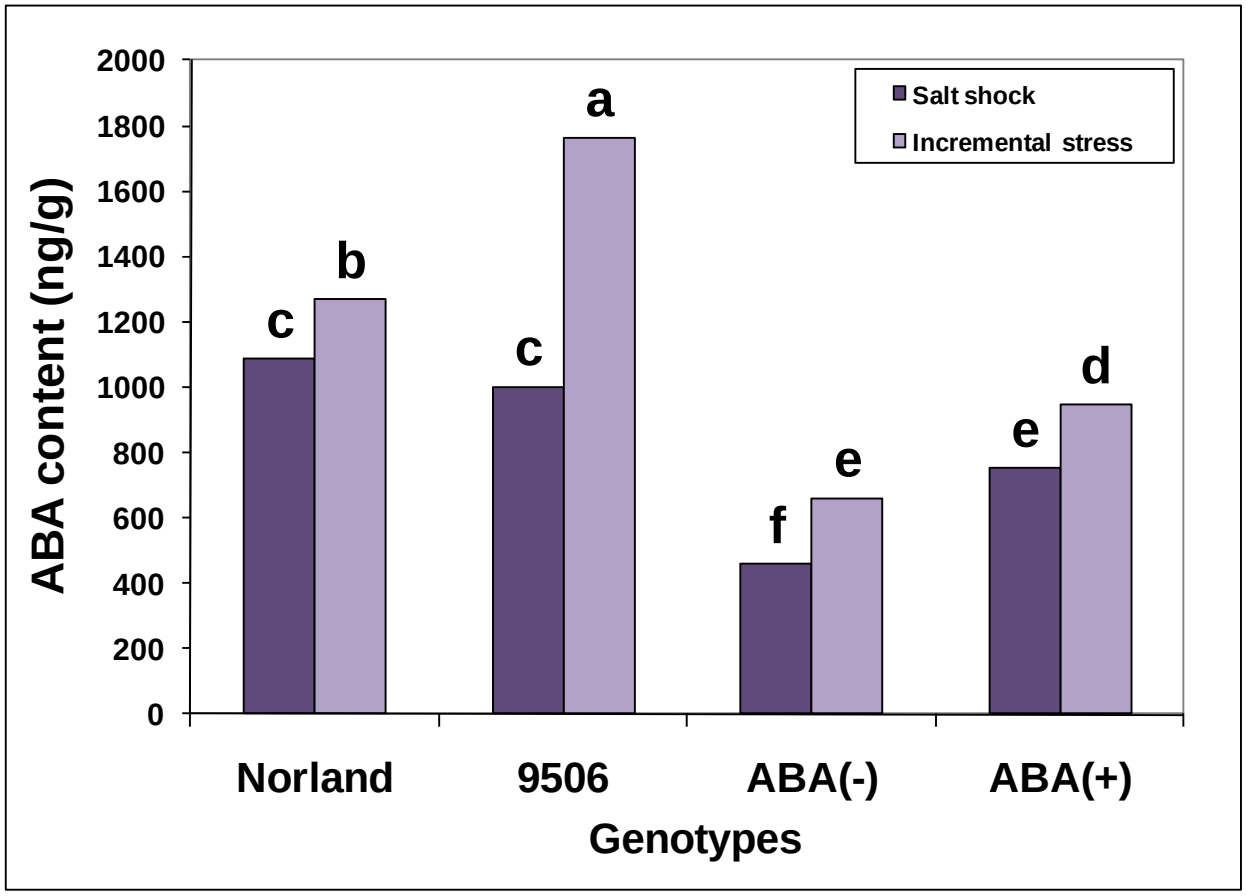

Figure 3.2 The effects of incremental salt stress pre-treatments (75-100 mM NaCl) on abscisic acid (ABA) content of four potato genotypes measured after 3 weeks of salt stress (150-180 mM NaCl). Means with the same letters are not significantly different (LSD *P = 0.05*).

Exposure to incremental salt stress reduced shoot dry weight accumulation compared to salt-shocked plants. Silviera *et al*. (2001) showed that treatment of cowpea with 50 mM NaCl for 8 days resulted in a 25% reduction in shoot dry mass compared to non-stressed control plants. NaCl-adapted *Sorghum* plants also had reduced shoot dry weight compared to control plants (Amzallag *et al*., 1990b; Amzallag, 1996; De Lacerda *et al*., 2001). In soybean, leaf dry weights were reduced slightly to severely by salt pre-treatment, depending on the salt concentration used during the pre-treatment (Umezawa *et al*., 2000). Shoot dry weight in bean (*Phaseoulus vulgaris*) (Bayuelo-Jiménez *et al.*, 2003) was also reduced.

After exposure to salt stress, shoot biomass was higher in the salt-tolerant genotypes ('Norland' and 9506) than in the salt-susceptible group (ABA-deficient mutant and its ABA-normal sibling) irrespective of the salt stress regime. Mandal and Singh (2001), who screened rice genotypes, and Umezawa *et al*. (2001), who screened soybean, found similar genotypic differences in response to salt stress. The greater shoot weights of salt-tolerant safflower (Ashraf and Fatima, 1995), *Sorghum* (De Lacerda *et al*., 2001) and sugarcane (Akhtar *et al*., 2003) under gradually increasing NaCl salinity levels are consistent with our results.

ABA accumulated to a varying extent in all genotypes of our study in response to incremental salt stress. ABA accumulation also varied with the salt stress regime. The incremental salt stress treatment induced significantly higher ABA levels than did salt shock. Salt stress-induced accumulation of endogenous ABA has been reported in many crops including rice (Asch *et al*., 1995) (60 mM NaCl), tomato (Dunlap and Binzel, 1996; Mulholland *et al*., 2003) (50 mM NaCl), *Brassica* (He and Cramer, 1996; Verslues *et al*., 2006) (85 mM NaCl), bean (Sibole *et al*., 1998) (75 mM NaCl), soybean (Umezawa *et al*., 2001) (50 mM NaCl), barley (Jia *et al*., 2002) (100 mM NaCl) and *Arabidopsis* ABA mutants (Cramer, 2002) (80 mM NaCl). The ranking of salt stress resistance after incremental salt stress treatment application followed the ranking for production of endogenous ABA (ABA-deficient mutant < ABA- normal sibling < 'Norland' ≤ 9506). Salt-tolerant genotypes maintained higher concentrations of ABA than did salt sensitive genotypes (Figure 3.2) following incremental salt stress. 'Norland' and the 9506 genotype had similar ABA profiles under salt shock conditions but incremental salt stress treatment induced higher levels of ABA in the more salt-tolerant 9506 genotype (76% increase) than in 'Norland' (17% increase). The higher endogenous ABA levels

under incremental salt stress could be the result of any one or a combination of factors including: elevated ABA biosynthesis, reduced ABA catabolism, reduced ABA export from the sampled tissues. The main pathways that alter ABA catabolism include: conjugation of ABA to form ABA glucose ester (ABAGE), or oxidation yielding phaseic acid (PA), and dihydro-PA (DPA) (Hartung *et al*., 2005). Studies using inhibitors of ABA biosynthesis (fluridone, naproxen, and tungstate) on ABA-deficient tomato mutants (*notabilis*, *flacca*, and *sitiens*) indicate that ABA biosynthesis is influenced, probably through stimulated cleavage of xanthophylls to xanthoxal in shoot tissue (Hansen and Grossmann. 2000).

Although able to accumulate ABA to a limited extent (Coleman and Schneider, 1996; Mulholland *et al*., 2003), the ABA-deficient mutants of potato could not readily accumulate ABA and could not convert ABA aldehyde to ABA (Duckham *et al*., 1989). These lines are salt sensitive (Shaterian *et al*., 2005a) and were unable to close their stomata in response to water deficit (Quarrie, 1982). In our study, under incremental salt stress the ABA-deficient mutant failed to increase its stress resistance to the same extent as the other genotypes, thus the presence of ABA appears to be important in the development of salt stress resistance in the potato genotypes tested. Cramer (2002) observed the protective role of ABA on growth of the *Arabidopsis* ABA-deficient mutant (*aba1-3*) under high salt intensity. Exogenous ABA also increased the rate of acclimation to salinity by *Sorghum* (Amzallag *et al*., 1990a). In the absence of exogenous ABA, *Sorghum* required 20 days of saline pre-treatment for acclimation to occur but when exogenous ABA was applied, acclimation was achieved within approximately one week. Acceleration of salt acclimation by accumulation of ABA was also reported in *Sorghum bicolor* (Amzallag, 1996) and tobacco cells (LaRosa *et al*., 1985).

Plant injury and physiological responses of various potato genotypes to salt stress differed depending on how the salt stress was applied. This difference needs to be considered when developing experimental protocols for assessing stress responses and when developing screening programs. The incremental salt stress regime produced greater tolerance to subsequent exposure to high salt levels than did salt shock. Incremental salt stress appears to induce an acclimation response. Generally, the ability to produce ABA was related to the degree of salt stress resistance, with higher ABA levels induced under incremental salt stress compared to those induced under salt shock. Amzallag and Lerner (1994) showed that pre-existing stress resistance and acclimation to stress are different phenomena and that the ability to acclimate to

stress (selected under incremental stress) does not necessarily relate to the inherent degree of stress tolerance (selected under a salt shock regime). Phytohormones, particularly ABA, appear to be involved in the acclimation phenomenon (Amzallag *et al*., 1990a).

3.6 Conclusion

Plant injury and physiological responses of various potato genotypes to salt stress differed depending on how the salt stress was applied. Salt shock did not permit full expression of potential salt stress resistance. However, the relative ranking of salt stress resistance of the four potato genotypes tested was similar in the salt shock regime as in the regime when the salt stress was applied in a more gradual "lifelike" manner. ABA deficiency also limited the degree of salt acclimation. The type of salt stress regime applied has a significant impact on outcome and should be carefully considered when developing experimental protocols and screening programs.

4.0 The method of ABA application affects salt stress responses in salt stress resistant and sensitive potato lines

4.1 Abstract

The phytohormone abscisic acid (ABA) has been proposed to act as a mediator in plant responses to a range of stresses, including salinity. Most studies of ABA responses apply ABA as a single dose. This does not accurately represent the prolonged increases in endogenous ABA levels that occur naturally in association with slowly increasing salinity stresses. Salt stress responses as influenced by the method of ABA application were examined in four potato genotypes of varying salt stress resistance: (1) an ABA-deficient mutant, (2) its normal sibling, (3) a salt stress resistant genotype line (9506) and (4) a commercial cultivar 'Norland' which has moderate resistance to salt stress. ABA was applied prior to salt stress as a root drench at 0, 50, 75 or 100 μM concentrations through a single dose, or by applying multiple ABA doses at gradually increasing concentrations. The study was conducted in a sand-based growing system under greenhouse conditions. Salt tolerance was evaluated after two weeks' exposure to 150–180 mM NaCl stress. Plant responses to the method of ABA application were differentiated according to (a) growth rate, (b) root water content and (c) apparent shoot growth. The method of ABA application had a marked effect on the responses to salt stress. With a single dose of ABA the change in plant height increased in all genotypes exposed to salt stress, while multiple applications of ABA maintained a more stable change in plant height. Percent root water content was elevated in two genotypes by multiple ABA doses, while none of the single-dose treatments induced any change in root water content. The single ABA dose enhanced vertical shoot growth, while the multiple ABA dose applications enhanced lateral shoot growth. The ABA-deficient mutant consistently expressed the lowest root fresh and dry weights and water content; the lowest shoot fresh and dry weights; and the lowest change in plant height, suggesting that the presence of ABA is important for these parameters. Growth parameters of the ABA-deficient mutant were generally enhanced under slowly increasing multiple doses of ABA, with less response to a single dose. This study indicates that the method of ABA application can alter plant responses to salt stress.

4.2 Introduction

Plants are frequently subjected to environmental stresses such as water deficit, freezing, heat or salt stress. The phytohormone ABA has been suggested as playing a role in stress responses and/or adaptation (Thomas and Eamus, 1999; Gómez-Cadenas *et al*., 2003; Gusta *et al*., 2005; Sharma *et al*., 2005; Shaterian *et al*., 2005a). Following a stress event, ABA content increases within a few minutes to several hours, depending upon the type and severity of the stress (Cramer and Quarrie, 2002; Jia *et al*., 2002; Liu *et al*., 2003; Fricke *et al*., 2004; 2006). Increased ABA concentrations trigger the expression of specific genes; gene induction can also be triggered in unstressed plants by the application of ABA (Moons *et al*., 1997; Ma and Bohnert, 2006; Srivastava *et al*., 2006).

ABA is known to play an important role in enhancing plant water use efficiency. Root systems are critical for plant growth; they give support and take up nutrients and water. In plants coping with drought, ABA plays an important role in root-to-shoot signalling and stomatal regulation, resulting in slowing of shoot growth but maintaining elongation of the primary root (Sharp and LeNoble, 2002; Hartung *et al*., 2005). Normally, the application of ABA slows shoot and root growth under non-stress conditions (Griffiths *et al*., 1997; Chen *et al*., 2006a). There is, however, evidence that the ABA that accumulates during salt stress may actually enhance root growth (Mulholland *et al*., 2003). A recent study demonstrated the positive impact of applied ABA on root growth, morphology and regulation of ion accumulation (Chen *et al*., 2006b). The negative effect of NaCl salt on root nodule dry weight of common bean has also been shown to be alleviated by an exogenous ABA (Khadri *et al*., 2006; 2007). ABA contributed to the increase of xylem water potential and water uptake in the presence of salt (Fricke *et al*., 2004). Overproduction of ABA is associated with increased transpiration efficiency and root hydraulic conductivity, and positively influences leaf expansion (Thompson *et al*., 2007).

ABA modulates stomatal opening, thereby regulating transpiration and CO_2 assimilation (Gómez-Cadenas *et al*., 2003) by slowing down metabolism and Cl^- uptake and accumulation in the leaves. ABA delayed the slowing of CO_2 assimilation under adverse conditions and decreased the inhibitory effects of NaCl on the rate of CO_2 fixation (Popova *et al*., 1995; Arbona *et al*., 2006). The apparent effects of ABA may be partially due to its effects on photosynthate supply (Liu *et al*., 2004). Alleviation of the water stress by ABA was positively correlated with

increases in leaf area, leaf dry weight, shoot length and shoot dry weight (Sansberro *et al*., 2004). By mitigating the negative effects of salt stress, ABA has been implicated in promoting leaf growth (Fricke *et al*., 2004). Exogenous ABA enhanced drought tolerance in turf grass through osmotic adjustment, cell turgor maintenance and reduced damage to cell membranes and the photosynthetic systems (Wang *et al*., 2003).

Near-isogenic mutants with altered endogenous ABA levels are useful tools in the investigation of ABA responses in plants (Fambrini *et al*., 1995). Several mutants with reduced ability to produce or respond to ABA have been identified: *notabilis* (*not*), *flacca* (*flc*) and *sitiens* (*sit*) mutants of tomato (Neil and Horgan, 1985; Borsani *et al*., 2002), potato (Quarrie 1982; De Jong *et al*., 2001), pea (Wang *et al*., 1984; Kof *et al*., 2006), *Arabidopsis thaliana* (Koornneef *et al.,* 1982; Umezawa *et al.,* 2006), *Helianthus annuus* (*nd-1*; *w-1*) (Fambrini *et al.,* 1995; 2004), viviparous corn mutants (Robichaud *et al.,* 1980; Suzuki *et al.*, 2006) and wheat (Holappa *et al.*, 2005). The ABA-deficient mutant of barley (*Az34*) exhibited much-reduced rates of leaf expansion compared to its near-isogenic wild type. When ABA was supplied to ABA-deficient mutants, leaf expansion was increased to levels seen in the wild type (Mulholland *et al*., 1996). In an ABA-deficient mutant of tomato (*sitiens*), ABA levels in the leaves were only 10% of the levels found in the normal type, regardless of salt treatment (Dunlap and Binzel, 1996). Treatment with exogenous ABA resulted in phenotypic reversion of the mutant to the wild type, accompanied with an increase in hydraulic conductance.

A study of wild type and mutant of maize, *Vp5*, showed that ABA restricted ethylene production for maintenance of root elongation at low water potential (Spollen *et al*., 2000). The observed high mortality of ABA-deficient mutants of *Arabidopsis*, *aba*1-3 under high salinity and high light intensity supported the idea that ABA protected plants from damage against these stresses (Cramer, 2002). Shaterian *et al*. (2005a) confirmed the involvement of ABA in salt stress tolerance using the ABA-deficient mutant of potato (*droopy*). The *Arabidopsis thaliana* ABA-deficient mutant, *aba*2-1 was used to explore the regulatory role of ABA in proline (Pro) accumulation and osmotic adjustment at low water potentials (Verslues and Bray, 2006). It was concluded that ABA is required for Pro accumulation at low water potentials. The limited growth of the ABA-deficient mutant tomato (*notabilis*) under salinity was due to its inability to produce ABA as efficiently as the wild type (Mulholland *et al*., 2003). This was verified when supplemental synthetic ABA added to the rooting medium, increased leaf and root growth of the

mutant to wild-type levels. Differences between salt tolerances of genotypes could also be attributed to variation in ABA concentrations (Umezawa *et al*., 2001; Chen *et al*., 2002c; Sibole *et al*., 2003; Chang *et al*., 2006).

A close and connected interrelationship between ABA accumulation and tolerance of osmotic, salt and chilling stresses has been proposed by Liu *et al*. (2003). Since ABA production increases with the degree of salt stress shock (Jia *et al*., 2002), under the slowly increasing salinity stress that typically occurs under natural conditions, ABA elevation will likely be gradual. Therefore, ABA treatments involving slow increases in ABA concentrations may provide a better simulation of ABA responses to real-world stresses. Single doses of ABA may actually represent a shock to the plant.

The objective of this work was to determine if the method of ABA application influences plant responses to salt stress and to investigate the potential role of ABA in alleviating salt stress injury using genotypes differing in their ability to produce ABA.

4.3 Materials and Methods

4.3.1 Genotypes resources

Four potato genotypes were used: (1) 9120-05, a highly salt-sensitive ABA-deficient mutant; (2) 9120-18, the ABA-normal sibling of 9120-05 and only moderately sensitive to salt stress; (3) 9506, which is highly salt resistant; and (4) a commercial variety, 'Norland', which is moderately salt resistant (Shaterian *et al*., 2005b; Chapter 3). See 3.3.1. for detailed description of these genotypes. These genotypes were clonally propagated using stem cuttings (apices) in Ottawa sand (75.5% 1 - 2 mm diameter, very coarse sand; 24.4% 0.5 - 1 mm, course sand; and less than 0.1% less than 0.5 mm sand) in a mist chamber at the University of Saskatchewan, College of Agriculture greenhouses. Ottawa sand provides an inert and stable medium which resists the accumulation of salts. A single uniform-sized rooted cutting was placed into a 1.5-L pot filled with white Ottawa sand. The pots were arranged in 20 x 40 x 60 cm diameter plastic trays, with four pots (one of each line) per tray in the greenhouse. The pots were irrigated by flooding the trays 2 - 3 times a day with a solution containing 1.7 g L^{-1} of a complete nutrient solution (20-20-20 N-P-K plus micro-

nutrients, Plant Products Co. Inc., 314 Orinda Road, Brampton, Ontario, LGT 1J1). The percentage of micro- and macro-nutrients of 20-20-20 N-P-K fertilizer are summarized in detail in appendix I. The EC and pH of the nutrient solution were checked weekly. The EC and pH of the 0-180 mM NaCl treatment solutions ranged between 1.22-22.16 dS m^{-1} and 6.52-6.96, respectively (Appendix II). The tubs were flooded for 3-5 minutes each time. The trays were drained completely to prevent salt accumulation in the root area. A photoperiod of 14 hours was provided by supplementing natural light with high-pressure sodium halogen lamps providing an average intensity between 500 – 900 μM s^{-1} m^{-2}, with a 25/18^{o}C day/night temperature regime. The main stem and lateral shoots of each line were supported vertically with bamboo sticks.

4.3.2 ABA pre-treatment

ABA was applied after 3 weeks of growth and prior to stolon initiation. The ABA was applied in two different experiments, as either (1) a single dose of 0, 50, 75 or 100 μM ABA applied only once; or (2) by multiple doses ("Multiple applications"), where the ABA was applied in five increasing levels to reach a final concentration of 50, 75 or 100 μM (Table 4.1). For example, in the 50 μM ABA treatment, ABA was increased by 10 μM every three days (i.e; 10, 20, 30, 40, 50, by day 12). +/- ABA (Sigma Chemical Co. Inc., St. Louis, MO) was dissolved in a small amount of NaOH (1 N) and diluted to the desired concentration with distilled water. The pH of the ABA solution was adjusted to 6.5 by adding HCl or NaOH. An aliquot of 150 ml of the ABA solution was applied to the plants as a root drench between 9:00 and 10.00 a.m. Control plants were watered with distilled water and NaOH (1 N), with the pH adjusted to the same level as the treated solutions. Since the nutrient solutions were re-circulated back to individual tanks, the solutions were changed one day after each ABA treatment to prevent the accumulation of ABA in the tank.

Table 4.1. Multiple application treatments for different final concentrations of ABA (μM).

Day 1	Day 3	Day 6	Day 9	Day 12	Final
10	10	10	10	10	50
-15	15	15	15	15	75
20	20	20	20	20	100

One week after the ABA treatments were completed, the salt stress regime began. This involved adding NaCl to the nutrient solution until it contained 180 mM of NaCl for 'Norland' and the salt-resistant 9506 line and 150 mM NaCl for the ABA-mutant line and its normal sibling. This salt stress was applied for two weeks.

Treatments were a Factorial combination (4 genotypes x 4 ABA treatments) arranged in a Randomized Complete Block with 4 replications per treatment and three plants per replication, with one plant per pot. Pooled analysis of variance (ANOVA) for a factorial RCBD was performed by the general linear model (GLM) in SAS (2002-2003). Means were compared using LSD at $P = 0.05$. The ANOVA tables of all parameters are presented in Appendix III.

The effect of two weeks of salt stress on leaf necrosis of the lowest non-wilted leaf and the youngest fully expanded leaves was visually monitored. Leaf necrosis was scored on a scale of 1-5: 1 - (0% leaf area necrosis), 2 - (1-25% leaf area necrosis), 3 - (26-50% leaf area necrosis), 4 - (51-75% leaf area necrosis), 5 - (76-100% leaf area necrosis) (Shaterian *et al*., 2005b). Also, leaf water content, stomatal conductance and osmotic potential of the fourth and fifth fully expanded leaves (one leaf) were recorded between 10 a.m. and 12 p.m. At the end of the two-week salt stress treatment, water content was determined by measuring leaf fresh and dry weight (one leaf) after drying in a hot-air oven for 48 hours at 75^{o}C. Water content was calculated as: (FW- DW) / FW x 100. After two weeks of salt stress, fresh and dry weights of shoot and root and also shoot and root water content were recorded. The roots were washed with tap water to remove any sand, rinsed with water, and dried with paper towel before being oven dried. Stomatal conductivity (one leaf) was measured at the end of the two-week salt stress treatment using a Steady State Porometer (Li-Cor Inc. Li 1600) between 9 a.m. and 11 a.m. Plant height was measured based on the distance from plant collar to the tallest point of the main stem. Plant height was monitored every week. Change in plant height was calculated by the following formula:

$$\text{Change in plant height} = \frac{\text{height at T2 - height at T1}}{\text{T2 - T1}}$$

T1 = before stress

T2 = after stress

Osmolality of the sap was quantified by a Wescor vapour pressure osmometer (Wescor Model 5500, U.S.A) from samples (one-two leaves) taken after two-week salt stress, obtained by crushing frozen leaf tissue with a hand press. Osmotic potentials were calculated by the formula $\Psi s = - CiRT$. Ψs = osmotic potential, C = concentration of the solution (moles of solute per kg H_2O), i = a constant that accounts for the ionization of a solute, R = the universal gas constant (0.00831 kg. MPa $mole^{-1}$ K^{-1}), T = absolute temperature (K) = degree C + 273.

4.4 Results

The method of ABA treatment, either through a single or multiple doses (slowly increasing ABA concentration), produced significantly different responses in potato genotypes exposed to salt stress. The responses were both ABA-treatment and genotype-dependent.

4.4.1 'Norland'

In 'Norland', a single dose of ABA produced plants with less leaf necrosis after two weeks of salt stress compared to control plants (Figure 4.1); however, a single treatment was not as effective at reducing leaf necrosis as multiple ABA applications. With a single dose, only the 75 μM treatment level was effective in significantly reducing leaf necrosis. By contrast, multiple ABA applications culminating in 50 or 75 μM final concentrations significantly reduced leaf necrosis after exposure to salt stress. The highest dose of ABA tested (100 μM) was ineffective in reducing leaf necrosis under either single or multiple ABA applications.

A single (75 and 100 μM) ABA dose increased root fresh and dry weight (Table 4.2). Slowly increasing multiple applications from as little as the 50 μM final ABA concentration also elevated root dry weight, with no change in root water content. However, the 100 μM multiple application treatment resulted in a significant reduction of root fresh weight. This was likely as a function of a dramatic reduction in root water content (Table 4.2).

A single ABA dose at all concentrations stimulated shoot growth as expressed by shoot fresh weight (Table 4.3). The maximum shoot growth response to a single ABA application was observed with the 75 μM treatment. Multiple ABA applications also induced elevated shoot fresh weights, with the treatment leading the highest shoot fresh weight. The enhanced shoot fresh weight resulted from an elevation in shoot water content and was not due to increased

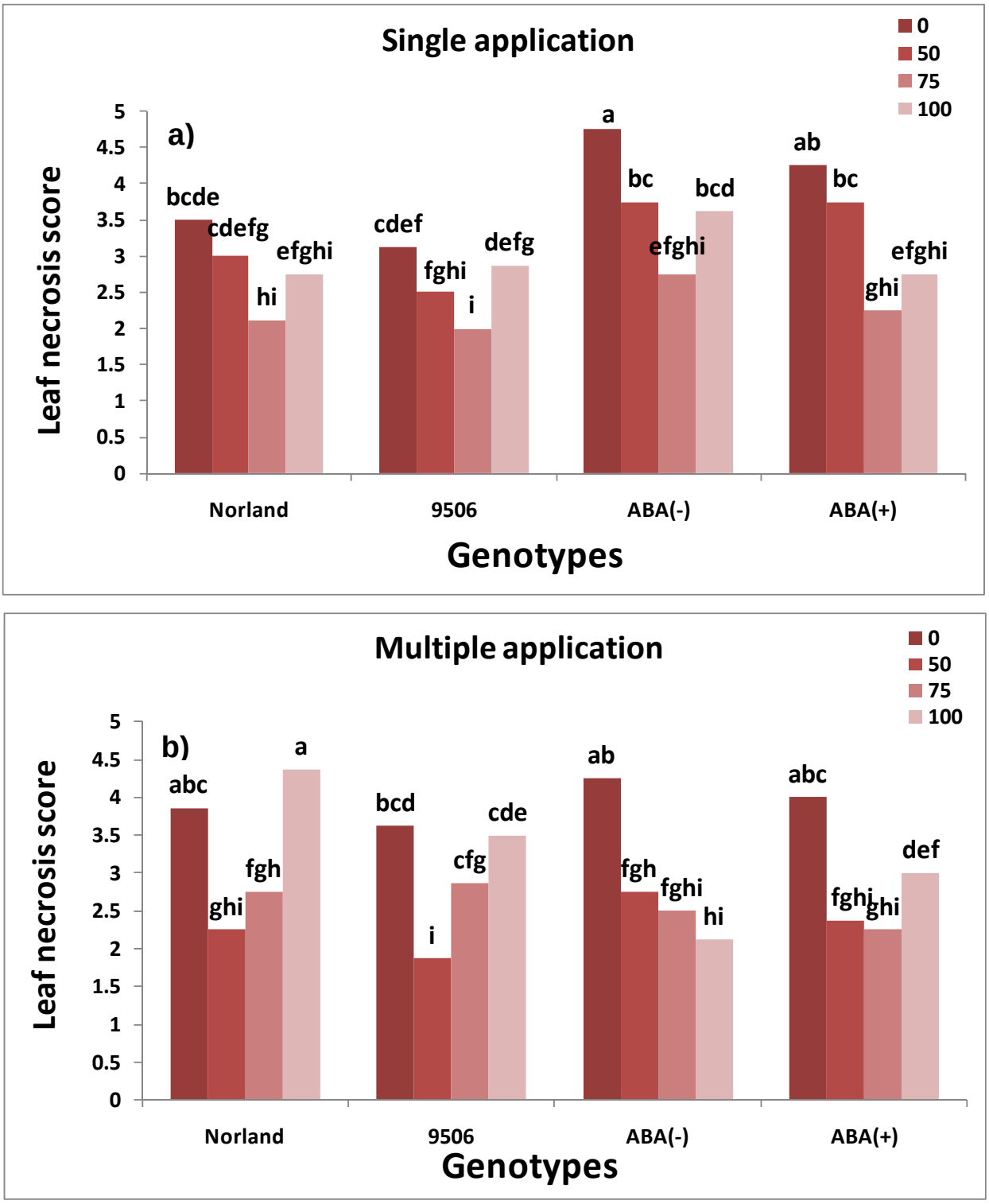

Figure 4.1 The effects of single (a) and multiple (b) ABA applications (µM) on leaf necrosis scores of four potato genotypes after 2 weeks of salt stress (150-180 mM NaCl). Leaf necrosis score, 1-5: 1- (0% leaf area necrosis), 2- (1-25% leaf area necrosis), 3- (26-50% leaf area necrosis), 4- (51-75% leaf area necrosis), 5- (76-100% leaf area necrosis). Means with the same letters for each ABA application method are not significantly different (LSD *P = 0.05*).

Table 4.2. The effects of single and multiple ABA applications on root fresh and dry weights (g) and root water content (%) of four potato genotypes after 2 weeks of salt stress (150-180 mM NaCl). Means with the same letters for each application method are not significantly different (LSD $P = 0.05$).

Root fresh weight

Treatments		**Single**	**application**			**Multiple**	**applications**	
ABA (µM)	'Norland'	9506	ABA-mutant	Normal sibling	'Norland '	9506	ABA-mutant	Normal sibling
0	24.27 cd	18.82 f	0.68 j	10.44 i	24.04 c	9.09 g	0.99 h	8.94 g
50	24.51 bcd	19.69 ef	2.14 j	12.34 hi	26.77 b	17.32 d	2.04 h	12.78 e
75	26.93 ab	26.61 abc	2.90 j	15.31 g	30.57a	23.89 c	2.48 h	11.19 efg
100	28.41a	21.98 de	2.25 j	13.61 gh	9.95 fg	11.93 ef	1.95 h	8.97 g

Root dry weight

0	4.61 cd	4.19 de	0.21 h	2.68 g	3.61 c	3.47 c	0.41 f	1.90 de
50	5.13 bc	4.83 bcd	0.51 h	2.72 fg	4.28 b	3.36 c	0.45 f	2.18 d
75	5.42 bc	6.59 a	0.87 h	3.54 ef	5.23 a	3.88 bc	0.40 f	2.03de
100	5.52 b	6.47 a	0.66 h	2.41 g	3.58 c	2.48 d	0.39 f	1.47e

Root water content

0	80.89 a	77.76 ab	67.73 d	73.21 abcd	85.00 a	60.81 c	57.96 c	78.72ab
50	80.23 a	75.42 abcd	68.16 d	77.49 abc	84.09 ab	80.17 ab	77.81 b	82.76 ab
75	80.58 a	75.19 abcd	68.30 d	77.86 abc	82.74 ab	83.81 ab	83.18 ab	81.67 ab
100	80.55 a	69.83 cd	70.28 cde	79.72 a	59.43 c	78.96 ab	78.83 ab	83.68 ab

Table 4.3. The effects of single and multiple ABA applications on shoot fresh and dry weights (g) and shoot water content (%) of four potato genotypes after 2 weeks of salt stress (150-180 mM NaCl). Means with the same letters for each application method are not significantly different (LSD $P = 0.05$).

Shoot fresh weight

Treatments		**Single**	**application**			**Multiple**	**applications**	
ABA (μM)	'Norland'	9506	ABA-mutant	Normal sibling	'Norland'	9506	ABA-mutant	Normal sibling
0	102.50 d	68.60 h	20.68 i	75.36 gh	155.10 e	140.40 f	9.45 i	54.13 j
50	122.98 c	88.88 ef	26.19 i	83.05 fg	270.60 b	170.36 d	26.54 k	85.15 i
75	159.09 b	173.90 a	29.91 i	116.30 c	317.08 a	201.11 c	40.05 k	87.94 hi
100	125.15 c	117.62 c	24.08 i	98.95 de	125.62 g	101.24 h	33.20 k	74.62 i

Shoot dry weight

0	24.87 a	22.40 bc	5.21 f	16.46 e	39.05 b	43.13 a	2.09 j	15.16 e
50	23.36 ab	23.11 abc	4.74 f	16.86 e	39.65 b	27.15 c	4.30 ij	12.70 ef
75	22.21 bc	23.64 ab	4.82 f	19.77 d	41.40 ab	26.30 c	6.87 hi	10.35 fg
100	21.05 cd	19.27 d	4.50 f	16.34 e	24.80 c	18.61 d	4.68 ij	8.74 gh

Shoot water content

0	75.49 fg	67.01 h	73.51 g	78.01 ef	74.51 ij	69.14 k	77.39 hi	71.77 jk
50	80.78 cde	72.62 g	81.52 cd	79.49 de	85.25 abcde	84.02 cdef	83.48 defg	84.82 bcdef
75	85.98 ab	86.39 a	83.75 abc	82.87 bc	86.93 abc	86.65 abcd	83.03 efg	88.10 ab
100	83.14 abc	83.43 abc	80.88 cde	83.37 abc	80.27 gh	81.70 fg	85.67 abcde	88.15 a

shoot dry weight in either single or multiple ABA applications. However, multiple ABA applications leading to the 50 and 75 μM final concentrations induced a two fold higher shoot fresh weight than the corresponding single ABA application. Multiple applications of ABA that produced a 100 μM final concentration significantly depressed shoot fresh weight.

Changes in plant heights for 'Norland' were distinctly different for single versus multiple doses of ABA (Figures 4.2, 4.3). A single ABA dose significantly increased changes in plant heights, with the highest changes in plant height occurring at 75 μM. By contrast, none of the multiple ABA applications induced any change in the changes in plant heights (Figures 4.3). Stomatal conductivity also responded differently depending upon the method of ABA treatment. When the ABA was applied as a single dose, leaf stomatal conductance after two weeks of salt stress markedly increased relative to the control (Table 4.4). This increase in conductance may have contributed to the observed increase in change in plant height (Figure 4.2). For multiple ABA applications, stomatal conductance both prior to (data not shown) or after salt stress showed only a marginal elevation in response to the 75 and 100 μM ABA treatments (Table 4.4). Leaf water content after salt stress was not changed by the single ABA dose (Table 4.5) and leaf osmotic potentials were also stable (Table 4.5). Under multiple ABA applications, neither leaf water content nor osmotic potential before stress changed. However, after salt stress, leaf water content increased for all multiple ABA concentrations, while leaf osmotic potential remained constant, except at the 100 μM level, where it decreased (Table 4.5). Stem water content greatly increased under multiple ABA applications (Table 4.6). No data for stem water content are available on single ABA application.

4.4.2 9506 genotype

For the single dose of ABA, only the 75 μM treatment reduced leaf necrosis in the 9506 genotype under salt stress compared to non-treated plants (Figure 4.1). Where multiple ABA applications were used, only the 50 μM ABA final concentration treatment was effective in reducing leaf necrosis in this genotype.

A single ABA dose at 75 and 100 μM increased root fresh weight, reflecting an increase in root dry weight but not water content (Table 4.2). Multiple ABA applications with final concentration as low as 50 μM increased root fresh weight, with the highest response at 75 μM

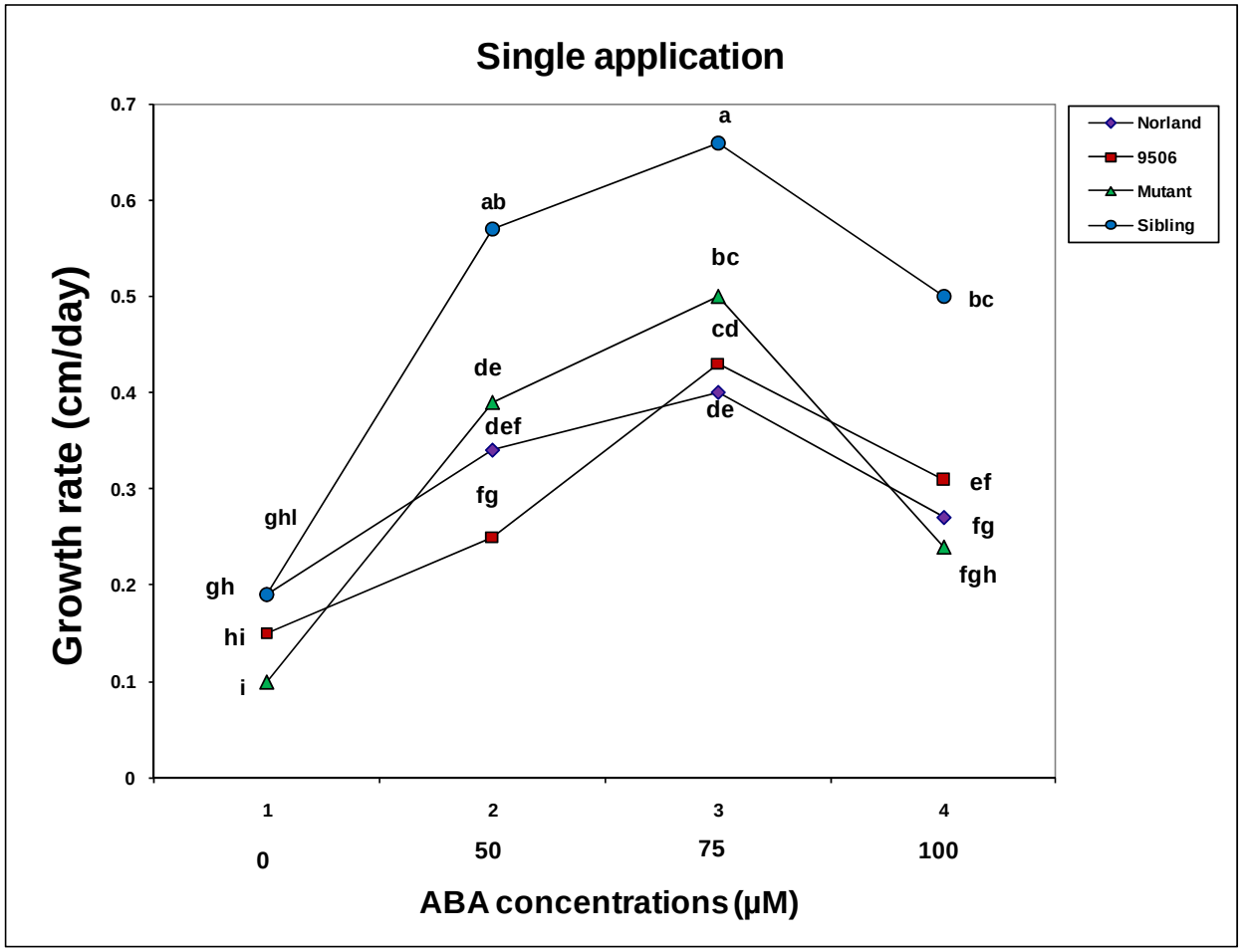

Figure 4.2 The effects of single application of differing concentrations of ABA on change in plant height (growth rate) of four potato genotypes over 2 weeks of salt stress (150-180 mM NaCl). Means with the same letters are not significantly different (LSD *P = 0.05*).

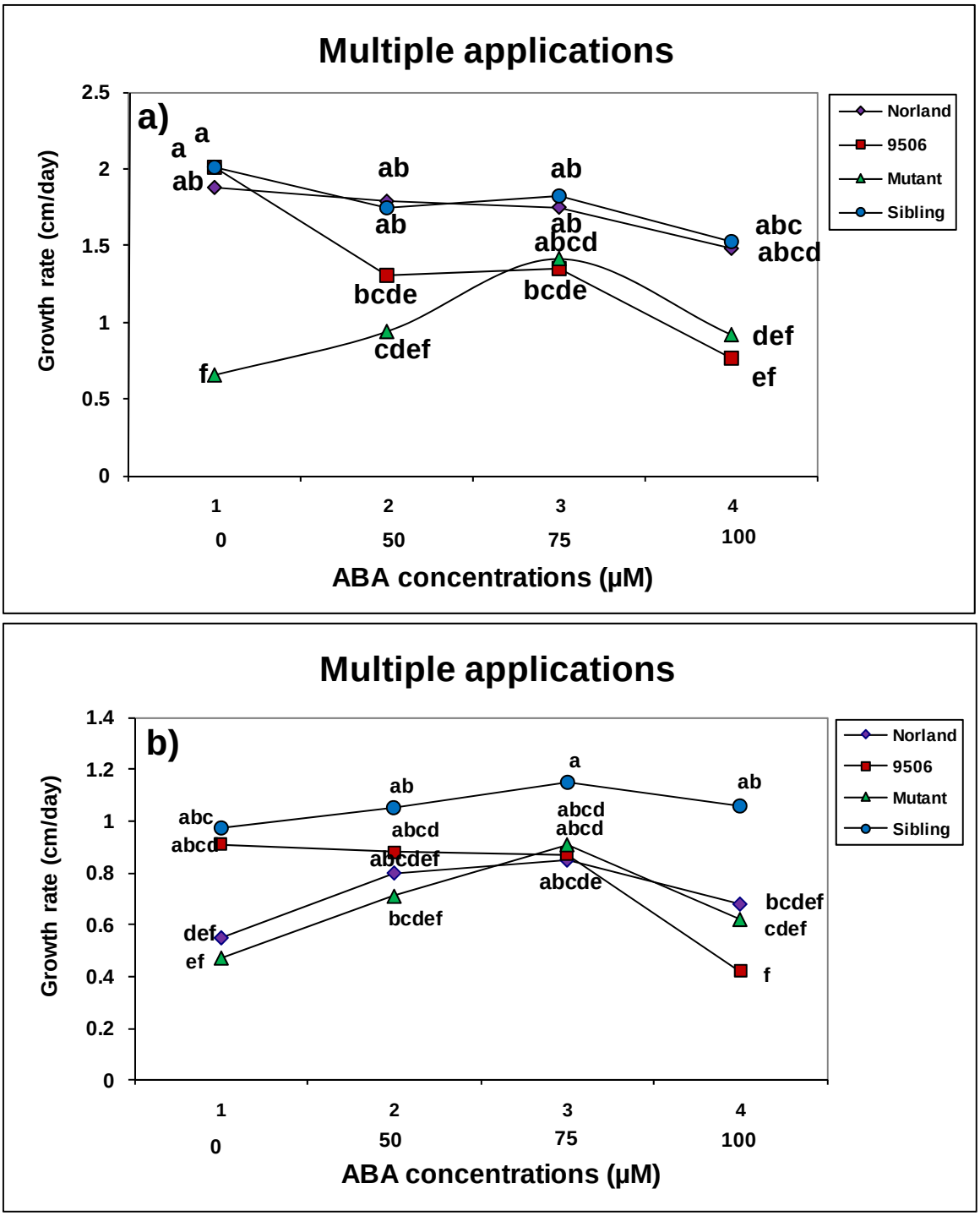

Figure 4.3 The effects of multiple application of differing concentrations of ABA on changes in plant heights (growth rates) of four potato genotypes before (a) and over 2 weeks (b) of salt stress (150-180 mM NaCl). Means with the same letters are not significantly different (LSD *P = 0.05*).

Table 4.4. The effects of single and multiple ABA applications on leaf stomatal conductivity (Cm s^{-1}) after 2 weeks of salt stress (150-180 mM NaCl). For each type of application, means with the same letters are not significantly different (LSD *P = 0.05*).

Single application

ABA (µM)	'Norland'	9506	ABA mutant	Normal sibling
0	0.07 i	0.10 hi	0.12 gh	0.15 fg
50	0.18 def	0.21 cd	0.18 def	0.16 efg
75	0.35 a	0.26 b	0.21 cd	0.26 b
100	0.25 bc	0.22 bcd	0.20 de	0.18 def

Multiple applications

0	0.03 b	0.04 c	0.02 a	0.04 c
50	0.03 b	0.04 c	0.03 b	0.04 c
75	0.05 d	0.04 c	0.06 e	0.06 e
100	0.05 d	0.05 d	0.06 e	0.04 c

Table 4.5. The effects of single and multiple ABA applications on leaf water content (%) and leaf osmotic potential (Ψs=MPa) of four potato genotypes after 2 weeks of salt stress (150-180 mM NaCl). Means with the same letters for each application method are not significantly different (LSD $P = 0.05$).

Single application

	Leaf water content				**Leaf osmotic potential**			
	'Norland'	9506	ABA-mutant	Normal sibling	'Norland'	9506	ABA-mutant	Normal sibling
0	83.24 ab	77.36 bc	42.02 f	72.45 cd	-2.32 def	-2.54 efg	-3.22 h	-3.00 gh
50	83.40 ab	81.69 ab	67.59 d	80.57 ab	-2.14 bcde	-1.70 abc	-2.75 fgh	-1.77 abc
75	84.78 a	79.84 ab	84.56 a	83.86 ab	-1.78 abcd	-1.50 a	-1.77 abc	-1.56 a
100	85.56 a	77.78 bc	55.34 e	81.41 ab	-2.23 cdef	-1.69 abc	-2.55 efg	-1.66 ab

Multiple applications

	Leaf water content				**Leaf osmotic potential**			
	'Norland'	9506	ABA-mutant	Normal sibling	'Norland'	9506	ABA-mutant	Normal sibling
0	58.52 e	56.66 e	68.19 d	70.57 d	-2.13 ef	-2.39 f	-2.29 f	-2.27 f
50	88.03 a	87.35 a	71.45 cd	82.96 ab	-2.20 f	-1.20 a	-2.36 f	-1.59 bc
75	85.60 a	80.16 abc	84.52 ab	86.86 a	-2.31 f	-1.35 ab	-2.40 f	-1.65 cd
100	83.81 ab	70.37 d	75.64 bcd	84.54 ab	-3.06 g	-1.87 cde	-2.16 ef	-1.89 de

Table 4.6. The effects of multiple ABA applications on stem water content (%) of four potato genotypes after 2 weeks of salt stress (150-180 mM NaCl). Means with the same letters are not significantly different (LSD $P = 0.05$).

Stem water content (%)

ABA (µM)	'Norland'	9506	ABA-mutant	ABA-sibling
0	76.76 b	77.14 b	72.68 b	85.38 a
50	88.78 a	87.69 a	87.31 a	91.98 a
75	92.32 a	89.19 a	87.14 a	92.16 a
100	85.69 a	85.40 a	90.95 a	92.01 a

ABA. In contrast to the single ABA treatment, the observed root fresh weight increase under multiple ABA applications was due to an elevated root water content rather than to increased root dry weight.

All concentrations of ABA applied as a single dose enhanced shoot fresh weight relative to the control (Table 4.3). The greatest increase in shoot fresh weight occurred with 75 μM ABA**.** These increases in shoot fresh weight were only due to an elevated shoot water content and did not reflect an increase in shoot dry matter. In fact, a decrease in shoot dry weight was observed in the 100 μM treatment. Multiple ABA applications at 50 and 75 μM levels increased the shoot fresh weight, while the 100 μM treatment depressed this growth parameter. As in the single dose, the most effective concentration in the multiple treatments was 75 μM ABA. The increased shoot fresh weight was again attributed to an increase in water content. Shoot dry weight was again reduced by increasing concentrations of ABA.

Significant increases in stomatal conductance were observed for all single doses of ABA (Table 4.4). Under multiple ABA applications, stomatal conductivity (after stress) increased at 100 μM (Table 4.4). All concentrations of the single-dose ABA treatment also significantly enhanced the change in plant height under salt stress (Figure 4.2). Similar to 'Norland', changes in plant heights after salt stress were distinctly different for single versus multiple doses of ABA for the 9506 genotype (Figures 4.2, 4.3). A single ABA dose significantly increased changes in plant heights, with the highest change in plant height occurring at 75 μM. By contrast, under multiple ABA applications, change in plant height did not change with multiple ABA applications at 50 and 75 μM and diminished under the 100 μM ABA treatment (Figure 4.3). Although ABA had no effect on leaf water content of 9506 under a single dose (Table 4.5), all single ABA applications increased leaf osmotic potential (Table 4.5). All multiple ABA applications raised both the leaf water content and leaf osmotic potential after salt stress (Table 4.5) but had no effect prior to salt stress treatment (data not shown). Stem water content also significantly increased with all multiple ABA applications in the 9506 genotype (Table 4.6).

4.4.3 ABA-deficient mutant

In the absence of applied ABA, compared to all other ABA normal genotypes, the ABA-deficient mutant consistently had the lowest: root fresh and dry weight, shoot fresh and dry

weights, and change in plant height following exposure to salt stress (Tables 4.2, 4.3; Figures 4.2, 4.3).

Leaf necrosis of the ABA-deficient mutant was reduced by all concentrations of ABA applied as a single dose, with 75 μM ABA inducing the greatest effect (Figure 4.1). Similarly, all multiple ABA applications also significantly reduced leaf necrosis (Figure 4.1).

The distinction between a single and multiple ABA dose response was observed in the enhanced root water content under all multiple doses, but this did not occur with the single dose treatment (Table 4.2). In addition, a single dose of ABA induced only an elevation of shoot water content while multiple doses elevated shoot fresh weight, dry weight and water content (Table 4.3).

All single and multiple ABA doses significantly increased leaf stomatal conductance (Table 4.4). However, changes in plant heights differed between the two methods of ABA application. With a single ABA dose, the change in plant height was increased by all ABA concentrations, peaking at the 75 μM level (Figure 4.2). Changes in plant heights did not differ after salt stress following multiple ABA applications (Figure 4.3). In the single ABA treatment, and particularly at 75 μM, leaf water content increased appreciably relative to the controls (Table 4.5). Leaf osmotic potentials were also less negative under these ABA treatments (Table 4.5). Only the multiple ABA application to 75 μM enhanced leaf water content (Table 4.5). Leaf osmotic potentials were also unaffected by multiple ABA treatments (Table 4.5). As seen in the 'Norland' and 9506 genotypes, stem water content of the ABA-deficient mutant increased under multiple ABA applications (Table 4.6).

4.4.4 ABA-normal sibling

With a single ABA dose, only the 75 and 100 μM concentrations reduced leaf necrosis of the ABA-normal sibling genotype relative to the control plants (Figure 4.1). However, with multiple ABA applications there was a marked reduction in leaf necrosis at all ABA concentrations tested.

A single ABA dose only increased both root fresh weight and dry weight at the 75 μM level (Table 4.2). By contrast, under multiple ABA applications, root fresh weight was only

augmented at the 50 μM level (Table 4.2). In contrast to the ABA mutant, multiple ABA doses had no effect on root water content of the ABA-normal sibling.

While only the 75 μM single dose ABA treatment enhanced shoot fresh and dry weight, as well as shoot water content, all concentrations of ABA applied as multiple doses generally elevated these responses relative to the controls (Table 4.3). The single 100μM dose also enhanced shoot fresh weight and water content.

For both single and multiple ABA applications, leaf stomatal conductance after salt stress was only increased by the 75 μM ABA treatment (Table 4.4). As observed in all other genotypes, changes in plant heights were increased by all single ABA doses but not by the multiple ABA applications (Figures 4.2, 4.3). Leaf water content increased under all single- and multiple-dose treatments after salt stress (Table 4.5). A parallel increase in leaf osmotic potential was observed (Table 4.5). Unlike the other genotypes, mean stem water content of the ABA-normal sibling was not significantly increased by multiple applications of ABA (Table 4.6).

4.5 Discussion

The method of ABA treatment, as either a single dose or slowly multiple doses with increasing concentration of ABA, produced significantly different responses in potato genotypes subsequently exposed to salt stress, according to their tolerance levels. Therefore, the two salt stress tolerant genotypes: 'Norland' and 9506, and the two salt stress sensitive genotypes: ABA-deficient mutant and ABA-normal sibling are discussed in different sections. Although the responses were both ABA treatment- and genotype-dependent; several consistent trends emerged.

4.5.1 Norland' and 9506

Wahome *et al*. (2001) reported that leaf tip necrosis was the first sign of NaCl salt injury in rose. Udovenko (1995) further characterized growth rate as an index with high sensitivity to stress. Growth, as measured through vertical height, reacted rapidly to salt stress and could be used to determine parameter stress resistance of potato clones (Shaterian *et al*., 2005b). Both single and slowly increasing multiple ABA applications reduced leaf necrosis following exposure to salt stress in the relatively salt tolerant lines, Norland and 9506. In our study, the

shoot growth response varied between the two methods of ABA application. A single ABA dose enhanced vertical shoot growth, while multiple ABA applications partitioned the growth into lateral rather than vertical growth. This conclusion was based on the observation that: a) vertical height of the multiple ABA application did not change and b) fresh and dry matter accumulation was higher following multiple ABA application than treatment with single ABA application. Growth enhancement by ABA treatment under both normal and salt stress condition is widely reported in the literature (Sharp *et al*., 2000; Sansberro *et al*., 2004; Khadri *et al*., 2006; 2007). The ABA treatment nearly doubled the total leaf area, leaf and shoot dry weight and stem height of well watered the ABA-deficient mutant of tomato (*flc)* (Sharp *et al*., 2000). ABA treatments were shown to increase leaf area, leaf dry weight, shoot length and shoot dry weight of *Ilex paraguariensis* under water stress (Sansberro *et al*., 2004). Plant biomass and root/shoot ratio also improved in salinized plants by ABA application (Khadri *et al*., 2006; 2007). ABA may interact with other hormones to coordinate whole plant growth and other responses to salinity stress in tomato (Mulholland *et al*., 2003). ABA could have been interacting with ethylene to regulate growth in both roots and shoots under salinity stress. The synergistic interaction between ABA and the ethylene in root growth inhibition (Beaudoin *et al*., 2000) is embeded in a specific and more complex web of multi-hormonal regulation. For example, ABA application facilitated full recovery of root elongation of maize ABA-deficient mutant at low water potential, by restricting ethylene production (Spollen *et al*., 2000). However spraying *flacca* with an ethylene inhibitor resulted in only limited recovery of shoot growth (25% restoration in total shoot dry weight) in comparison with that of RR (Rhienlands Ruhm), indicating that the inhibited shoot growth in *flacca* plants may only be partly attributed to ethylene (Sharp and LeNoble, 2000). While ABA and ethylene seem to be the primary factors in controlling growth under salinity, cytokinins and auxins seem better candidates in explaining shoot growth impairment and changes in biomass partitioning (Albacete *et al*., 2003). For instance, in tomato, ABA and ethylene concentrations strongly increased in the roots, xylem sap, and leaves after salinization, but cytokinins and IAA were dramatically decreased when plants exposed to salinity (Albacete *et al*., 2003). Treatment of *Sorghum bicolor* with ABA during the first week of salinization with 150 mM NaCl enhanced growth and accelerated adaptation to higher levels of salinity (300 mM NaCl) (Amzallag *et al*., 1990a). Chen *et al*. (2003b) concluded

that exogenous ABA applied at low concentration tends to improve plant growth (root, hypocotyls, shoot) but at high levels served to inhibit plant growth.

The ABA treatment increased shoot fresh weight largely through increased shoot water content. ABA has been reported to be involved in plant water uptake, thereby resulting in increased xylem water potential in the presence of salt (Fricke *et al*., 2004). The apparent mechanism of continued water uptake in the presence of NaCl salt differed between the two methods of ABA application. Water uptake by line 9506 treated with a single dose of ABA appears to be via the transpiration stream, since the stomata stayed open during salt stress, as indicated by a pronounced increase in stomatal conductivity. Although ABA is known to induce stomatal closure (Wright, 1978; Montero *et al*., 1998; Sibole *et al*., 2000; Mishra *et al*., 2006), there are also reports of higher stomatal conductance in ABA-treated plants than in non-treated plants under single (Tenhunen *et al*., 1994; Ruan *et al*., 2005) and multiple ABA treatment (Gómez-Cadenas *et al*., 2003). Correia *et al*., (1997) indicated that high leaf water potential, low temperature and high cytokinin activity possible explanations for high stomatal conductivity during the first hours of the day, despite the occurrence of high concentration of ABA in the xylem sap.

By contrast, water uptake with multiple ABA applications may have occurred through the symplastic route since those plants exhibited low stomatal conductivity. Water uptake could be facilitated through osmoregulation (Wang *et al*., 2003) and includes the transcellular pathway through aquaporins, as well as through osmoregulation. Increased water availability to the plant is another known outcome of ABA treatment (Hose *et al*., 2000; Chen *et al*., 2002b; Sansberro *et al*., 2004; Sharma *et al*., 2005). ABA applied as multiple doses facilitated water uptake into the roots as soil drying started, especially under non-transpiring conditions, when the apoplastic path of water transport was largely excluded (Hose *et al*., 2000). ABA treatment also resulted in increased relative water content in *Ilex paraguariensis* (Sansberro *et al*., 2004). A rapid increase in ABA can lead to a rise in xylem water potential, which favours water uptake into the plant (Fricke *et al*., 2004). The contribution of higher endogenous ABA content in enhanced root hydraulic conductivity has also been demonstrated (Vysotskaya *et al*., 2004a; 2004b).

ABA enhanced salt tolerance by also altering root growth. However, the response differed depending upon both the salt stress regime and the level of salt resistance of the genotype. ‘Norland’ is of intermediate salt resistance compared to the salt-tolerant 9506

genotype (Shaterian *et al*., 2005a; 2005b). Under a single dose of ABA, both genotypes responded similarly in that enhanced root fresh weight was largely attributable to increased root dry weight but not water content. By contrast, multiple ABA applications distinguished 'Norland' from 9506, in that increased root fresh weight was largely a result of increased root water content in the more salt-tolerant 9506 clone. For both the single or multiple ABA doses, in 'Norland' the increased root fresh weight was largely attributable to enhanced root dry weight. The impact of ABA on root growth and development and formation of root hair and lateral roots has recently been reported (Chen *et al*., 2006b). Root length, root number and root cortex thickness of salt-stressed rice seedlings were increased as a result of pre-treatment with ABA before exposure to soil salinity (Cha-um *et al*., 2007). ABA accumulation maintained root elongation of the primary roots of maize at low water potentials (Spollen *et al*., 2000; Steffens *et al*., 2006). The ABA pre-treatment prior to salt stress treatment of common bean improved root and shoot growth and increased nodule weight (Khadri *et al*., 2007).

4.5.2 ABA-deficient mutant and ABA-normal sibling

Since the ABA-deficient mutant consistently expressed the lowest root fresh dry weight and water content, lowest shoot fresh and dry weights, and lowest change in plant height, this suggests that the presence of ABA is important for these functions. ABA is known to be important in root and shoot growth (e.g., Sharp and LeNoble, 2002). Application of external ABA, particularly multiple applications, enhanced growth habit of the ABA-deficient mutant, but could not achieve the growth habit of its normal sibling. The responsiveness of the ABA-deficient mutant to multiple ABA concentration is consistent with the result of Lenzi *et al*. (1995), who reported that in ABA-deficient mutant of sunflower (*w-1*), large amounts of exogenous ABA were required to supplement the endogenous hormonal level to reach the same growth level of the wild type (*W-1*). By contrast, root growth in the *notabilis* mutant of tomato was markedly increased at 10 µM ABA and was comparable to the wild type (Mulholland *et al*., 2003). There is also a report that exogenous ABA in roots of *notabilis* resulted in structural changes similar to those of wild type under normal conditions and this might result in enhanced resistance to soil drying (Unyayar *et al*., 2004).

Sharp *et al*. (2000) demonstrated that normal levels of endogenous ABA are required to maintain shoot development. They found shoot and root growth were greatly reduced in the

ABA-deficient mutant tomato, *flacca* and in *notabilis* mutants grown under controlled-humidity conditions, yet their leaf water potentials were equal to or higher than those of well-watered wild-type plants throughout development. Similarly in our study, under both single and multiple ABA doses, leaf water content in the ABA-deficient mutant and its normal sibling genotypes were generally equivalent throughout salt stress, yet root and shoot growth were reduced in the mutant genotype. Conversely, leaf osmotic potentials were more negative in the ABA-deficient mutant. Shoot growth was also increased in the ABA-deficient mutant potatoes (*droopy*) exposed to two weeks of salt stress if they were treated with ABA prior to the stress (Shaterian *et al*., 2005a). Additional experiments with *flacca* showed that shoot growth substantially recovered when levels of ABA seen in wild type plants were restored by exogenous applications of ABA. It was hypothesized that the poorer growth of the ABA-deficient mutant was due to its inability to produce ABA at the wild-type levels (Mulholland, *et al*., 2003). In our earlier study (Chapter 3), endogenous ABA levels were lower in the ABA-deficient mutant and incremental salt stress did not elevate internal ABA to the levels seen in the ABA normal type, or the 9506 genotype.

Prior to ABA application, the degree of leaf necrosis seen in the ABA-deficient mutant and its normal sibling were not different. While endogenous ABA levels were lower in the mutant compared to the sibling after incremental salt stress (Chapter 3), those differences were not large and were consistent with Coleman and Schneider (1996), who found no evidence for the role of ABA in regulation of water stress in the ABA-deficient mutant of tomato *flacca*. However, both single and multiple ABA applications did prevent leaf necrosis in both genotypes. This may have been manifested through a salt dilution effect in that both single and multiple ABA applications increased the leaf water content in both genotypes and the stem water content in the ABA-deficient mutant. Since leaf stomatal conductivity also increased under both ABA application regimes and particularly in the mutant, enhanced water uptake may be occurring through the transpiration stream. As was previously mentioned, there are reports of higher stomatal conductance in ABA-treated plants than in non-treated plants under stress (Tenhunen *et al*., 1994; Gómez-Cadenas *et al*., 2003; Ruan *et al*., 2005). This apparent dichotomy in ABA responses might be explained by the signalling factor from the root to the shoot. Studies indicate that ABA is produced in the leaves after signal induction from roots exposed to stress (Ali *et al*., 1999; see Jackson, 2002 for a review). If the roots are the primary

sensing organ of salt and drought stress, then direct application of ABA to the root zone may induce direct root-related changes, thereby by-passing the ABA source from the leaf. Conceivably, this may produce more salt stress-resistant roots and thus, less signal transmission to the leaves. ABA, either in a single or multiple dose application to the root zone, induced pronounced increases in both root water content and dry weight, which may have assisted in stress resistance.

4.5.3 Stem water content

Following multiple ABA application, stem water content was increased in all genotypes except the ABA-normal sibling, in which the percentage of stem water content was elevated but too variable to detect significant differences. Stem water potential was confirmed as a more reliable indicator of water stress than leaf water potential (Garnier and Berger, 1985; Olien and Lakso, 1986; McCutchan and Shackel, 1992; Novello and de Palma, 1997). The stem serves as a reservoir of water during drought stress in several plant species (Larcher, 2003). Similarly, under the influence of elevated ABA levels, the stem may increase its water content capacity to alleviate salt stress.

Except for the ABA-deficient mutant, ABA applied in single doses was generally required in higher concentrations than were multiple doses to elicit responses. However, based on the impaired growth parameters under higher concentrations of ABA (100 μM) in the two salt tolerant genotypes, 'Norland' and 9506, it was concluded that lower levels of ABA are required to maintain plant growth under salt stress. In 'Norland', applications of ABA that produced a final concentration of 100 μM, either through single or multiple doses were ineffective in reducing leaf necrosis, but reduced root and shoot fresh weights and leaf osmotic potential. In addition to the shoot fresh weight, a decrease in shoot dry weight and a change in plant height were also observed in the 9506 genotype under the 100 μM treatment. Therefore, it is possible that high endogenous ABA inhibited plant growth parameters in these two genotypes, possibly because high endogenous ABA causes stress response of plant growth. Indeed, Chen *et al*. (2003) in their study with tomato wild type and *flacca*, have found the inhibitory effect of high levels of endogenous ABA on shoot growth.

An accumulation of ABA was likely under multiple doses. Traditional single dose-response experiments may overestimate the actual ABA response threshold in plants. Exogenous ABA can produce inconsistent results (Freundl *et al*., 1998; 2000). Our study further indicates that the method of ABA application regime in itself can alter plant responses under salt stress.

4.6 Conclusion

The method of ABA application, either as a single dose or as a multiple application, induces marked differences in plant responses to salt stress. Root water content was generally enhanced by multiple applications of ABA, whereas there was no change under a single application of ABA. Single dose appeared to enhance vertical growth, while multiple ABA applications increased lateral growth. Stem water content was consistently increased by ABA treatment. The mechanism of continued water uptake in the presence of NaCl salt may also be different between the two methods of ABA application. The ABA-associated molecular signals/response mechanisms to induce lateral shoot growth under stress and the importance of the stem as a water storage mechanism under salt stress merit further study. While a single application of ABA is to a great extent easier and faster than multiple ABA applications, it may not simulate the actual salt stress responses in the field or in nature.

5.0 Scion and rootstock effects on ABA-mediated plant growth regulation and salt tolerance of acclimated and non-acclimated potato genotypes

5.1 Abstract

Tolerance of salt stress in potato (*Solanum tuberosum* L.) increased when the plants were pre-exposed to low concentrations of salt (salt acclimation). This acclimation was accompanied by increased levels of abscisic acid (ABA) in the shoot. To further study the role of roots and shoots in this acclimation process, reciprocal grafts were made between a salt-tolerant potato line (9506) and salt-sensitive ABA-deficient mutant lines, as well as an ABA-normal sibling potato genotype. The grafted plants were salt acclimated by exposure to 75 or 100 mM NaCl for three weeks and then exposed to 150-180 mM NaCl, depending on the salt tolerance of the rootstock. After two weeks exposure to the salt stress, the acclimated and non-acclimated plants were compared for physiological and morphological parameters. The response to the salt stress was strongly influenced by the rootstock. The salt-tolerant 9506 rootstock increased the salt tolerance of scions of both the ABA-deficient mutant and its ABA-normal sibling. The salt tolerance induced by the rootstock was primarily modulated by salt acclimation and was manifested in the scion as increased plant water content, stem diameter, dry matter accumulation, stomatal conductivity, osmotic potential and an associated alleviation of leaf necrosis. There was also a pronounced scion effect on the rootstock. Using salt-tolerant 9506 as a scion significantly increased root fresh and dry weight and stem diameter as well as root water content of ABA-deficient mutant rootstocks. Specific evidence was found for the role of exogenous ABA in the enhancement of water status in grafted plants under salt stress beyond that of grafting alone. This was verified through NMR imaging, which showed more positive stomatal conductivity and upward water flow in ABA-treated grafted and non-grafted plants and the absence of upward water flow in non-treated grafted plants. Grafting using either salt-tolerant scions or rootstocks with inherently high ABA levels may positively modify subsequent responses of the plant to salt stress.

5.2 Introduction

The progressive natural and anthropogenic salinization of arable lands is a major limiting factor in crop production (Parida and Das, 2005) and agricultural sustainability (Waisel, 2001). Potatoes are the fourth most important food crop globally (CIP, 2007). While halophytic wild types exist in potato species (Shaterian *et al*., 2005b), most cultivated potato genotypes are relatively salt sensitive (Katerji *et al*., 2003) with soil salinity levels as low as 2.3 dS m^{-1} reducing both growth and tuber yield (Katerji *et al*., 2003). The responses of potato cultivars and wild species vary following exposure to elevated levels of chloride and sulphate, which are the most common salts in saline soils (FAO, 2006). However, relatively little has been reported on the mechanisms of salinity tolerance of potato.

While salt shock is uncommon in nature (Maas and Grattan, 1999), most research on salt stress reports on the effects of a short-term salt exposure on non-acclimated plants. The nature of this salt stress may produce very different responses to those observed under real-world conditions (Gusta *et al*., 2005). In nature, plants are typically subjected to a gradual build-up of soil salinity due to fertilizer application for crop growth and/or increase in soil solution concentration as soil water is depleted (Eilers *et al*., 1995).

Adapted plants have an inherent level of salinity resistance and that level of resistance can significantly increase through a process of acclimation (Amzallag and Lerner, 1995). Acclimation results from pre-exposure to low, non-lethal levels of salt stress (Strognov, 1964; Matthews and Boyer, 1984; Conroy *et al*., 1988; Guy, 1990). Acclimated plants will grow at salt concentrations that are lethal to non-acclimated plants (Amzallag *et al*., 1990b). For acclimation to occur, the cell, organ and organism must be in a proper or receptive physiological state (Amzallag and Lerner, 1995). The capacity for acclimation varies considerably among plant species (Baker *et al*., 1986) and may also vary among genotypes of the same species (Durrant, 1981; Amzallag *et al*., 1993; Azevedo Neto *et al*., 2004). Virtually all plant species can acclimate to salt stress if the stress is imposed gradually (Amzallag *et al*., 1990b; Hasegawa *et al*., 1994). Enhanced salt tolerance following NaCl pre-treatment was observed in bell pepper (*Capsicum annuum* L.) (Bethke and Drew, 1992), jojoba (*Simmondsia chinensis*) (Ben Raïs *et al*., 1993), maize seedlings (*Zea mays*) (Rodríguez *et al*., 1997), rice (*Oryza sativa*) (Hassanein, 2000; Djanaguiraman *et al*., 2006), soybean (*Glycine max*) (Umezawa *et al*., 2000) and cowpea (*Vigna unguiculata*) (Silveira *et al*., 1999; 2001).

ABA is a plant hormone involved in coordinating the growth of roots and shoots of plants (Sharpe and LeNoble, 2002) and regulating adaptation responses to a number of stresses including water and salt (Thomas and Eamus, 1999; Gómez-Cadenas *et al*., 2003; Shaterian *et al*., 2005a). For example, the negative effect of NaCl salt on root nodule dry weight of common bean was alleviated by exogenous ABA (Khadri *et al*., 2006; 2007). ABA contributed to the increase of xylem water potential as well as water uptake to the plant in the presence of salt (Fricke *et al*., 2004). Simultaneous exposure of plants to salinity and ABA treatment resulted in stimulation of shoot growth at all ABA concentrations compared to plants exposed only to salinity (Cachorro *et al*., 1995). Furthermore, overproduction of ABA is associated with increased transpiration efficiency and root hydraulic conductivity, and increased leaf expansion (Thompson *et al*., 2007). Abscisic acid accelerated salt acclimation (Amzallag *et al*., 1990a), while cytokinin (CK) and gibberellic acid (GA) interfered with this process (Amzallag *et al*., 1992). Shaterian *et al*. (2005b) showed the importance of ABA in salt stress resistance in potato. Exposure to exogenous ABA in the absence of stress can induce acclimation to various stresses (salt, water, cold) (Amzallag and Lerner, 1995). Following a stress event, ABA content increased within a few minutes to several hours, depending upon the type and severity of the stress (Cramer and Quarrie, 2002; Jia *et al*., 2002; Liu *et al*., 2003; Fricke *et al*., 2004; 2006).

Several practices in agricultural systems are directed toward overcoming salinity. Selection for salt tolerance has shown merit in some potato-breeding programs (Elkhatib *et al.,* 2004; Shaterian *et al*., 2005b). Gene transformation has recently been used in developing salt-tolerant potato genotypes (Hmida-Sayari *et al*., 2005a; Behnam *et al*., 2006; Teixeira *et al*., 2006). Salt-tolerant transgenic potato lines that were introduced through genetic engineering expressed substantially more of the DREB1A transgenes than non-transgenic lines (Behnam *et al*., 2006). Desirable properties of the root of salt-resistant rootstocks can be supplied to the shoot of salt-sensitive scions through transcription of genes in the root, the products of which go onto act in the shoot (Pardo *et al*. 1998).

Grafting may represent a means of introducing desirable qualities without the controversy associated with genetic engineering. The use of grafted vegetables has increased in the past few years (Lee and Oda, 2003), particularly in tomato plants subject to salinity (Fernández-García *et al*., 2002; Santa-Cruz *et al*., 2002; Chen *et al*., 2003a).

Grafting represents a useful tool for determining the relative roles of rootstocks versus scions in physiological processes such as the response to salt stress. When grafting was used to assess the role of roots and shoots in regulating salt tolerance in chickpea (*Cicer arietinum*), the scions of a sensitive genotype grafted onto a salt-tolerant rootstock died after exposure to salt stress, while tolerant scions grafted onto sensitive rootstocks remained tolerant of salinity (Dua, 1997). This suggests that the scion plays a vital role in salt tolerance of grafted plants. In soybean, both the shoot and the root have been reported to affect salt tolerance. Velagaleti *et al*. (1990) suggest that the root is dominant in determining salinity tolerance of soybean. The importance of the root system in regulation of salt stress tolerance was also documented in salt-sensitive and -tolerant potato genotypes (Shaterian *et al*., 2005a). By contrast, Abd-Alla *et al*. (1998) concluded that shoot factors were of primary importance in determining salt tolerance of soybean. Schmutz and Lüdders (1999) showed that the scions also play a role in salt tolerance of grafted mango plants. In tomato genotypes, rootstocks generally regulated accumulation of salt ions into the leaves (Santa-Cruz *et al*., 2001; 2002), but properties of the rootstock that were important in inducing salt tolerance of the shoot were also dependent on the shoot genotypes (Santa-Cruz *et al*., 2002). Scion genotypes played an important role in the growth of grafted tomato plants, regardless of the salinity of the growth media, whereas rootstock had little influence (Chen *et al*., 2003a).

ABA-deficient mutants have been found in a number of plant species including tomato (Tal, 1966; Tal and Nevo, 1973; Neill and Horgan, 1985; Borsani *et al*., 2002), potato (Quarrie 1982; De Jong *et al*., 2001), pea (Wang *et al*., 1984; Kof *et al*., 2006), *Arabidopsis thaliana* (Koorneef *et al*., 1982; Umezawa *et al*., 2006), *Helianthus annuus* (Fambrini *et al*., 1995; 2004), viviparous corn mutants (Smith *et al*., 1978; Robichaud *et al*., 1980; Suzuki *et al*., 2006) and wheat (Holappa *et al*., 2005). Reciprocal grafting of ABA-deficient mutants is a useful method for studying the functions of ABA (Chen *et al*., 2002b). Grafting studies with tomato identified a factor in the root of the ABA-deficient mutant (*flacca*) that was involved in opening the stomata of the leaves of the ABA normal scions (Tal, 1967). This study also indicated that the shoot genotype was dominant in determining stomatal aperture (closing/opening), although grafting ABA-deficient scions onto ABA-normal rootstock could cause a slight decrease in the stomatal conductance of ABA-deficient scions (Jones *et al*., 1987). While ABA-mutant scions grafted onto ABA-normal rootstocks reverted to a relatively normal phenotype, the ABA-

normal scion maintained its phenotypic characteristic when grafted onto a mutant rootstock (Cornish and Zeevaart, 1988). Shaterian *et al*. (2005b) showed that ABA-normal rootstocks were important in inducing calreticulin mRNA in the leaves of potato exposed to salt stress.

Nuclear Magnetic Resonance microscopy (NMRM) is a unique and promising tool in plant science (Ishida *et al*., 2000; Köckenberger *et al*., 2004). Holbrook *et al*. (2001) were able to use NMRM to monitor the water status of individual xylem vessels of plants such as grape (*Vitis vinifera*). Peuke *et al*. (2001) took simultaneous measurements of water flow velocity in xylem and phloem, monitored CO_2 and H_2O concentrations in castor bean seedlings by using the FLASH imaging capabilities of NMRM. The location of xylem and phloem in the stem, the total amount of water, the amount of stationary and flowing water, the linear velocity of the flowing water and the volume flow in cucumber were examined by NMR flow-imaging experiments (Scheenen *et al*., 2002). Water movement in inner and outer xylem in the shoot and leaf were also studied using various NMR spectroscopy and imaging techniques (Schneider *et al*., 2003). Velikanov and Belova (2005) examined the effect of ABA using NMR imaging. They showed that exogenous ABA affects water permeability of the vacuolar symplast in the root cells of maize seedlings by increasing water permeability of the tonoplast.

In spite of the global importance of potatoes in developing and developed countries, investigations into ABA and salt stress responses in grafted potato have not been widely performed. Thus, the objective of this study was to examine scion and rootstock effects on ABA-mediated salt tolerance responses of acclimated and non-acclimated potato genotypes.

5.3 Materials and Methods

5.3.1 Genotypes

Three diploid potato lines were obtained from Agriculture and Agri-Food Canada, Fredericton (Dr. H. De Jong). Line 9506 (*S. chacoense, X S. microdontum* X *S. tuberosum* X S. *Phureja*) is salt stress resistant (Shaterian *et al*., 2005b; Chapter 3). Line 9120-05 is an ABA-deficient mutant [*Solanum tuberosum* L. group *Phureja* (*phu*)], containing the (*dr*) mutant gene (Simmonds, 1965), and is salt sensitive (Shaterian *et al*., 2005b; Chapter 3). Line 9120-18 (*Drdr*) is the ABA-normal sibling of 9120-05 and is moderately sensitive to salt (Shaterian *et*

al., 2005b; Chapter 3). The commercially produced tetraploid potato 'Norland' was included for comparison purposes for the endogenous ABA experiment.

5.3.2 Grafting

Shoot tip cuttings of the various potato genotypes were rooted in Ottawa sand (75.5% 1 - 2 mm diameter, very coarse sand; 24.4% 0.5 - 1 mm, course sand; and less than 0.1%, less than 0.5 mm). Ottawa sand has minimal ion-binding capacity, which reduces interference by ion absorption in salinity or fertility trials. After five weeks, the rooted cuttings were transferred to 400-mL pots filled with Ottawa sand. The seedlings were grown in a greenhouse with 25/20^{o}C day/night temperature, 60-85% relative humidity and 600-800 $\mu Ms^{-1}m^{-2}$ light intensity (PAR) for 14-16 hours (combination of natural light and artificial light provided by high-pressure sodium halogen lamps).

Grafting occurred when the stem attained a length of 15-20 cm. Attempts to graft at other stages of growth were unsuccessful. Scions of the ABA-deficient mutant and its ABA-normal sibling were grafted onto 9506 resistant rootstocks [ABA(-)/9506] and [ABA(+)/9506]. Reciprocally, 9506 scions were grafted onto rootstocks of the ABA-deficient mutant or its ABA-normal sibling [9506/ABA(-)] and [9506/ABA(+)]. A cleft graft was used in all cases. The graft unions of the autografts (ABA-/ABA-) of the ABA-deficient mutant and the ABA-normal sibling broke a few days after grafting. Other grafting studies on the same plants using autografted material found significant rootstock effects independent of an autografting response (Shaterian *et al*., 2005a). Therefore, non-grafted plants were used as comparative controls. The scion and rootstock were held together and protected from desiccation by wrapping with a paraffin-embedded plastic film (Parafilm, American National Can Menasha). Newly grafted plants were held in a mist chamber for two weeks. The plants were irrigated with water containing 1.27 g L^{-1} 20-20-20 N-P- K, plus micronutrients (Plant Products Co. Ltd., 314 Orinda Road, Brampton, Ontario, Canada) three times a day. The percentage of micro- and macro-nutrients of 20-20-20 N-P-K fertilizer are mentioned in detail in the appendix I. To prevent foliar diseases, the plants were sprayed with fungicide three times per week.

After six weeks, the grafted plants were transferred to 1.5-L pots filled with Ottawa sand. Four pots were used for each scion/rootstock combination. One pot of each scion/rootstock

combination was placed in to a tray (20 x 40 x 60 cm). Three to five times each day, each tray was flooded for 5 minutes with a fertilizer solution (1.27 g L^{-1} [20-20-20 N-P-K, plus micro-nutrients], Plant Products Co. Ltd., 314 Orinda Road, Brampton, Ontario, Canada), augmented with differing amounts of NaCl (see below). The EC and pH of the nutrient solution were checked weekly. The EC and pH of the 0-180 mM NaCl treatment solutions ranged between 1.22-22.16 dS m^{-1} and 6.52-6.96, respectively (Appendix II). Sprouts developing from nodes on the rootstocks were continuously removed to maintain just the grafted scion.

5.3.2.1 Experiment 1- Salt acclimation treatment

To determine the effects of salt acclimation on subsequent salt stress tolerance, graft combinations (scion/rootstock) were pre-treated with low concentrations of NaCl salt for three weeks. Based on previous studies of the salt tolerance of the rootstocks, the salt acclimation treatments were as follows:

ABA-deficient mutant, ABA-normal sibling, 9506/ABA(-), 9506/ABA(+) = 25 mM in the first week, 50 mM in the second week and 75 mM in the third week.

ABA(-)/9506, ABA(+)/9506, 9506 = 33.3 mM in the first week, 66.6 mM in the second week and 100 mM in the third week.

Non-acclimated plants received fertilizer nutrient solution without NaCl salt during the acclimation period. After three weeks of salt acclimation, all acclimated and non-acclimated plants were exposed to two weeks of salt stress. On the basis of the salt tolerance of the rootstock, the NaCl salt concentrations used in the salt stress regimes were:

ABA-deficient mutant, ABA-normal sibling, 9506/ABA(-), 9506/ABA(+) = 150 mM
ABA(-)/9506, ABA(+)/9506, 9506 = 180 mM

Physiological parameters evaluated after two weeks exposure to salt stress were: leaf injury, change in plant height, stem diameter, shoot and root fresh and dry weights, degree of leaf greenness (chlorophyll content), leaf water content, leaf osmotic potential and shoot and root water contents. Leaf injury was ranked from 1-5: 1 - (0% leaf area necrosis), 2 - (1-25% leaf area necrosis), 3 - (26-50% leaf area necrosis), 4 - (51-75% leaf area necrosis) and 5 - (76-100% leaf area necrosis) (Shaterian *et al*., 2005b). Leaf injury ratings were performed on the

most recently fully expanded and the lowest non-wilted leaves. Plant height (stem collar to shoot tip, apex) was monitored on a weekly basis over the two weeks of salt stress to allow calculation of the change in plant height:

$$\text{Change in plant height} = \frac{\text{height at T2 - height at T1}}{\text{T2 - T1}}$$

T1 = before stress

T2 = after stress

To determine leaf water content and leaf osmotic potential at the end of salt tress event, tissue samples were taken from the fourth and fifth fully expanded leaves between 10 a.m. and 12 p.m. Osmolality of the sap was quantified by a Wescor vapour pressure osmometer (Wescor Model 5500, U.S.A) from samples taken after two-week salt stress, obtained by crushing frozen leaf tissue with a hand press. Osmotic potentials were calculated by the formula $\Psi s = - CiRT$. Ψs = osmotic potential, C = concentration of the solution (moles of solute per kg H_2O), i = a constant that accounts for the ionization of a solute, R = the universal gas constant (0.00831 kg. MPa $mole^{-1}$ K^{-1}), T = absolute temperature (K) = degree C + 273.

A SPAD Meter (Model Minolta-502) was used to measure the degree of leaf greenness (approximates chlorophyll content) on the same leaves used for the necrosis evaluations. Duplicate readings were performed at two positions. Stem diameter was measured using Electronic Digital Calipers (Model SCM DIGV-6) at 5 cm below and above the graft union. Stomatal conductivity was measured using a Steady State Porometer (Li-Cor Inc. Li 1600) between 9 a.m. and 11 a.m. after two weeks of salt stress. Root and shoot fresh and dry weights and water content were also determined. The number of samples for each treatment in all parameters mentioned above was six.

The 7 graft combinations x 2 salt acclimation factorial of treatments was arranged in a Randomized Complete Block Design with three replicates and two plants per replicate, with one plant per pot. Fisher's protected LSD ($P = 0.05$) was used for mean comparisons. The ANOVA tables of all parameters are presented in Appendix III.

5.3.2.2 Experiment 2- ABA application

To test the effect of ABA on salt stress tolerance of grafted potato genotypes, the plants were supplied with exogenous ABA before the salt stress treatment. A racemic mixture of (+/-) ABA (Sigma Chemical Co. Inc., St. Louis, MO, USA) was applied at the pre-stolon initiation stage (five weeks of growth of the grafted cuttings) in all genotypes. ABA was applied at increasing concentrations every three days to reach a final concentration of 75 µM. To reach this final concentration ABA was increased by 15 µM every three days (i.e; 15, 30, 45, 60, 70, by day 12). The (+/-) ABA was dissolved in a small amount of NaOH (1N) diluted to the desired concentration with distilled water. The pH of the ABA solution was adjusted to 6.5 by adding HCl or NaOH. An aliquot of 150 ml of the ABA solution was applied to the plants as a root drench between 9:00 and 10.00 a.m. Control plants were watered with distilled water and NaOH (1N) with the pH adjusted to 6.5. Since solutions were circulated back to individual tanks, the water of the tanks was changed after two days to prevent the accumulation of ABA. The salt stress treatments started one week after the final ABA application and continued for two weeks. The salt stress treatment consisted of 150 mM NaCl for the ABA-deficient mutant and its normal sibling and 180 mM NaCl for 'Norland' and the 9506 line. The salt stress was applied as above. Treatments were arranged as a Factorial experiment with a Randomized Complete Block Design (7 genotypes x 2 ABA treatments) with 4 replications per treatment and three plants per replication. In this experiment, leaf injury, stem diameter, leaf water content, leaf osmotic potential and leaf stomatal conductivity were parameters that were monitored with the same procedure as was used in the previous experiment.

5.3.3 NMR imaging

The salt stress sensitive (ABA-deficient mutant) and the salt stress resistant (9506) genotype and their graft combinations [9506/ABA(-)] and [ABA(-)/9506] were used to examine the rate and direction of water flow in the stem. All plants were imaged 7 days after ABA application and again after two weeks of salt stress. NMR imaging of ABA treated and non-treated shoots was performed with a Siemens Magnetum Symphony 1.5 Tesla, whole-body medical scanner (Erlangen Germany). NMR imaging illustrated water distribution and transport in the shoots of plants grown under normal and salt stress conditions. NMR imaging

of water distribution was measured in intact potato plants. All imaging was done using a Siemens 1.5 Tesla head coil.

NMR sequences were acquired in a T1 (spin-spin relaxation) sequence in a transverse plane. Second a turbo spin echo T2 weighted (spin-lattice relaxation) sequence was acquired in a longitudinal plane. This acquired data was then post-processed into a NMR Image (Maximum Intensity Pixel). For T2 sequence: TR (repetition time) = 4000 ms, Te (echo time) = 95 ms, Ta (acquisition time) = 5' 25", Slice thickness = 2.0 mm, Field of view = 64 X 64 mm, The image matrix = 189 X 256.

For the flow sensitive sequence: Flow quantification parameter (3dfisp, 3 dimension, fast imaging steady state precision): TR = 32 ms, Te = 7 s, Ta = 6.34 s, Field of view = 260 X 260, The image matrix = 256 X 512. Temperature Temperatures 20 ^{o}C.

NMR physics –In the magnetic environment the hydrogen proton (water) is aligned north and south in the magnet. The application of radio frequency energy (RF) energy to the hydrogen proton causes a temporary loss in its N-S orientation. The realigning and aligning of the hydrogen proton causes the emittance of RF energy. This RF pulse is collected by a RF coil. This acquired data was then post-processed into a MIP (Maximum Intensity Pixel) image.

Pooled analysis of variance (ANOVA) for factorial RCBD was performed by the general linear model (GLM) in SAS and means of lines were compared using LSD. The ANOVA tables of all parameters are also presented in Appendix III.

5.4 Results

5.4.1 Leaf necrosis and chlorophyll content

After salt stress both the non-acclimated and the acclimated plants of the resistant 9506 genotype showed less leaf necrosis than the ABA-deficient mutant (Fig. 5.1). When acclimated, the 9506 genotype showed less leaf necrosis compared to the ABA-deficient mutant and ABA-normal sibling types after salt stress. However, the impact of the 9506 as a rootstock, independent of acclimation, was more significant on inducing salt tolerance of the scions. When 9506 was used as a rootstock for relatively more salt-sensitive scions: the ABA-deficient mutant and the ABA-normal sibling (ABA(-)/9506 and ABA(+)/9506 graft combinations) leaf necrosis

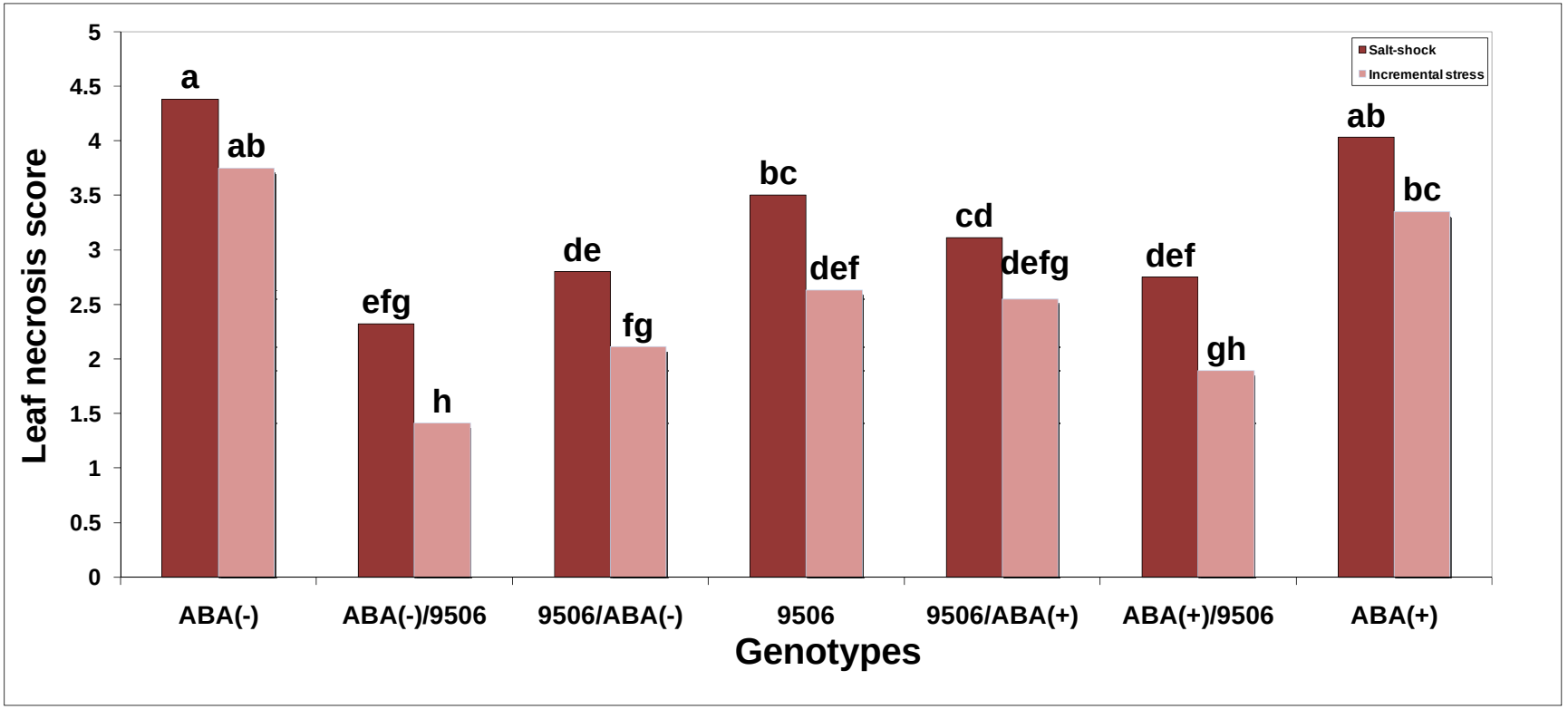

Figure 5.1 Influence of salt acclimation (75-100 mM NaCl) on leaf necrosis scores of potato genotypes and their graft combinations (scion/rootstock) measured after 2 weeks of salt stress (150-180 mM NaCl). Leaf necrosis score, 1-5: 1- (0% leaf area necrosis), 2- (1-25% leaf area necrosis), 3- (26-50% leaf area necrosis), 4- (51-75% leaf area necrosis), 5- (76-100% leaf area necrosis). Means with the same letters are not significantly different (LSD *P = 0.05*). 9506 = Salt stress resistant, ABA(-) = ABA-deficient mutant, ABA(+) = ABA normal sibling.

was reduced (Fig. 5.1). Salt acclimation also reduced the proportion of shoots with injured leaves in these graft combinations. In addition, salt acclimation decreased leaf necrosis in resistant 9506 scions in the reciprocal 9506/ABA(+) and 9506/ABA(-) graft combinations to the same extent as in the 9506 plants alone.

ABA application significantly reduced leaf necrosis after the salt stress treatment in all genotypes except in the already-resistant 9506 line (Fig. 5.2). Grafting the ABA-deficient mutant onto the resistant 9506 rootstock in combination with exogenous ABA application decreased leaf necrosis beyond the effect seen with grafting alone. This ABA response was not observed for the ABA(+)/9506 grafting combination.

Grafting onto the salt-resistant 9506 rootstock also increased greenness (chlorophyll content) of scions of the ABA-deficient mutant after exposure to salt stress (Fig. 5.3). Salt acclimation improved this parameter in non-grafted plants of the ABA-deficient mutant.

5.4.2 Plant biomass

In both non-acclimated and acclimated treatments, using resistant 9506 as a scion increased root fresh and dry weights of the ABA-deficient mutant rootstock compared to non-grafted ABA-deficient mutants (Table 5.1). Using the ABA-deficient mutant as a scion significantly reduced the root fresh and dry weights of the salt stress resistant 9506 rootstocks in both the salt-acclimated and non-acclimated treatments. Using the ABA-normal sibling as a scion increased root dry weight of the resistant 9506 rootstock. While the salt acclimation treatment had no effect on root fresh weights of the ABA-deficient mutant, ABA(-)/9506 and 9506 resistant genotypes compared to the salt shock treatment, the salt acclimation treatment reduced root fresh weights in the other genotypes and graft combinations tested. The highest root weights were observed in the 9506 genotype and the ABA(+)/9506 graft combination under both salt acclimated and non-acclimated conditions.

Grafting salt-sensitive scions onto the salt-tolerant 9506 rootstock resulted in increased shoot fresh and dry weights of the ABA(-)/9506 and ABA(+)/9506 graft combinations relative to non-grafted plants (Table 5.2). Correspondingly, reciprocal grafts of 9506/ABA(-) and 9506/ABA(+) reduced shoot fresh weight of the resistant 9506 scions. The salt acclimation treatments reduced shoot fresh weight in all genotypes and graft combinations tested, except in

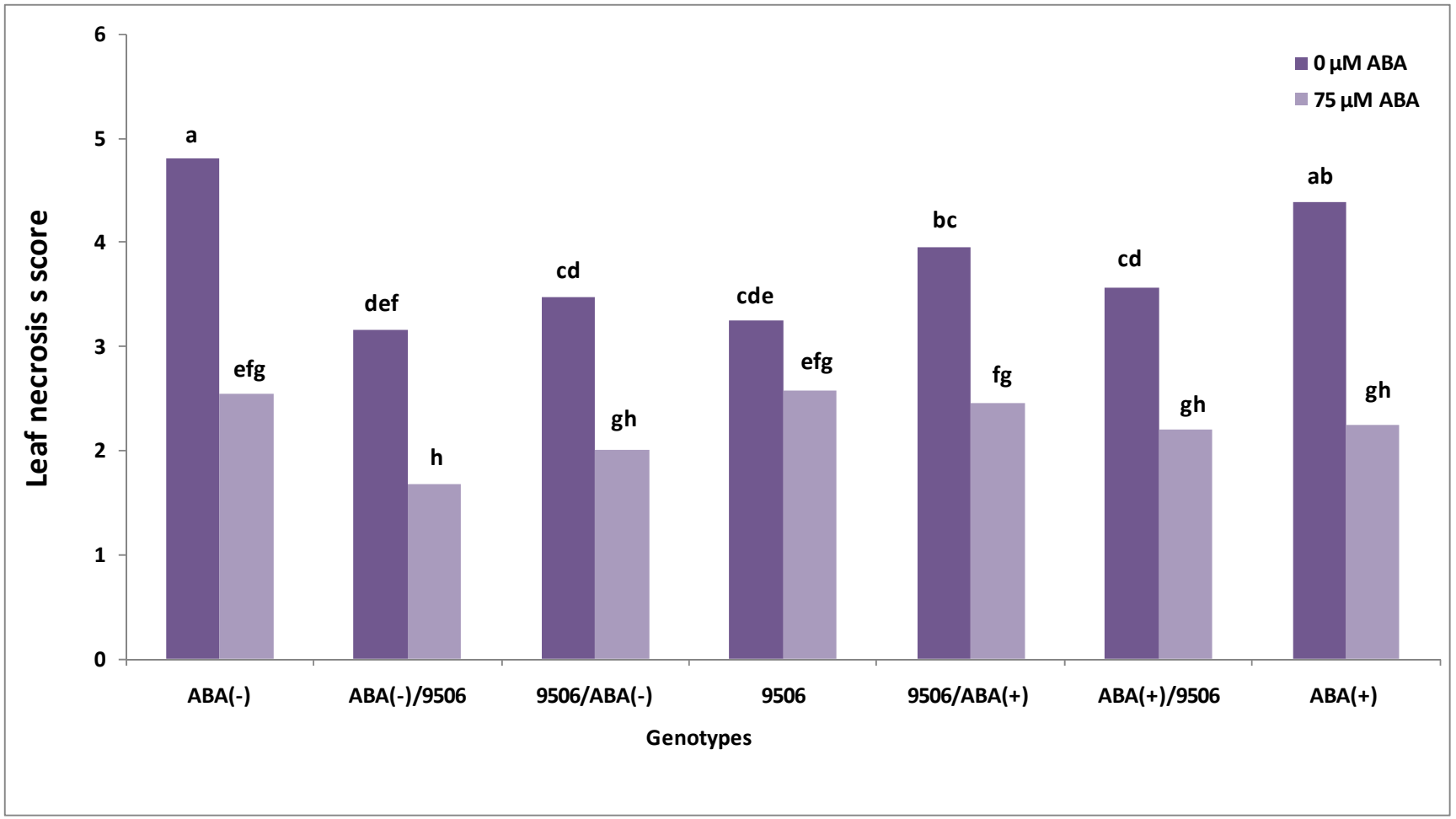

Figure 5.2 Influence of ABA application on leaf necrosis scores of potato genotypes and their graft combinations (scion/rootstock) measured after 2 weeks of salt stress (150-180 mM NaCl). Leaf necrosis score, 1-5: 1- (0% leaf area necrosis), 2- (1-25% leaf area necrosis), 3- (26-50% leaf area necrosis), 4- (51-75% leaf area necrosis), 5- (76-100% leaf area necrosis). Means with the same letters are not significantly different (LSD *P = 0.05*). 9506 = Salt stress resistant, ABA(-) = ABA-deficient mutant, ABA(+) = ABA-normal sibling.

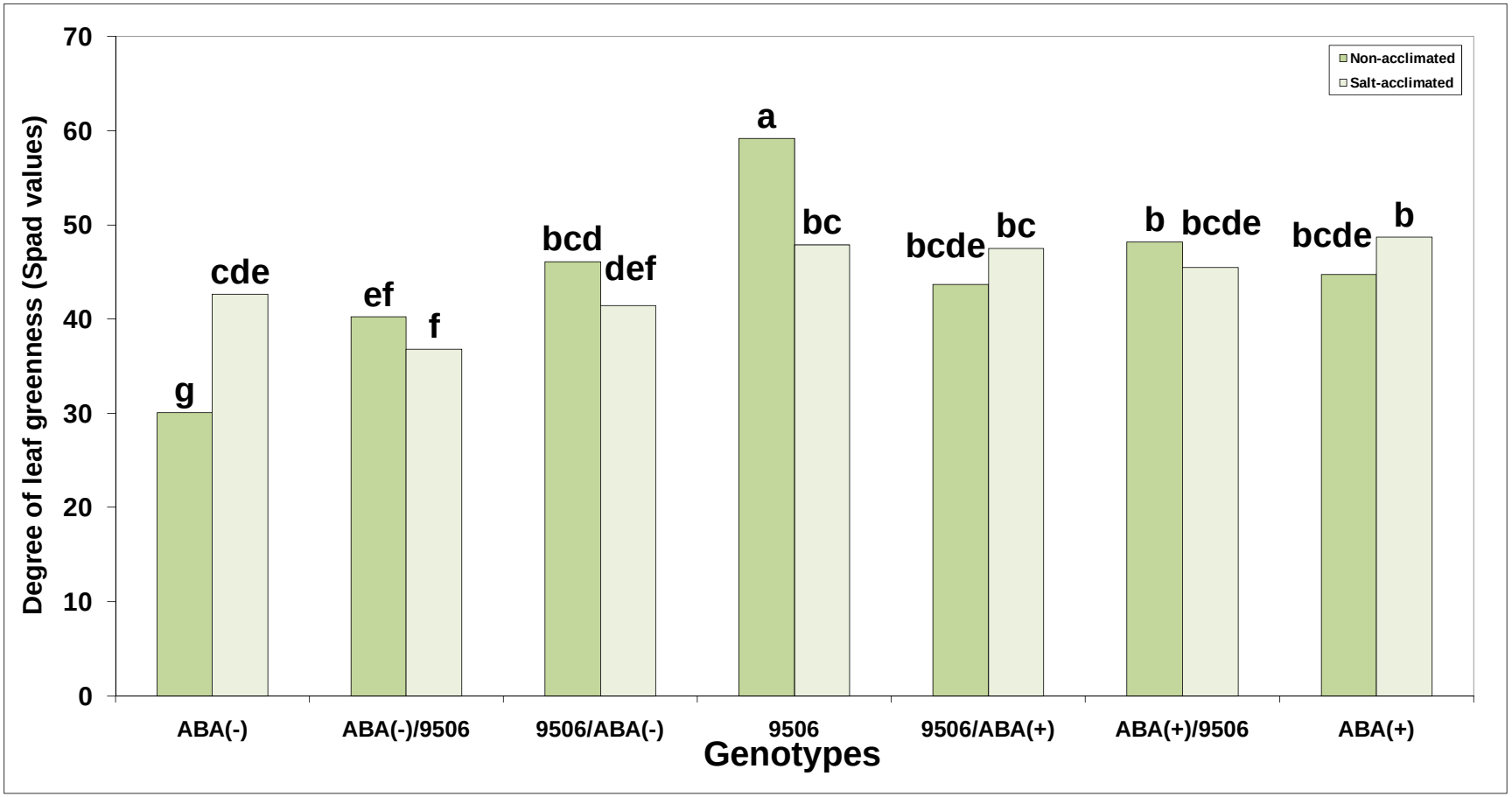

Figure 5.3 Influence of salt acclimation (75-100 mM NaCl) on degree of leaf greenness (SPAD values) of potato genotypes and their graft combinations (scion/rootstock) measured after 2 weeks of salt stress (150-180 mM NaCl). Means with the same letters are not significantly different (LSD P = 0.05). 9506 = Salt stress resistant, ABA(-) = ABA-deficient mutant, ABA(+) = ABA normal sibling.

Table 5.1. Mean root fresh and dry weights and water content of salt acclimated (75-100 mM NaCl) and non-acclimated potato genotypes and their graft combinations (scion/rootstock) measured after 2 weeks of salt stress (150-180 mM NaCl). Means with the same letters are not significantly different (LSD $P = 0.05$). 9506 = Salt stress resistant, ABA(-) = ABA-deficient mutant, ABA(+) = ABA normal sibling.

Responses	**Treatments**	**ABA(-)**	**ABA(-)/9506**	**9506/ABA(-)**	**9506**	**9506/ABA(+)**	**ABA(+)/9506**	**ABA(+)**
Root fresh weight	Non-Acclimated	1.11 f	5.66 e	10.87 bc	14.07 a	9.76 c	14.53 a	8.65 cd
(g)	Acclimated	0.90 f	4.44 e	6.67 de	12.40 ab	5.66 e	10.75 bc	5.51 e
Root dry weight	Non-Acclimated	0.22 g	0.93 f	1.87 d	2.59 c	1.62 de	4.74 a	1.42 e
(g)	Acclimated	0.21 g	0.79 f	0.69 f	1.97 d	0.90 f	3.12 b	0.84 f
Root water content	Non-Acclimated	80.00 b	83.10 ab	82.12 ab	81.40 b	83.21 ab	83.44 ab	67.24 c
(%)	Acclimated	70.88 c	81.44 b	89.43 a	83.76 ab	83.27 ab	84.58 ab	70.49 c

Table 5.2. Mean shoot fresh and dry weights and water content of salt acclimated (75-100 mM NaCl) and non-acclimated potato genotypes and their graft combinations (scion/rootstock) measured after 2 weeks of salt stress (150-180 mM NaCl). Means with the same letters are not significantly different (LSD $P = 0.05$). 9506 = Salt stress resistant, ABA(-) = ABA-deficient mutant, ABA(+) = ABA normal sibling.

Responses	**Treatments**	**ABA(-)**	**ABA(-)/9506**	**9506/ABA(-)**	**9506**	**9506/ABA(+)**	**ABA(+)/9506**	**ABA(+)**
Shoot fresh weight	Non-Acclimated	18.76 i	42.83 h	109.70 d	177.75 a	123.08 c	136.12 b	91.53 e
(g)	Acclimated	16.06 i	44.96 h	80.32 f	124.59 c	60.61 g	141.75 b	57.08 g
Shoot dry weight	Non-Acclimated	3.53 e	17.58 c	18.28 c	28.02 b	17.61 c	38.73 a	15.80 c
(g)	Acclimated	3.02 e	11.29 d	10.79 d	19.06 c	11.69 d	28.96 b	9.76 d
Shoot water content	Non-Acclimated	59.26 f	81.18 bcd	83.25 abcd	84.22 abcd	85.70 ab	82.42 abcd	71.53 e
(%)	Acclimated	74.20 e	81.26 bcd	86.55 a	84.70 abc	80.87 cd	82.92 abcd	79.51 d

the ABA(-), ABA(-)/9506 and the ABA(+)/9506 combination in which there was no change (Table 5.2).

5.4.3 Stem diameter

Grafting had a significant impact on stem diameter under salt stress (Table 5.3). The stem diameter of ABA-deficient mutant scions grafted onto resistant 9506 rootstocks increased relative to non-grafted plants in both the non-acclimated and salt acclimation treatments (Table 5.3A). This grafting response was only observed following salt acclimation when scions from the ABA-normal sibling were grafted on 9506 rootstocks. The diameter of the 9506 resistant scions did not change except when grafted onto the ABA-normal sibling rootstock, in which case the diameter of 9506 scions was significantly reduced under non-acclimated conditions. The 9506 rootstock stem diameter was not affected by grafting with scions of the ABA-normal sibling; however, the ABA-deficient mutant scion reduced 9506 rootstock diameter under both non-acclimated and acclimated conditions (Table 5.3).

Application of ABA generally had no significant effect on stem diameter of any of the genotypes or graft combinations (Table 5.4).

5.4.4 Water status

When the resistant 9506 genotype was used as a scion for grafting onto rootstocks of either the ABA-deficient mutant or ABA-normal sibling, root water content significantly increased under salt acclimation (Table 5.1). Salt acclimation alone increased shoot water content of the ABA-deficient mutant and ABA-normal sibling after salt stress (Table 5.2). In both the non-acclimated and salt acclimation treatments, the resistant 9506 rootstock increased shoot water content in the ABA(-)/9506 graft combination compared to the non-grafted ABA-deficient mutants (Table 5.2). In the ABA(+)/9506 graft combination, this 9506 rootstock effect was observed only under non-acclimated conditions.

Salt acclimation also significantly increased stem water content of both the ABA-deficient mutant and ABA-normal sibling (Table 5.3). Even in the absence of acclimation, using 9506 as a rootstock increased stem water content in scions of the ABA-deficient mutant and ABA-normal sibling compared to non-grafted plants (Table 5.5). ABA application significantly increased stem water content of the scion and rootstocks of the ABA-deficient mutant and

Table 5.3. Mean stem diameters above and below the graft union of salt acclimated (75-100 mM NaCl) and non-acclimated potato genotypes and their graft combinations (scion/rootstock) measured after 2 weeks of salt stress (150-180 mM NaCl). Means with the same letters are not significantly different (LSD $P = 0.05$). 9506 = Salt stress resistant, ABA(-) = ABA-deficient mutant, ABA(+) = ABA normal sibling.

Stem diameter	**Treatments**	**ABA(-)**	**ABA(-)/9506**	**9506/ABA(-)**	**9506**	**9506/ABA(+)**	**ABA(+)/9506**	**ABA(+)**
A) Above graft union	Non-Acclimated	1.75 e	4.16 d	5.83 abc	6.83 ab	5.08 cd	5.33 bcd	5.16 cd
(mm)	Acclimated	1.33 e	4.16 d	5.83 abc	6.00 abc	5.00 cd	7.16 a	4.00 d
B) Below graft union	Non-Acclimated	1.66 g	4.00 def	4.00 def	5.16 abc	3.50 ef	5.83 a	4.66 bcd
(mm)	Acclimated	1.08 g	3.33 f	3.83 def	5.33 ab	4.33 cde	5.50 ab	4.33 cde

Table 5.4. Mean stem diameters (mm) above (a) and below (b) the graft union, and mean stem water content (%) above (c) and below (d) the graft union of ABA treated (75µM) and non-treated (0 µM) potato genotypes after 2 weeks of salt stress (150-180 mM NaCl). Means with the same letters are not significantly different (LSD P = 0.05). 9506 = Salt stress resistant, ABA(-) = ABA-deficient mutant, ABA(+) = ABA normal sibling.

Responses	**Location**	**Treatments**	**ABA(-)**	**ABA (-)/9506**	**9506 /ABA(-)**	**9506**	**9506/ABA(+)**	**ABA(+)/9506**	**ABA(+)**
Stem diameter	a) Above graft union	ABA(-)	3.71 e	4.75 de	6.85 ab	6.74 ab	6.07 abc	6.89 ab	5.26 cd
		ABA(+)	4.10 e	4.68 de	7.14 a	6.71 ab	6.15 abc	6.87 ab	5.79 bcd
	b) Below graft union	ABA(-)	3.45 e	6.86 a	6.38 abc	6.54 ab	6.34 abcd	6.97 a	5.25 d
		ABA(+)	3.90 e	5.32 cd	6.52 ab	6.55 ab	6.59 ab	6.77 a	5.63 bcd
Stem water content	c) Above graft union	ABA(-)	66.43 c	72.60 bc	84.38 a	80.71 ab	83.61 a	65.44 c	77.89 ab
		ABA(+)	78.38 ab	83.99 a	85.47 a	84.36 a	85.36 a	83.98 a	83.64 a
	d) Below graft union	ABA(-)	52.32 c	77.23 ab	79.15 ab	82.51 a	81.91 a	69.68 b	83.04 a
		ABA(+)	80.25 ab	83.16 a	81.15 a	85.17 a	85.07 a	85.80 a	85.31 a

Table 5.5. Mean leaf water content, leaf osmotic potential, stem water content and leaf stomatal conductivity of salt acclimated (75-100 mM NaCl) and non-acclimated potato genotypes and their graft combinations (scion/rootstock) measured after 2 weeks of salt stress (150-180 mM NaCl). Means with the same letters are not significantly different (LSD $P = 0.05$). 9506 = Salt stress resistant, ABA(-) = ABA-deficient mutantABA(+) = ABA normal sibling.

Responses	**Treatments**	**ABA(-)**	**ABA(-)/9506**	**9506/ABA(-)**	**9506**	**9506/ABA(+)**	**ABA(+)/9506**	**ABA(+)**
Leaf water content	Non-Acclimated	75.13 cd	81.75 ab	78.77 abc	83.26 ab	81.57 abc	79.72 abc	83.63 a
(%)	Acclimated	70.36 d	77.51 abc	77.59 abc	82.86 ab	78.31 abc	76.83 bcd	80.25 abc
Leaf osmotic potential	Non-Acclimated	-2.06 f	-2.38 g	-1.29 abc	-1.36 abc	-1.51 cd	-1.63 de	-1.74 e
(Ψs = MPa)	Acclimated	-1.81 e	-1.48 bcd	-1.28 ab	-1.27 ab	-1.41 abcd	-1.31 abc	-1.22 a
Stem water content	Non-Acclimated	56.92 e	70.11 d	76.54 abcd	76.96 abc	76.26 abcd	79.81 ab	61.35 e
(%)	Acclimated	71.59 cd	70.81 cd	73.66 bcd	76.46 abcd	76.23 abcd	78.44 ab	81.23 a
Leaf stomatal conductivity	Non-Acclimated	0.011 ef	0.010 f	0.115 b	0.115 b	0.118 b	0.021 e	0.018 ef
($Cm\ s^{-1}$)	Acclimated	0.015 ef	0.058 c	0.135 a	0.130 a	0.125 ab	0.051 c	0.035 d

ABA(+)/9506 graft combination and the scion of ABA(-)/9506 (Table 5.4). After ABA application, the stem water content became similar in all genotypes and graft combinations under salt stress conditions.

Using 9506 as a rootstock significantly increased leaf water content of the ABA-deficient mutant scion under both acclimation as well as non-acclimated treatments (Table 5.5). Exogenous ABA application did not influence leaf water content in the absence of stress (Table 5.6). However, after stress, leaf water content was significantly increased in ABA-treated plants of the ABA-deficient mutant, its ABA-normal sibling and the ABA(-)/9506 and ABA(+)/9506 combinations.

5.4.5 Leaf osmotic potential

Without acclimation, using the resistant 9506 genotype as a rootstock induced a more negative leaf osmotic potential in scions of the ABA-deficient mutant but had no effect on scions of the ABA-normal sibling (Table 5.5). Salt acclimation (Table 5.5) and exogenous ABA application (Table 5.6) to the ABA-deficient mutant, ABA-normal sibling and the ABA(-)/9506 and ABA(+)/9506 plants induced a less negative leaf osmotic potential under salt stress.

5.4.6 Leaf stomatal conductivity and water flow

Salt acclimation increased leaf stomatal conductivity of all genotypes and graft combinations during subsequent salt stress except for non-grafted plants of the ABA-deficient mutant and the 9506/ABA(+) combination (Table 5.5). Grafts using the resistant 9506 line as a rootstock increased leaf stomatal conductivity in ABA-deficient mutant and ABA-normal sibling scions; however, this occurred only under salt acclimation. Similarly, ABA application increased leaf stomatal conductivity before stress in the ABA-deficient mutant, ABA(-)/9506, 9506/ABA(+) and ABA(+)/9506 grafted plants (Table 5.7). Moreover, after stress, all ABA treatments induced higher stomatal conductivity compared to the untreated controls.

Applying ABA to ABA-deficient mutant and ABA(-)/9506 grafted plants altered water flow from a net downward flow to a more upward flow (Table 5.8). This response was not observed when ABA-deficient mutant scions were grafted onto 9506 rootstocks.

Table 5.6. Mean leaf water content (%) and leaf osmotic potential (Ψs=MPa) of ABA treated (75µM) and non-treated (0 µM) potato genotypes before and after 2 weeks of salt stress (150-180 mM NaCl). Means with the same letters are not significantly different (LSD *P* = 0.05). 9506 = Salt stress resistant, ABA(-) = ABA-deficient mutant, ABA(+) = ABA normal sibling.

Responses	**Time**	**Treatments**	**ABA(-)**	**ABA(-)/9506**	**9506/ABA(-)**	**9506**	**9506/ABA(+)**	**ABA(+)/9506**	**ABA(+)**
Leaf water content	Before stress	ABA(-)	74.12 a	76.67 a	80.22 a	83.61 a	74.01 a	77.24 a	83.09 a
(%)		ABA(+)	79.90 a	81.07 a	79.32 a	78.56 a	78.46 a	83.73 a	82.55 a
	After stress	ABA(-)	34.41 f	42.41 f	72.25 bcde	76.42 abc	70.07 cde	70.39 cde	70.27 cde
		ABA(+)	65.92 e	79.75 ab	74.95 abcd	76.68 abc	66.58 de	81.75 a	82.57 a
Leaf osmotic potential	Before stress	ABA(-)	-1.50 d	-1.16 c	-1.05 abc	-0.94 abc	-1.09 bc	-1.01 abc	-1.00 abc
(Ψs = MPa)		ABA(+)	-0.99 abc	-0.82 a	-0.89 ab	-0.95 abc	-0.98 abc	-0.88 ab	-0.97 abc
	After stress	ABA(-)	-2.95 g	-2.42 f	-1.13 ab	-1.47 cd	-1.22 bc	-2.56 f	-1.97 e
		ABA(+)	-1.80 e	-1.51 d	-0.95 a	-1.48 cd	-1.31 bcd	-1.51 d	-1.48 cd

Table 5.7. Mean leaf stomatal conductivity (Cm s^{-1}) of ABA treated (75µM) and non-treated (0 µM) potato genotypes before and after 2 weeks of salt stress (150-180 mM NaCl). Means with the same letters are not significantly different (LSD $P = 0.05$). 9506 = Salt stress resistant, ABA(-) = ABA-deficient mutant, ABA(+) = ABA normal sibling.

Before salt stress

Treatments	**ABA(-)**	**ABA(-)/9506**	**9506/ABA(-)**	**9506**	**9506/ABA(+)**	**ABA(+)/9506**	**ABA(+)**
ABA(-)	0.09 def	0.03 g	0.13 bcd	0.11 cdef	0.09 def	0.08 f	0.11 cdef
ABA(+)	0.19 a	0.15 abc	0.17 ab	0.13 bcd	0.16 ab	0.16 ab	0.14 abc

After salt stress

ABA(-)	0.01 f	0.01 f	0.04 c	0.03 d	0.03 d	0.01 f	0.02 e
ABA(+)	0.04 c	0.04 c	0.05 b	0.08 a	0.04 c	0.05 b	0.05 b

Table 5.8. Mean shoot water flow (μL h^{-1}) of ABA treated (75μM) and non-treated (0 μM) grafted and non-grafted potato genotypes before and after 2 weeks of salt stress (150-180 mM NaCl). Means with the same letters are not significantly different (LSD $P = 0.05$). 9506 = Salt stress resistant, ABA(-) = ABA-deficient mutant.

Before salt stress

Treatments	**ABA(-)**	**ABA(-)/9506**	**9506**	**9506/ABA(-)**
ABA(-)				
	-0.35 d	-0.41 d	+0.07 b	-0.32 cd
ABA(+)				
	-0.07 bc	+0.44 a	+0.38 a	+0.01 b

After salt stress

ABA(-)				
	-0.27 cd	-0.34 d	+0.05 a	-0.19 bcd
ABA(+)				
	-0.03 ab	-0.09 abc	+0.02 ab	-0.02 ab

+ = Upward - = Downward

5.5 Discussion

In various crops both scions and rootstocks have been shown to influence salinity tolerance, with the majority of research focused on the influence of the rootstocks (Schmutz and Lüdders, 1999; Santa-Cruz *et al*., 2001; Fernández-García *et al*., 2002; Chen *et al*., 2003a). This response was most pronounced when halophytic rootstocks were employed (Chen *et al*., 2003a; Estañ *et al*., 2005; Shaterian *et al*., 2005a). In our present study on potato, the ability of the ABA-accumulating salt-resistant 9506 line used as either rootstock or scion to enhance the salt tolerance of the otherwise salt-sensitive genotypes was examined. The subsequent impact of ABA and translocation of the acclimation response between rootstock and scions was also observed.

5.5.1 Rootstock effects

Plant biomass is the most widely used index in defining the impact of grafting on salt stress tolerance. Higher fresh matter accumulation (Santa-Cruz *et al.,* 2002; Chen *et al.,* 2003a) and dry matter accumulation in grafted relative to non-grafted plants was reported in tomato/tomato (Fernández-García *et al.,* 2004a), tobacco/tomato (Ruiz *et al.,* 2005) and watermelon (Colla *et al.,* 2006a).

Under salt stress conditions growth of both the ABA-deficient line and its normal sibling scions were positively affected by grafting onto rootstocks of the highly vigorous salt stress resistant 9506 line. This effect of grafting was consistent with the work of Chen *et al*. (2003a), who reported that shoot growth of ABA-deficient mutant tomato (*flacca*) scions grafted onto ABA-normal rootstocks was superior to growth of *flacca* grafted on its own rootstock, regardless of the salinity level. They attributed this improvement in growth to the supply of ABA from the rootstock to the shoots. Stress-induced ABA produced in the rootstock improved stomatal control in *flacca* scions grafted on ABA-normal rootstock and resulted in better control of water status and enhanced growth (Chen *et al*., 2003a). Phenotype reversion of ABA-deficient shoots on ABA-normal wild-type roots was reported with grafted tomato (Cornish and Zeevaart, 1988) and sunflower (Fambrini *et al*., 1995). Shoot dry weight was also enhanced by root genotype in grafted faba bean (*Vicia faba*) (Barbera *et al*., 1998). Using the salt-tolerant

9506 rootstock increased stomatal conductivity of scions of both salt-stress-sensitive potato lines in this trial. The observed increase in growth in these two sensitive genotypes might be due to this increase in stomatal conductivity. This type of rootstock effect on stomatal conductivity has also been evidenced in tomato (Fernández-García *et al*., 2004b). Holbrook *et al*. (2002) indicated that root signals controlled stomatal conductance in tomato through changes in apoplastic ABA levels in leaves.

The higher leaf and shoot water content characteristic of the resistant 9506 line was transmissible to scions of the ABA-deficient mutant. This change was accompanied by increased leaf osmotic potential of the ABA-deficient mutant scion in the ABA(-)/9506 graft combination. Graft unions are not a physical barrier to water transport from rootstock to scion (Fernández-García *et al*., 2002) and in fact they are structurally and chemically completely functional in herbaceous plants such as tomato (Fernández-García *et al*., 2004a). The water flow in grafted potato plant shoot systems grown under normal and salt stress conditions was affirmed through our microimaging NMR studies. The effect of salt stress resistant rootstocks on increasing leaf water content (Santa-Cruz *et al*., 2001, 2002; Estañ *et al*., 2005) and increased leaf osmotic potential of the scion was significant particularly at high salinity levels (Santa-Cruz *et al*., 2002). Shoot water content was enhanced when scions of the ABA-deficient mutant of tomato (*flacca*) were grafted onto ABA-normal wild-type rootstocks relative to grafting *flacca* scions on its own rootstock (Chen *et al*., 2002b). An increase in water content (Fricke *et al*., 2004; 2006) and leaf osmotic potential (Wilkinson and Davies, 1997; Zhu *et al.,* 2005) appeared to be related to ABA accumulation in the leaf. The increase in water content will increase tissue succulence and under saline conditions this may facilitate the dilution of salt within the tissue (Ottow *et al.,* 2005). Cherian and Reddy (2000) reported that the large increase in shoot fresh weight of *Suaeda nudiflora* in response to salt stress was mainly due to plant water content. Shoot dry weight, total dry weight and plant water content were also positively correlated to each other (Basal *et al.,* 2006).

Chlorophyll content, a readily measured objective indicator of leaf health and growth potential (Percival, 2005) was also explored in this study. Chlorophyll content showed varying responses to the salt stress and grafting treatments. Grafting of ABA-deficient mutant scions onto salt-resistant rootstock increased chlorophyll content of the scion following two weeks exposure to salt stress. These results are in agreement with those of Romero *et al*. (1997) and

Fernández-García *et al*. (2002) who demonstrated that, under saline conditions, leaf pigments and chlorophyll content in grafted plants were determined by the genotype used as the rootstock. Using the salt-sensitive ABA-deficient line as a rootstock reduced chlorophyll concentrations in the stress-resistant 9506 scion. This might be due to low vigour of the root systems resulting in limited uptake and translocation of ions, photosynthates and plant hormones to the scions (Lee and Oda, 2003) or of loss of chlorophyll content of salt- susceptible genotypes caused by increasing salt stress (Mandal and Singh, 2001).

5.5.2 Scion effects

While much work has been done on rootstock-to-scion responses, fewer reports are available on scion-to-rootstock effects. Our study in potato indicates a significant influence of the scion on the rootstock.

Relative to non-grafted plants, biomass of the ABA-deficient mutant and ABA-normal sibling roots were increased by grafting onto the resistant 9506 scions. This finding might be due to higher photosynthesis rates in the more vigorous, salt-tolerant 9506 scions, leading to greater potential for partitioning of assimilates to the rootstock (Chen *et al.,* 2003a). Chen *et al*. (2003a) also indicated that the scion determines the growth rate of grafted plants, and that growth was positively correlated with shoot ABA concentration. In our study, when the ABA-deficient mutant was grafted onto the resistant 9506 rootstock, the reduced root growth of the resistant 9506 rootstock might also be due to low photosynthetic rate of the scion and reduced production and distribution of photosynthate. This might also explain the increased stem diameter of ABA-deficient mutant rootstocks grafted onto resistant 9506 scions.

Holbrook *et al.* **(**2002) showed that the control of shoot physiology under water stress resided in the shoots rather than in the roots in reciprocal grafts of wild-type and ABA-deficient mutants of tomato. They suggested that the scion may be sending a hormonal signal to the root system. Dunlap and Binzel (1996) reported that ABA levels were higher in leaves than in roots. This may be particularly significant in light of our findings of greater endogenous ABA levels in the 9506 genotype and further, the subsequent induction of higher ABA levels in the resistant 9506 line compared to all other genotypes after salt acclimation and salt stress. A large proportion of ABA (70%) transported in the xylem originated in the shoot and was subsequently

recycled back to the shoot (Chen *et al.,* 2002b). Chen *et al*. (2002b) believed that an unknown "phenotype reversion factor" (PRF) was produced in wild-type shoots (rather than in roots) that determined phenotype reversion of *flacca* in Ws/Fr (wild-type scion/*flacca* rootstock) combination. They also found a positive significant linear relationship between biomass production and ABA levels in the scion.

5.5.3 Acclimation

Plant responses, including dry matter production, stem diameter, water status, hormonal balance and other physiological and biochemical reactions are prime indicators of salinity tolerance. Our study showed that alterations of some of these factors were induced in the rootstock by salt acclimation and in some cases these responses were transferred to the scion. Reduction of leaf necrosis, a sensitive indicator of salt tolerance in grafted plants (Wahome *et al.,* 2000; 2001), was transmitted to the sensitive scions grafted onto salt-tolerant rootstocks by salt acclimation treatment prior to a salt stress event.

Our study further indicates that the salt acclimation that occurs in resistant scions can be transferred to the rootstock. Salt-acclimated resistant 9506 scions increased both the root biomass and diameter when grafted onto ABA-deficient mutant rootstock. Biomass of shoots and roots of ABA-deficient mutant plants grafted with the resistant 9506 genotype increased in comparison with non-grafted plants; the enhanced salt tolerance might be attributed to the higher ABA levels observed in this salt-tolerant genotype after salt acclimation.

Acclimation itself plays a role in salt-stress tolerance of potato genotypes and their grafted combinations. When non-grafted ABA-deficient mutant plants were salt acclimated, leaf greenness actually increased during subsequent salt stress. This is consistent with Djanaguiraman *et al*. (2006), who found that rice plants which had been gradually salt treated had higher chlorophyll contents than those of control plants. In this project salt acclimation treatments reduced shoot dry weight accumulations of potato genotypes compared to those of non-treated plants. Similarly, Silveira *et al*. (2001) showed that treatment of cowpea with 50 mM NaCl for eight days resulted in a 25% reduction in shoot dry mass compared to control plants. Compared with control plants, NaCl-adapted *Sorghum* plants also had reduced shoot dry weight (Amzallag *et al.,* 1990b; Amzallag, 1996; De Lacerda *et al.,* 2001). In soybean, leaf dry weights were slightly to severely reduced by salt pre-treatment, depending on the salt

concentration used during the pre-treatment (Umezawa *et al.,* 2000). Shoot dry weight in bean (*Phaseoulus vulgaris*) (Bayuelo-Jiménez *et al.,* 2003) was also reduced by NaCl salinity.

In this experiment, salt acclimation increased stem water content in the non-grafted ABA-deficient mutant and its normal sibling. Ramoliya and Pandey (2003) found that stems of *Cordia rothii* were the most salt-tolerant tissues, followed by leaves. The high volume of water stored in the stem might enable the dilution of salt ions during a stress event. Accumulation of salts in the shoots and prevention of absorption by photosynthetic and actively growing leaves is a salt-tolerance mechanism of many plants (Reddy *et al.,* 1992).

Salt-acclimated potato genotypes had higher leaf stomatal conductivity and leaf water content after two weeks of salt stress relative to non-acclimated plants. Higher stomatal conductance in salt-acclimated plants has also been reported in rice (Djanaguiraman *et al.,* 2006). The increase in leaf osmotic potential in non-grafted ABA-deficient mutant, ABA(-)/9506 and ABA(+)/9506 under salt acclimation were also similar to the salt accumulation responses seen in sunflower (Steduto *et al.,* 2000).

5.5.4 ABA-mediated responses

Application of ABA significantly reduced leaf necrosis after a salt stress event in the genotypes that normally accumulated little ABA, particularly the ABA-deficient mutant. Exogenous ABA did not alter leaf necrosis, leaf water content or leaf osmotic potential in the salt-stress-resistant 9506 genotype. This genotype had the highest level of endogenous ABA induced after salt acclimation (Chapter 3). As a rootstock, the 9506 line also had the most significant ability to enhance the salt stress resistance of stress-sensitive scions. Although ABA was not measured in grafted plants after salt stress, other work has shown that a signal translocated from the root to the shoot induces ABA biosynthesis in the leaves. Even though the roots of ABA-deficient tomato *flacca* had some endogenous ABA, little of this ABA was transported to the shoots (Sagi *et al.,* 1999). Under stress conditions, the shoots and roots of this mutant were unable to accumulate ABA (Grillo *et al.,* 1995; Bray *et al.,* 1999). In the ABA-deficient mutant of tomato, *Flacca (Fs),* Chen *et al*. (2002b) showed that when *flacca* scions were grafted onto the ABA-normal rootstock (Wr), (Fs/Wr), the scions exhibited higher ABA content, lower transpiration rate and higher water content than when the mutant was grafted onto its own rootstock (Fs/Fr). Using ABA-deficient mutant of tomato (*sitiens* and *flacca)* with their

near-isogenic ABA-normal types in three other grafting experiments (reciprocal grafting, split system and grafting under drought stress), Holbrook *et al*. (2002) concluded that a chemical signal in the root led to a change in apoplastic ABA levels within the scions and this signal caused stomatal closure. To study the effect of rootstock on salt stress tolerance, a grafting experiment with three potato genotypes (1) an early-maturing salt-sensitive (EMS), (2) an early-maturing salt-tolerant (EMT) and (3) a late-maturing salt-tolerant (LMT) was performed (Shaterian *et al.,* 2005a). Salt tolerance of the EMS scion showed a greater increase when grafted on the LMT rootstock than when grafted on the EMT rootstock. Grafting of the EMS genotype on the LMT rootstock also resulted in a more positive leaf osmotic potential than grafting on the EMT rootstock.

The precise mechanism of ABA-enhanced salt stress resistance is not clear but may be regulated by stomatal opening during salt stress, enabling more water uptake into the leaves and shoots. Direction of water flow was altered to a more net upward flow following ABA treatment in our trials. This response is consistent with the observed increase in stomatal conductance. NMR studies have demonstrated an interrelationship between transpiration rate and calculated water flow rates (Kuchenbrod *et al.,* 1996; Köckenberger *et al.,* 1997). Magnetic resonance spectrometry (Peuke *et al.,* 2001) confirmed that greater water loss from leaves was compensated for by a greater water supply via the xylem. In 2005, Velikanov and Belova using NMR imaging showed that exogenous ABA (100 μM) also increased water permeability of the vacuolar symplast in the root cells of maize.

This study suggests that salt tolerance is controlled by both root and shoot factors. The enhanced salt tolerance of ABA(-)/9506 resistant genotype and ABA(+)/9506 resistant genotype under salt stress relative to the ABA-deficient mutant and ABA-normal sibling suggested that root parameters may represent a good criterion for evaluating plant responses to salt stress. Using the resistant 9506 genotype as a rootstock seemed to increase the salt stress resistance of sensitive scions by enhancing water content, possibly via increased stem diameter, leaf water content, leaf osmotic potential, leaf stomatal conductivity and more positive upward water flow. These responses were probably mediated by ABA. Assessment of endogenous ABA levels in potato genotypes as a rapid screening tool for salt stress resistance should be further explored. A scion-to-rootstock effect was also shown by our studies and this effect significantly

influenced salt tolerance of potato genotypes under salt stress conditions. Further observations on performance of grafted material under field conditions are required to verify these results.

5.6 Conclusion

Under salt stress assessment, grafting incorporated with salt acclimation provided greater potential in growth and salt tolerance than salt acclimation alone. Salt-tolerant genotypes transmitted their salt tolerance when used either as a scion or as a rootstock. Scion/rootstock transmissible changes in salt tolerance were probably mediated by ABA as suggested by exogenous ABA experiments and through NMR imaging.

6.0 The effect of $CaCl_2$ and NaCl salt acclimation in stress tolerance and its potential role in ABA and scion/rootstock-mediated salt stress responses

6.1 Abstract

Previously (Chapter 5), we demonstrated that the response of potato to salt stress was influenced by both the scion and the rootstock, and that this response was modulated by NaCl salt acclimation and ABA. However, Ca^{2+} is also widely acknowledged to increase salinity tolerance in plants and Ca^{2+} is considered to be a secondary messenger of ABA. $CaCl_2$ acclimation has not been examined in salt stress responses of potato. Thus, we investigated both NaCl and $CaCl_2$ salt acclimation pre-treatment responses in potato genotypes, ABA(-), ABA(+), 9506, 'Norland', of contrasting salt stress resistance. Subsequently, the effects of rootstocks and scions in NaCl and $CaCl_2$ salt acclimation were evaluated using reciprocal grafting techniques between salt-sensitive and resistant potato genotypes. Plants were grown in a hydroponic nutrient sand culture under greenhouse conditions and were subjected to: (a) no pre-treatment; (b) NaCl pre-treatment; (c) $CaCl_2$ pre-treatment; and (d) combined $CaCl_2$ + NaCl pre-treatment. All plants were then stressed with higher levels of NaCl salt for two weeks. Pre-treatment with NaCl-based acclimation generally enhanced salt stress resistance to a greater extent than when $CaCl_2$ was used in the acclimation. This difference was associated with a specific Na^+ ion effect rather than a non-specific EC-dependent response. That the ABA(-) genotype was unable to exclude Na^+ from the shoot relative to the ABA(+) and other genotypes, and that the $CaCl_2$ pre-treatment enhanced shoot K^+ except in the ABA(-) genotype, suggests that both ABA and $CaCl_2$ are requirements for this mode of salt stress defence. The salt tolerant rootstock positively influenced water content in the more salt sensitive scions after salt stress as reflected through stomatal conductivity, osmotic potential, shoot growth and water content of the scions. Grafting salt stress resistant scions onto the ABA(-) rootstock also led to a pronounced increase in root growth. Grafting of salt-tolerant scions under salt acclimation treatments may be an alternative approach to increasing stress resistance in commercially important potato cultivars. While the $CaCl_2$ acclimation pre-treatment was not as effective as NaCl pre-treatment, the importance of calcium in stress acclimation cannot be excluded, particularly since low levels of Ca^{2+} existed in the background nutrient and NaCl salt stress solutions. Further, calcium and ABA appear to be

involved in increasing the ratio of K^+/ Na^+ in the shoots which was associated with elevated salt stress resistance.

6.2 Introduction

The potato is a key part of the global sustainable food system – producing more food energy on less land than corn, wheat or rice (www.cipotato.org). More than half of global potato production is in developing countries, rendering it an important source of food and income to millions of farmers. However, potato is moderately susceptible to salinity, with both growth and yield being adversely affected when soil electrical conductivity (EC) exceeds 2.16 to 3.38 dSm^{-1} (Katerji *et al*., 2003). Salinization has the potential to negatively impact potato production in irrigated soils, with the most deleterious effects occurring in arid and semi-arid regions where high evapotranspiration rates are coupled with increasing demands on limited supplies of high quality water sources (Rengasamy *et al*., 2006). NaCl, the most detrimental salt in saline soils, has been shown to negatively impact growth of both cultivated and wild species of potato (Bilski *et al*., 1988a; 1988b; Li *et al*., 2006), and its impact is anticipated to increase as a function of global climate change (Hijmans, 2001).

The process whereby pre-exposure to lower levels of stress improves the ability of a plant to adapt to various environmental stresses is generally known as acclimation (Conroy *et al*., 1988; Guy, 1990). Salt acclimation is characterized by the ability to subsequently grow at salt concentrations which otherwise would be lethal to non-acclimated plants (Amzallag *et al*., 1990b). For acclimation to occur, the cell, tissues, organs and organism must be competent to undergo the change (Amzallag and Lerner, 1995). Pre-exposure to low levels of NaCl prior to NaCl salt stress increased subsequent salinity resistance in maize seedlings (Rodríguez *et al*., 1997), soybean (Umezawa *et al*., 2000), cowpea (Silveira *et al*., 2001), rice (*Oryza sativa*) (Hassanein, 2000; Djanaguiraman *et al*., 2006) and potato (Etehadnia *et al*., 2008a).

There is evidence that ABA is involved in salt acclimation (Amzallag *et al*., 1990a; Noaman *et al*., 2002). NaCl pre-treatment increases ABA levels in bush bean resulting in lower ion concentrations in the treated plants (Montero *et al*., 1997). In *Phaseolus vulgaris*, a Na^+ excluder, ABA mediated responses to Na^+ toxicity and the signaling of salt-induced water deficit (Sibole *et al*., 1998; 2000). Exogenous ABA resulted in an increase in salt stress

resistance and dry weight accumulation in corn seedlings under different salinity levels (Zhao *et al*., 1995). These changes were the result of enhanced osmotic adjustment activity, exclusion of Na^+ from the shoot and accumulation in the root. The role of ABA in coordinating growth of roots and shoots of plants and regulating tolerance responses to a number of stresses including water and salt has been reported (Sharpe and LeNoble, 2002).

Whereas ABA is widely demonstrated to be involved in many physiological responses, De Silva *et al*. (1985) proposed that calcium was the secondary messenger in ABA-mediated signalling of stomatal closure. Calcium is considered to be a ubiquitous secondary messenger in plant signalling (McAinsh *et al*., 1997). The requirement of Ca^{2+} for plants has long been known to increase as NaCl soil salinity increases (Gerard, 1971). Calcium elevates plant salt stress tolerance through mechanisms involving both re-adjustment of carbon metabolism and Na^+ regulation (Bolat *et al*., 2006). The calcium binding protein, calreticulin, was shown to be associated with salt stress tolerance and its expression in the scion was regulated by the rootstocks (Shaterian *et al*., 2005a).

Grafting is an integrative reciprocal process that contributes in transferring desirable properties in the scion/rootstock communication, allowing the examination of both scion and rootstock influence on salt tolerance (Pardo *et al*. 1998; Holbrook *et al*., 2002). Previous studies on diploid potato indicate that rootstocks play an important role in salt stress resistance not only through regulation of calreticulin expression (Shaterian *et al*., 2005a), but also through Na^+ exclusion from the shoots and Na^+ accumulation in the roots (Shaterian *et al*., 2005b). It was further demonstrated that the response of potato plants to salt stress was influenced by both the scion and the rootstock, and that this response was modulated by NaCl salt acclimation and ABA (Chapter 5). However, an examination of the effect of $CaCl_2$ salt acclimation in stress tolerance and its potential role in ABA and scion/rootstock-mediated salt stress responses has not been performed.

My specific objectives were to: (a) examine the effects of $CaCl_2$ salt acclimation in stress tolerance by comparing potato plant responses between $CaCl_2$, NaCl and combined NaCl + $CaCl_2$ salt acclimation pre-treatments; (b) determine if salt acclimation pre-treatment responses were related to a specific ion effect or a non-specific EC effect; (c) examine the effect of $CaCl_2$ salt acclimation in ABA and scion/rootstock-mediated salt stress responses.

6.3 Materials and Methods

Four potato lines of varying salt tolerance (Shaterian *et al*., 2005b) were used in this study; the diploid clones: 9506-resistant (a salt tolerant line,); 9120-05 (ABA(-), ABA-deficient mutant, a salt sensitive line), and 9120-18 (ABA(+), ABA-normal sibling of 9120-05, a moderately salt sensitive line). These lines were kindly provided by Dr. H. De Jong of Agriculture and Agri Food Canada, Fredericton. A tetraploid commercial cultivar, 'Norland' (moderately salt tolerant), was used as a check. Plants were propagated vegetatively under greenhouse conditions by stem tip cuttings in Ottawa sand (1-2 mm diameter, 75.5% very coarse sand; 0.5-1 mm, 24.4% course sand; and < 0.5 mm, < 0.1%) under a mist system.

After three weeks, rooted plants were transferred to 1 L standard plastic pots containing the same Ottawa sand medium. Ottawa sand has minimal ion-binding capacity, which reduces interference by ion absorption in salinity or fertility trials. The pots were placed into trays (20 × 40 × 60 cm) which were automatically irrigated three times a day with fertilized water containing 2 g L-1 20-20-20 N-P-K including micronutrients (Plant Products Co. Ltd., 314 Orinda Road, Brampton, Ontario, LGT-1J1). The percentage of micro- and macro-nutrients of 20-20-20 N-P-K fertilizer are discussed in detail in the appendix I. The EC and pH of the nutrient solution were checked weekly. The EC and pH of the 0-180 mM NaCl treatment solutions ranged between 1.22-22.16 dS m^{-1} and 6.52-6.96, respectively (Appendix II). Six pots per tray with a single plant per pot of salt-tolerant ('Norland', 9506) or relatively salt-sensitive [(ABA(-), ABA(+)] genotypes were placed in each tray. The entire pot was flooded from the bottom with nutrient solution for 5 min and drained over a 2-min period. Plants were grown under natural light supplemented with 500-600 $\mu Ms^{-1}m^{-2}$ light intensity, 14 h photoperiod, and 25/20 ^{o}C day/night temperature. To prevent tangling of stems, all plants were tied to bamboo stakes during the experiment.

Physiological responses to salt stress and acclimation abilities of the four potato genotypes were evaluated in a preliminary experiment. Based on observed relative physiological response and LD50, different levels of salt stress were selected for the salt-sensitive and salt-resistant genotypes. The relatively salt stress sensitive ABA(-) and its ABA(+) sibling were acclimated by increasing the concentration of NaCl in the nutrient solution by adding 16.66 mM per week, leading to a final concentration of 50 mM in 3 weeks. The more salt stress resistant

'Norland' and 9506 genotypes were treated in increments of 25 mM NaCl per week, reaching a final concentration of 75 mM in 3 weeks.

Accordingly, pre-treatments applied in this study were: 0 mM $CaCl_2$ and 0 NaCl; 20 mM $CaCl_2$ for one week followed by three weeks of NaCl salt acclimation; 20 mM $CaCl_2$ applied for four weeks; and 20 mM $CaCl_2$ for one week followed by three weeks of NaCl salt acclimation combined with $CaCl_2$ (20 mM). The salt pre-treatments were applied three times per day starting on the first day after transferring rooted plants to the hydroponic sand-based system. The EC and pH of the solutions were monitored weekly. The EC ranged from 1.22 for 0 mM NaCl to 13.44 dS m^{-1} for 75 mM NaCl + 20 mM $CaCl_2$, whereas the pH ranged between 7.6 for 0 mM NaCl, to 6.63 for 75 mM NaCl + 20 mM $CaCl_2$ (Appendix V). The calcium content in the tap water used to prepare nutrient solution is recorded (Appendix VI).

After exposure to the pre-treatments, two weeks of salinity stress were applied. According to preliminary trials, LD50 was attained at 150 mM NaCl for the ABA(-) and its ABA(+) sibling while LD50 was reached at 180 mM NaCl for the more stress resistant 'Norland' and 9506 genotypes. The salt stress treatments were applied three times per day via the irrigation system.

A range of phenotypical and physiological traits were measured after two weeks of salt stress. The number of samples per each treatment in all parameters that was recorded is 12. Leaf necrosis using a rating scale of the lowest non-wilted leaf and the youngest expanded fourth or fifth leaves was scored from 1-5: 1- (0% leaf area necrosis), 2- (1-25% leaf area necrosis), 3- (26-50% leaf area necrosis), 4- (51-75% leaf area necrosis), 5- (76-100% leaf area necrosis) (Shaterian *et al*., 2005b).

Leaf water content (fourth and fifth leaves from apex) was also measured after the two weeks of salt stress. Leaves were sampled between 9:00 a.m. and 12:00 p.m. Water content was determined by measuring leaf fresh weight (FW), then drying in a hot air oven (DW) for 48 hrs at 75°C. Water content was calculated by the formula: (FW-DW)/DW. Plant height (stem collar to shoot tip) was monitored on a weekly basis over two weeks of salt stress to calculate change in plant height:

$$\text{Change in plant height} = \frac{\text{height at T2 - height at T1}}{\text{T2 - T1}}$$

T1 = before stress

T2 = after stress

Osmotic potential (OP) was measured at the end of the salt stress treatment. Sampling was done between 10:00 a.m. and 12:00 p.m. on the fourth and fifth fully expanded leaves. The tissue samples were collected in Eppendorf test tubes and kept frozen at -20$^{\circ C}$ until analysis. Samples were thawed and homogenized in the tubes at room temperature. The sap was then loaded into a Wescor vapour pressure osmometer (Wescor-5000, U.S.A.) chamber for analysis. The Ψs was calculated by the formula: Ψs = - CiRT.

Stomatal conductance was also recorded after two weeks of salt stress. Using the abaxial leaf surface of the fourth and fifth fully expanded leaves stomatal conductivity was assessed between 10:00 a.m. and 11:00 a.m. with a Steady State Porometer (Li-Cor Inc. Li 1600, Lincoln, Nebraska, U.S.A.). At the termination of the project, shoot and root fresh and dry weight and shoot and root water content were measured as described above.

The roots were washed with tap water to remove any sand or residual salt and rinsed with distilled water for ion analysis. Total Ca^{2+}, Na^{+} and K^{+} concentrations in the shoots and roots were measured at the termination of the trial using atomic absorption spectrophotometry (Perkin Elmer-3100, Waltham, Mass., U.S.A.) according to Thomas *et al*. (1967), and expressed on a dry weight basis.

A randomized complete block design in a factorial (4 pre-treatments × 4 genotypes) experiment with four replicates was utilized with three plants per replicate. All data were analyzed by GLM program of SAS (SAS Institute, 2002-2003). Means were compared using the least significant difference (LSD) test at $P=0.05$.

6.3.1 Grafting Experiment

A reciprocal grafting experiment was performed using the ABA(-), ABA(+), and the 9506 genotypes. Shoot tip cuttings of the three genotypes were prepared and after 5 weeks, the rooted cuttings were transferred to 400-mL pots filled with Ottawa sand. The ABA(-) and its ABA(+) sibling were reciprocally grafted onto the 9506 resistant rootstock [ABA(-)/9506], [ABA(+)/9506] and [9506/ABA(-)], [9506/ABA(+)]. The graft unions were covered by a paraffin-embedded plastic film (Parafilm, American National Can Menasha) to avoid

desiccation. The grafted plants were transferred to a mist chamber where they were grown for two weeks. During this time, the plants were irrigated three times daily with water containing 1.27 g L-l 20-20-20 N-P- K plus micro-nutrients (Plant Products Co. Ltd., 314 Orinda Road, Brampton, Ontario, Canada). To provide enough humidity for healing of the graft unions, the grafted plants were covered by clear plastic bags for the first week after grafting. All plants were sprayed with fungicide three times per week to prevent foliar diseases. After a week, the plastic bags were removed and the plants were transferred to the greenhouse and acclimatized to the new conditions. To maintain only one grafted scion, any sprouts growing from the nodes of the rootstock were removed. Plants were grown in the sand and hydroponic system as previously described.

6.3.2 Sodium chloride and calcium chloride pre-treatments

Treatments were applied to the grafted plants to determine if there is a difference between NaCl and $CaCl_2$ acclimation pre-treatments on scion/rootstock responses. Based on the salt tolerance of the rootstock, appropriate levels of NaCl were applied as previously described. To study the effect of NaCl and $CaCl_2$ pre-treatments, different concentrations of NaCl and $CaCl_2$ at the same EC levels were utilized. In summary, the salt pre-treatments applied in this experiment consisted of:

1) a) 50 mM NaCl (EC = 5.0) or b) 75 mM NaCl (EC = 7.39):

a) 16.66 mM NaCl per week reaching 50 mM in 3 weeks applied to grafts on ABA(-), ABA(+) rootstocks

b) 25 mM NaCl per week reaching 75 mM in 3 weeks applied to the 9506-resistant rootstocks

2) a) 27 mM $CaCl_2$ (EC = 5.0) or b) 37 mM $CaCl_2$ (EC = 7.39)

a) 9 mM $CaCl_2$ per week, reaching 27 mM in 3 weeks applied to the ABA(-), ABA(+) rootstocks

b) 37 mM $CaCl_2$ (12.33 mM per week, reaching 37 mM in 3 weeks) treated on 9506-resistant rootstocks

Leaf necrosis, leaf water content, leaf osmotic potential, leaf stomatal conductance, shoot and root dry weight, and shoot and root water content were evaluated as previously described at the end of two weeks of salt stress. Degree of leaf greenness (which is an approximate estimate

of chlorophyll content) was also measured (on the same leaf as necrosis) by a SPAD Meter (Model Minolta-502).

Based on the relative salt tolerance of genotypes and rootstocks, two weeks of salinity stress were initiated after three weeks of salt pre-treatment. The salt stress consisted of 150 mM NaCl for the ABA(-) and ABA(+) genotypes/rootstocks and 180 mM NaCl for the 9506-resistant genotype/rootstock. The experiment was conducted as a randomized complete block design in a factorial experiment (7 graft combinations × 2 pre-treatments) with five replicates and one plant per replicate. All data were analyzed using the GLM program of SAS (SAS Institute, 2002-2003). Means were compared using the least significant difference (LSD) test at $P=0.05$.

6.4 Results and Discussion

6.4.1 Leaf Necrosis

In the absence of salt pre-treatments, both the ABA(-) and the ABA(+) genotypes expressed the same degree of leaf necrosis after exposure to salt stress (Fig. 6.1), suggesting that ABA may not be directly involved in alleviating the symptoms of leaf necrosis in these genotypes without acclimation. The responses to both $CaCl_2$ and NaCl salt pre-treatments were also similar for these two genotypes. However, the pre-treatments were more effective at reducing leaf necrosis when applied to the ABA(+) genotype compared to the ABA(-) and thus ABA may be involved in salt acclimation. A similar result was found with another ABA-deficient mutant, tomato (*sitiens*), in which the percentage of leaf injury was greater than its wild type at moderate salinity (Mäkelä *et al*., 2003).

6.4.2 NaCl and $CaCl_2$ pre-treatment effects

Leaf stomatal conductivity was reduced under all pre-treatments in the more salt resistant 9506 and 'Norland' genotypes (Table 6.1). However, none of the pre-treatments affected stomatal conductivity in either of the most salt sensitive ABA(-) and ABA(+) genotypes. There was no differential stomatal conductivity response between the $CaCl_2$ and NaCl pre-treatments except in the 9506 genotype in which stomatal conductivity increased under $CaCl_2$. Leaf osmotic potential generally became less negative under both $CaCl_2$ and NaCl

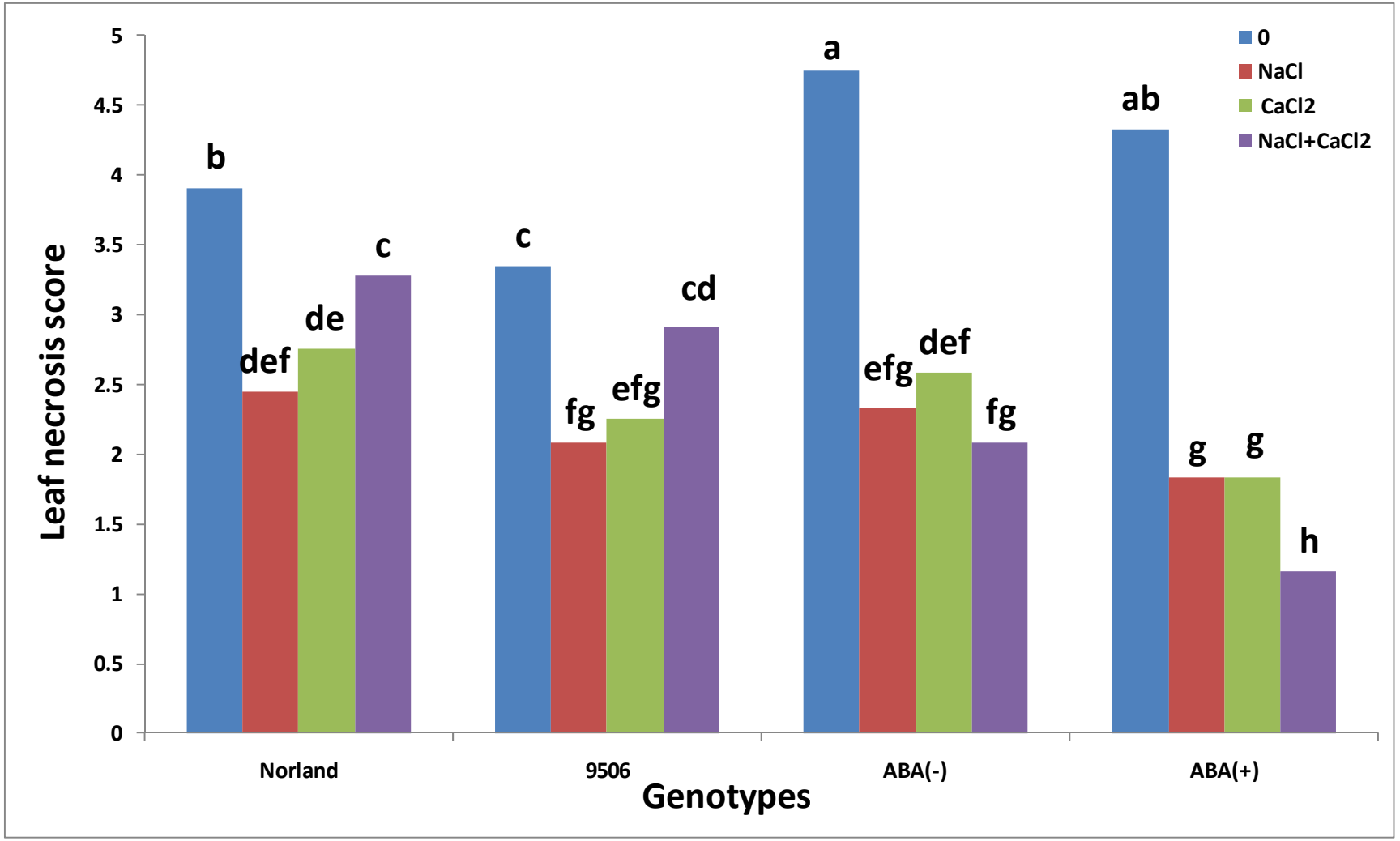

Figure 6.1 The effects of salt pre-treatment on leaf necrosis scores of four potato genotypes after two weeks of salt stress (150-180 mM NaCl). Leaf necrosis score, 1-5: 1- (0% necrosis), 2- (1-25% necrosis), 3- (26-50% necrosis), 4- (51-75% necrosis), 5- (76-100% necrosis). Means with the same letters are not significantly different (LSD $P = 0.05$). 9506 = Salt stress resistant, ABA(-) = ABA-deficient mutant, ABA(+) = ABA normal sibling.

Table 6.1. The effects of salt pre-treatments on stomatal conductivity (cm s^{-1}), leaf osmotic potential (Ψs=Mpa), and leaf water content (g H_2O/g dry weight) of four potato genotypes after two weeks of salt stress (150-180 mM NaCl). Means with the same letters are not significantly different (LSD $P = 0.05$). 9506 = Salt stress resistant, ABA(-) = ABA-deficient mutant, ABA(+) = ABA normal sibling.

Leaf stomatal conductivity

Pre-treatments	**'Norland'**	**9506**	**ABA(-)**	**ABA(+)**	**Mean**
0	0.048 b	0.074 a	0.014 fg	0.016 fg	0.038
NaCl	0.036 c	0.029 cd	0.011 g	0.017 fg	0.023
$CaCl_2$	0.036 c	0.047 b	0.011 g	0.020 ef	0.028
NaCl + $CaCl_2$	0.027 de	0.025 de	0.010 g	0.017 fg	0.019
Mean	0.036	0.043	0.011	0.017	
Leaf osmotic potential					
0	-1.94 efgh	-1.31 a	-2.42 i	-2.04 gh	-1.92
NaCl	-1.27 a	-1.30 a	-2.02 fgh	-1.65 bcd	-1.56
$CaCl_2$	-1.74 cdef	-1.39 ab	-1.73 cde	-1.49 abc	-1.58
NaCl + $CaCl_2$	-1.68 bcde	-1.81 defg	-2.14 hi	-1.68 bcde	-1.82
Mean	-1.65	-1.45	-2.07	-1.71	
Leaf water content (g H_2O/g dry weight)					
0	5.37 abc	4.10 abc	1.72 ab	2.73 abc	3.48
NaCl	6.29 bc	4.42 abc	4.11 abc	6.18 bc	5.25
$CaCl_2$	7.47 c	5.52 abc	5.92 bc	7.18 c	6.52
NaCl + $CaCl_2$	7.88 c	6.32 c	7.55 c	7.27 c	7.25
Mean	6.75	5.09	4.82	5.84	

pre-treatments when measured after 2 weeks of salt stress, except for the 9506 genotype in which there was no effect. The relative difference in osmotic potential response between $CaCl_2$ and NaCl pre-treatments was genotype-dependent in which $CaCl_2$ decreased (more negative) osmotic potential in 'Norland' while osmotic potential became less negative in ABA(-) compared to the NaCl salt pre-treatment. Leaf water content generally exhibited no differential response to any of the pre-treatments in any genotypes except in the most sensitive ABA(-) genotype in which the combined NaCl + $CaCl_2$ pre-treatment induced a significantly higher water content compared to the stressed plants without any pre-treatment.

A more consistent distinction between $CaCl_2$ and NaCl pre-treatments was observed in shoot and root responses (Tables 6.2, 6.3). By the end of the salt stress event, shoot fresh and dry weights were most elevated under the NaCl pre-treatment compared to the $CaCl_2$ salt pre-treatment in all genotypes except the ABA(-) genotype in which none of the pre-treatments induced a differential response. Shoot water content, root fresh and dry weights were consistently elevated under the NaCl pre-treatment but results were less consistent under the $CaCl_2$ pre-treatment. The observed enhancement of shoot water content appears to be associated with stem water since leaf water content did not significantly increase under the NaCl pre-treatment, even though leaf osmotic potential generally became less negative under this pre-treatment (Table 6.1). Saxena *et al*. (1989), who compared different salts including NaCl, $CaCl_2$, Na_2SO_4, and Na_2CO_3 on the germination and growth stages of alfalfa, also found NaCl to be the most effective in inducing salt tolerance. Root water content showed relatively little response to either $CaCl_2$ or NaCl pre-treatments.

6.4.3 The role of ABA, $CaCl_2$ and the K^+/ Na^+ ratio

ABA appears to be related to regulating the K^+/ Na^+ ratio in the shoots (Table 6.4). The ABA(-) genotype was unable to reduce shoot Na^+ or increase shoot K^+ levels under any of the pre-treatments compared to its ABA(+) counterpart, resulting in a non-significant difference in the K^+/ Na^+ ratio in the ABA(-) genotype as compared to an elevated ratio in the ABA(+) genotype. Accumulation of Na^+ in the roots with a reduction of Na^+ in the shoots appeared to be a consistent mode of salt stress resistance in all genotypes tested in this study and may have been the main factor resulting in reduced leaf necrosis of the pre-treated plants. Roots, the first organ of the plant to become exposed to salinity (Koyro, 1997), have been shown to play a role

Table 6.2. The effects of salt pre-treatments on shoot fresh and dry weight and shoot water content of four potato genotypes after two weeks of salt stress (150-180 mM NaCl). Means with the same letters are not significantly different (LSD $P = 0.05$). 9506 = Salt stress resistant, ABA(-) = ABA-deficient mutant, ABA(+) = ABA normal sibling.

Pre-treatments	**'Norland'**	**9506**	**ABA(-)**	**ABA(+)**	**Mean**
			Shoot fresh weight (g)		
0	110.86 de	120.34 d	35.83 i	81.59 fg	87.15
NaCl	238.43 b	261.97 a	56.54 h	174.46 c	182.85
$CaCl_2$	84.60 fg	128.10 d	40.51 hi	98.27 ef	87.87
NaCl + $CaCl_2$	79.26 g	81.66 fg	46.04 hi	125.88 d	83.21
Mean	128.28	148.01	44.73	120.05	
			Shoot dry weight (g)		
0	28.72 de	41.61 b	15.99 gh	21.39 fg	26.92
NaCl	34.42 c	51.15 a	10.87 hi	26.79 def	30.80
$CaCl_2$	26.29 ef	32.08 cd	10.20 i	17.62 g	21.54
NaCl + $CaCl_2$	20.53 g	21.45 fg	9.68 i	20.44 g	18.02
Mean	27.49	36.57	11.68	21.56	
			Shoot water content (g H_2O/g dry weight)		
0	2.88 bc	1.92 ab	1.40 a	2.84 abc	2.26
NaCl	5.95 f	4.14 cde	4.96 def	5.54 ef	5.14
$CaCl_2$	2.25 ab	5.66 f	2.76 abc	4.64 def	3.82
NaCl + $CaCl_2$	2.93 bc	3.74 cd	5.42 ef	5.34 ef	4.35
Mean	3.50	3.86	3.63	4.59	

Table 6.3. The effects of salt pre-treatments on root fresh and dry weights and root water content of four potato genotypes after two weeks salt stress (150-180 mM NaCl). Means with the same letters are not significantly different (LSD $P = 0.05$). 9506 = Salt stress resistant, ABA(-) = ABA-deficient mutant, ABA(+) = ABA normal sibling.

Root fresh weight (g)

Pre-treatments	**'Norland'**	**9506**	**ABA(-)**	**ABA(+)**	**Mean**
0	2.01 fghi	2.55 def	1.55 i	1.93 ghi	2.01
NaCl	3.28 bc	3.57 b	2.46 efg	3.54 b	3.21
$CaCl_2$	1.88 ghi	3.05 bcd	1.97 fghi	2.79 cde	2.42
NaCl + $CaCl_2$	1.79 hi	2.91 fgh	1.98 fghi	4.78 a	2.86
Mean	2.24	3.02	1.99	3.26	

Root dry weight (g)

0	0.63 de	0.89 cd	0.45 e	0.57 de	0.63
NaCl	1.25 ab	1.47 a	0.72 d	1.25 ab	1.17
$CaCl_2$	0.56 de	1.24 ab	0.64 de	1.10 bc	0.88
NaCl + $CaCl_2$	0.57 de	0.76 cde	0.64 de	1.46 a	0.85
Mean	0.75	1.09	0.61	1.09	

Root water content (g H_2O/g dry weight)

0	2.21 cd	1.42 a	2.33 cde	2. 02 bcd	1.99
NaCl	1.64 ab	1.86 abc	2.43 de	2.80 e	2.18
$CaCl_2$	2.37 de	1.51 a	2.03 bcd	1.86 abc	1.94
NaCl + $CaCl_2$	2.11 bcd	2.09 bcd	2.12 cd	3.63 f	2.48
Mean	2.08	1.72	2.22	2.57	

Table 6.4. The effects of salt pre-treatment on shoot and root Ca^{2+}, Na^{+}, K^{+} content (mg/g dry matter) and K^{+}/Na^{+} ratio of four potato genotypes after two weeks of salt stress (150-180 mM NaCl). Means with the same letters are not significantly different (LSD $P = 0.05$). 9506 = Salt stress resistant, ABA(-) = ABA-deficient mutant, ABA(+) = ABA normal sibling.

		Shoot		**Calcium**		**Root**		
Pre-treatments	**'Norland'**	**9506**	**ABA(-)**	**ABA(+)**	**'Norland'**	**9506**	**ABA(-)**	**ABA(+)**
0	7.92 g	6.19 h	4.45 i	4.49 i	4.62 def	4.46 def	2.34 gh	2.82 fgh
NaCl	4.17 i	5.87 h	4.21 i	4.82 i	5.76 d	3.75 efg	2.50 gh	1.81 h
$CaCl_2$	26.17 a	20.51 b	17.68 d	21.18 b	10.75 b	11.37 b	14.24 a	10.72 b
NaCl + $CaCl_2$	20.74 b	19.31 c	11.92 f	14.46 e	10.70 b	9.78 bc	8.32 c	4.73 de
		Shoot		**Sodium**		**Root**		
0	28.40 c	24.75 d	17.08 fg	35.71 a	21.57 e	18.20 f	13.98 h	16.70 g
NaCl	16.06 g	22.90 d	15.58 g	23.96 d	27.40 b	21.81 e	17.87 f	27.89 b
$CaCl_2$	23.98 d	16.44 fg	17.09 fg	20.29 e	34.83 a	20.91 e	21.42 e	21.35 e
NaCl + $CaCl_2$	34.97 a	31.24 b	15.17 g	18.61 ef	25.62 c	15.84 g	21.51 e	22.86 d
		Shoot		**Potassium**		**Root**		
0	54.89 bc	44.13 fgh	42.48 ghi	41.00 hi	7.21 g	3.63 k	3.80 jk	4.08 j
NaCl	55.06 bc	48.43 de	39.81 i	44.78 efgh	9.08 e	3.27 l	12.28 c	21.19 a
$CaCl_2$	59.84 a	48.62 de	45.45 efg	46.97 ef	5.27 h	2.97 l	4.53 i	8.74 f
NaCl + $CaCl_2$	58.93 ab	46.25 efg	45.07 efgh	52.54 cd	4.52 i	2.97 l	11.02 d	15.07 b
		Shoot		**Potassium /Sodium**		**Root**		
0	1.93 fgh	1.78 ghi	2.48 cde	1.14 j	0.33 f	0.19 ij	0.27 g	0.24 h
NaCl	3.42 a	2.11 efg	2.56 bcd	1.86 ghi	0.33 f	0.15 i	0.68 b	0.75 a
$CaCl_2$	2.72 bc	2.96 b	2.65 bcd	2.31 def	0.15 i	0.14 i	0.21 i	0.41 e
NaCl + $CaCl_2$	1.68 hi	1.48 ij	2.97 b	2.82 bc	0.17 k	0.19 ij	0.51 d	0.66 c

in exclusion of salt from the leaves (Heimler *et al*., 1995; Saqib *et al*., 2005). Shaterian *et al*. (2005b) reported that early-maturing potatoes such as 'Norland' tended to be Na^+ includers in the leaves compared to late-maturing types, which tend to be leaf Na^+ excluders. NaCl salt-adapted plants generally have significantly lower Na^+ in their leaves compared to non-adapted plants (Montero *et al*., 1997; Djanaguiraman *et al*., 2006). Huang and Liau (1998) and Umezawa *et al*. (2000) found higher Na^+ concentration in the root of soybean acclimated with salt than non-acclimated plants. The effect of salt pre-treatment on Na^+ accumulation in soybean and *Sorghum bicolor* appeared to be related to a re-translocation mechanism of Na^+ from the shoot (Amzallag and Lerner, 1994; 1995; Umezawa *et al*., 2000) to the root.

In the more salt stress resistant potato genotypes, greater accumulation of potassium in the shoots was observed (Table 6.4). K^+ is the most abundant and highly mobile cation in both the xylem and phloem. Potassium may serve to balance Na^+ thereby increasing the viability of leaves (Marschner, 1995; Pardo *et al*., 2006). The concurrent increase in shoot K^+ associated with a reduction in Na^+ is a consistent mechanism of salt stress avoidance (Greenway, 1962; Munns *et al*., 2000; Zhu *et al*., 2001). The reduction of Na^+ in the shoot of 'Norland' coincided with the increase of the K^+ ion concentrations in the root. A similar interplay between root K^+ levels and shoot Na^+ levels was observed by Bañuls *et al*. (1997) on *Citrus sinensis*, and Anil *et al*. (2005) on rice. K^+ plays an important role in enzymes, modulating ionic charge, balance of cytoplasm, and vacuole osmotic pressure (Maathuis and Amtmann, 1999).

The $CaCl_2$ pre-treatment appeared to shift the most salt stress resistant 9506 genotype from a shoot Na^+ includer to a Na^+ excluder (Table 6.4). In fact, the calcium-binding protein SOS3 (Salt-Overly-Sensitive 3) and the protein kinase SOS2 are activated by a NaCl-induced calcium signal which, in turn, activates the Na^+/H^+ antiport SOS1 (Zhu, 2003). The results of studies by Ehret *et al*. (1990) and Melgar *et al*. (2006) also indicated that Ca^{2+} participated in a Na^+ exclusion mechanism mainly by preventing transport of Na^+ to the shoot. Anil *et al*. (2005), working on two salt-sensitive and salt-tolerant genotypes of rice, demonstrated Ca^{2+}-dependent reduction in Na^+ transport to the shoot, which correlated with a decline in Na^+ bypass flow in the transpiration stream. The salt tolerant genotypes maintained lower Na^+ in its apoplast relative to the salt-sensitive genotypes by developing a regulatory mechanism of sequestration into intracellular compartments. That the $CaCl_2$ pre-treatment also enhanced shoot K^+ except in the ABA(-) genotype, and that the ABA(-) genotype was not able to exclude Na^+ from the shoot

relative to the ABA(+) and other genotypes (Table 6.4) further suggests that ABA and $CaCl_2$ are requirements for this mode of salt stress defense. Addition of calcium to the salt solution increased K^+ uptake and transport (Dabuxilatu and Ikeda, 2005) and improved the K^+/ Na^+ selectivity (Bolat *et al*., 2006). There is also evidence that K^+ levels can play a role in the homeostasis of Na^+ (Liu *et al*., 2000) and that calcium supply ($CaCl_2$) may take part in the Na^+ exclusion mechanism mainly by preventing Na^+ transport to the shoot (Melgar *et al*., 2006). Moreover, calcium content positively correlated with increasing levels of ABA in the plant (Staxen *et al*., 1999). Regulation

of Na^+ exclusion, as well as reduced Na^+ accumulation, has been reported to be induced by ABA in several studies (Sibole *et al*., 2003; Yan *et al*., 2006). The effect of exogenous ABA on Na^+ exclusion and reduced Na^+ accumulation has been studied (Wang, 1998; Sarwat *et al*., 2000). It has been proposed that in *A. chinense*, external ABA could promote the uptake of K^+ and reduce the toxicity of Na^+ (Wang *et al*., 1996; Wang, 1998). Exogenous ABA has also been shown to increase K^+ ion content by nearly twofold in xylem sap (Chen *et al*., 2006) mediated by K^+ channels (Roberts and Snowman, 2000).

While the combined NaCl + $CaCl_2$ pre-treatment reduced leaf necrosis after salt stress in both 'Norland' and 9506 genotypes compared to the control, this pre-treatment was the least effective of all pre-treatments and also induced the highest Na^+ accumulation in shoot tissue. The negative effect of mixed salts may have also been provoked by an increase in the Cl^- ion. The greater reduction in the osmotic potential of the solution may have prevented the root cells from obtaining water and essential nutrients (Çiçek and Çakirlar, 2002) or may have caused changes in membrane permeability (Cramer *et al*., 1985; Tobe *et al*., 2004). The high EC of the combined solution may have limited the supply of water to the plant resulting in a significant reduction of water in the leaf. Similar to our finding, significant reduction in plant growth parameters have been reported when EC of mixed salts exceeded 11 dS m^{-1} for wheat (Wilson *et al*., 2002) and 16.4 dS m^{-1} for pomegranate (*Punica granatum*) (Asrey and Shukla, 2003).

6.4.4 Grafting Responses

Grafting relatively salt sensitive scions [(ABA(-) and ABA(+)] onto the 9506 resistant rootstock was unable to decrease leaf necrosis beyond the salt pre-treatment effect alone. There was generally no difference between the NaCl and $CaCl_2$ pre-treatment effects in the leaf

necrosis response, except in the most salt sensitive ABA(-) genotype in which the NaCl pre-treatment was more effective than the $CaCl_2$ pre-treatment in alleviating leaf necrosis caused by subsequent exposure to salt stress (Fig. 6.2).

By contrast, leaf greenness, a reflection of chlorophyll content, was higher following the NaCl pre-treatment compared to the $CaCl_2$ pre-treatment and this effect was even more pronounced after the salt stress, particularly in the relatively more salt sensitive genotypes (Fig. 6.3). The 9506 salt stress resistant rootstock increased leaf greenness beyond the NaCl pre-treatment effect alone in both ABA(-) and ABA(+) scions (Fig.6.3). In a similar work with rice, Djanaguiraman *et al*. (2006) found that plants that had been gradually salt treated contained higher chlorophyll content than did control plants. Romero *et al*. (1997) and Fernández-García *et al*. (2002) also showed that leaf pigment and chlorophyll content in grafted plants exposed to salinity conditions were determined by the genotype of the rootstock.

Whenever a difference in either leaf stomatal conductivity (Table 6.5), leaf osmotic potential (Table 6.6), leaf water content (Table 6.7), shoot (Table 6.8) and root (Table 6.9) dry weight or water content was detected, NaCl pre-treatment consistently increased these parameters compared to the $CaCl_2$ pre-treatment. The higher leaf stomatal conductivity in the NaCl salt-acclimated potato genotypes and graft combinations during subsequent salt stress observed in this study is in accord with the results of Djanaguiraman *et al*. (2006) on salt-acclimated rice plants.

Although both acclimation treatments were applied at the same EC level, the NaCl pre-treatment generally produced a higher level of acclimation compared to the $CaCl_2$ pre-treatment. NaCl acclimation was more effective in alleviating negative salt stress responses compared to $CaCl_2$. A similar Na^+ specific effect on growth enhancement of *Suaeda maritime* following application of NaCl was also observed (Yeo and Flowers, 1980). This differential salt-specific response was particularly pronounced in stomatal conductivity and osmotic potential measurements after stress as well as leaf water content.

ABA levels in the rootstock also had a significant effect on influencing stomatal conductivity. The 9506 rootstock, which had the highest levels of endogenous ABA after salt acclimation (Etehadnia *et al*., 2008a), induced greater stomatal closure in the ABA(-) and ABA(+) scions both before and after salt stress compared to non-grafted plants (Table 6.5). Similarly, under NaCl pre-treatment when 9506 scions were grafted onto the ABA(-) or ABA(+)

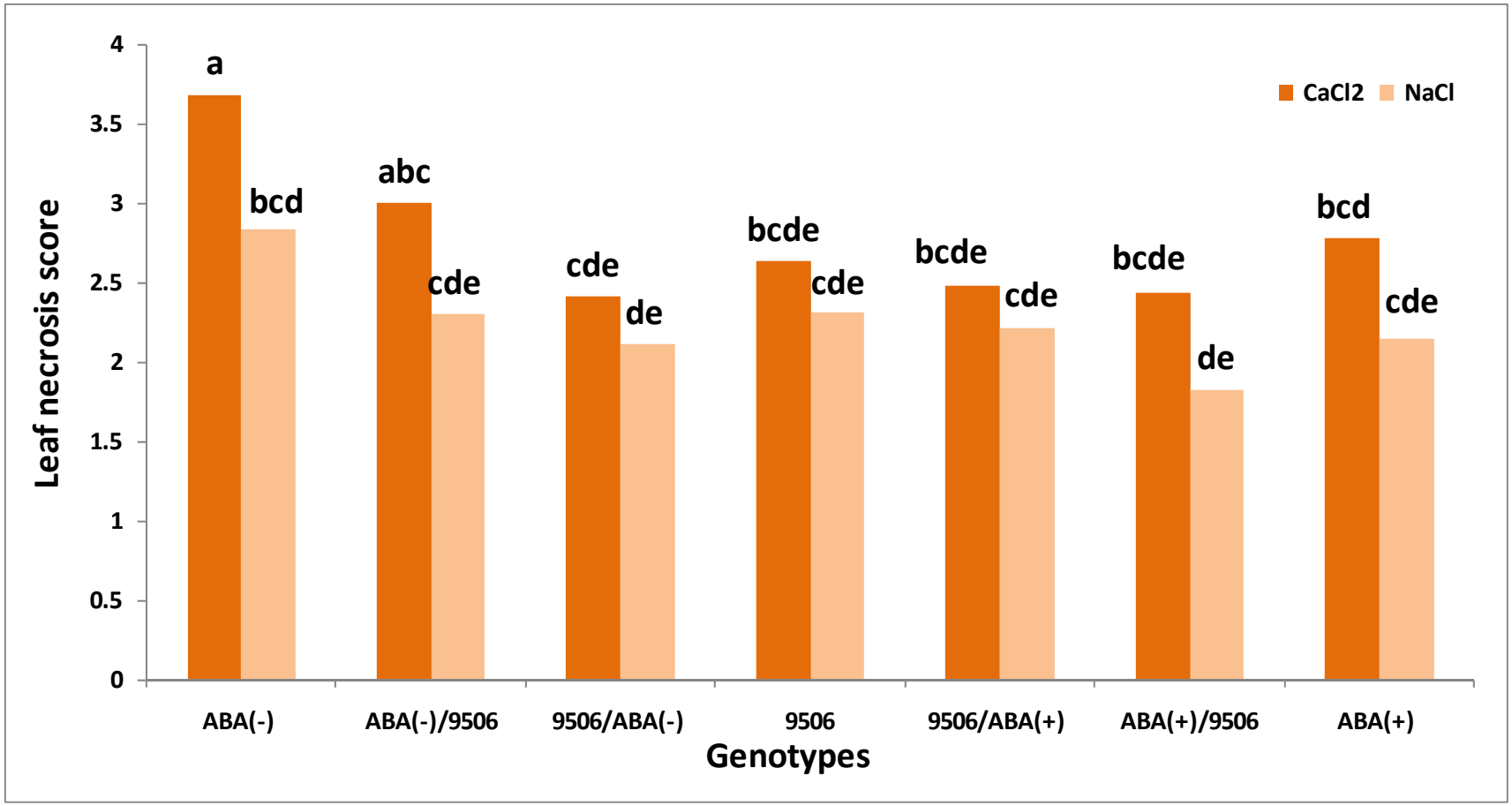

Figure 6.2 The effect of sodium chloride and calcium chloride pre-treatments on leaf necrosis of potato genotypes and their graft combinations (scion/rootstock) measured after two weeks of salt stress (150-180 mM NaCl). Leaf necrosis score: 1-5. 1- (0% necrosis), 2- (1-25% necrosis), 3- (26-50% necrosis), 4- (51-75% necrosis), 5- (76-100% necrosis). Means with the same letters are not significantly different (LSD $P = 0.05$). 9506 = Salt stress resistant, ABA(-) = ABA-deficient mutant, ABA(+) = ABA normal sibling.

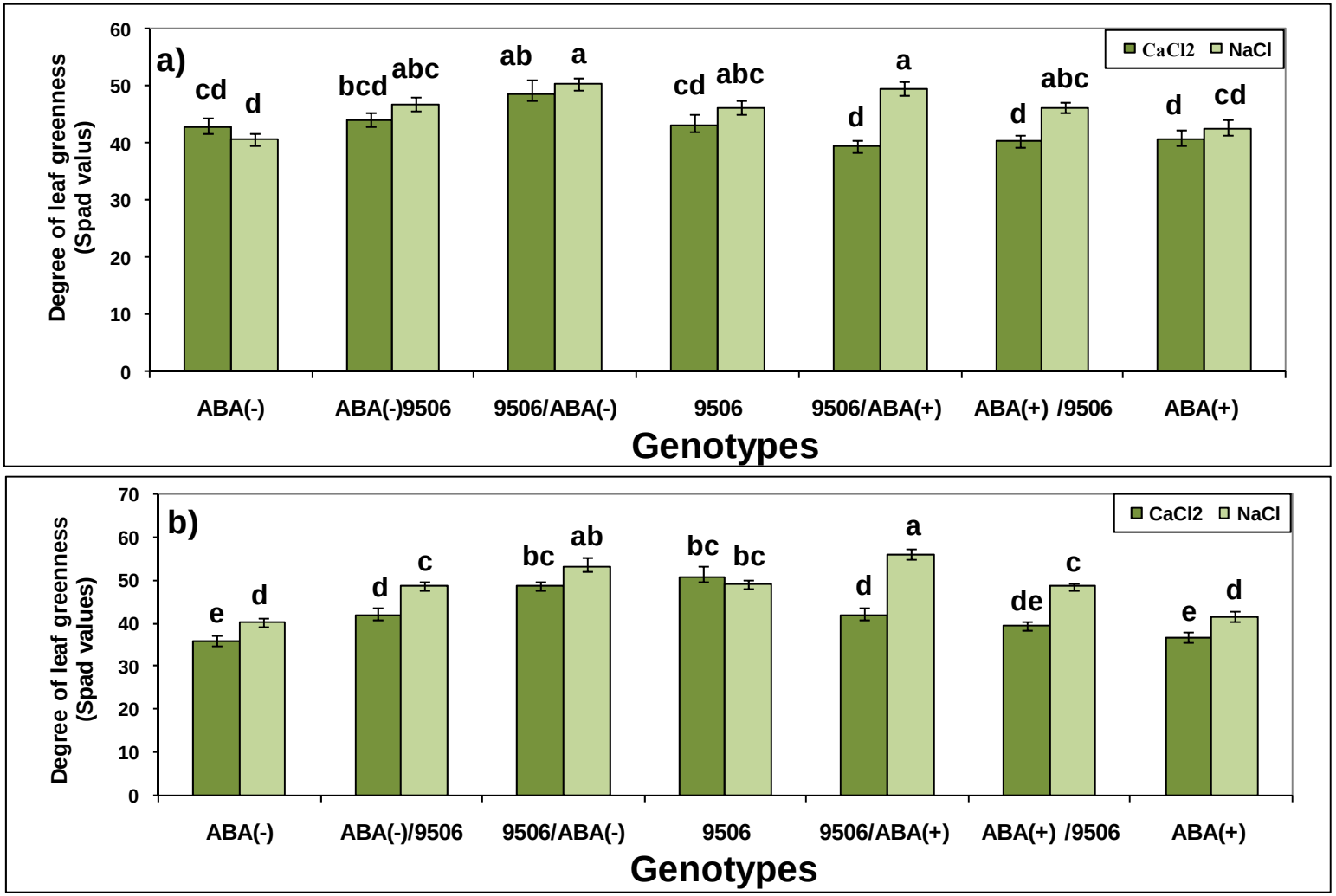

Figure 6.3 The effect of sodium chloride and calcium chloride pre-treatments on the degree of leaf greenness of different grafted and non-grafted potato genotypes (a) before salt stress and (b) after two weeks of salt stress (150-180 mM NaCl). Means with the same letters are not significantly different (LSD $P = 0.05$). 9506 = Salt stress resistant, ABA(-) = ABA-deficient mutant, ABA(+) = ABA normal sibling.

Table 6.5. The effect of NaCl and $CaCl_2$ on leaf stomatal conductivity (SC) (cm s^{-1}) of scion/rootstock combinations of different potato genotypes before and after two weeks of salt stress (150-180 mM NaCl). Means with the same letters are not significantly different ((LSD $P = 0.05$). 9506 = Salt stress resistant, ABA(-) = ABA-deficient mutant, ABA(+) = ABA normal sibling.

				Before stress			
Pre-treatments	**ABA(-)**	**ABA(-)/9506**	**9506/ABA(-)**	**9506**	**9506/ABA(+)**	**ABA(+)/9506**	**ABA(+)**
$CaCl_2$	0.04 c	0.02 a	0.06 e	0.04 c	0.02 a	0.04 c	0.05 d
NaCl	0.04 c	0.03 b	0.06 e	0.04 c	0.07 f	0.04 c	0.05 d
				After stress			
$CaCl_2$	0.02 b	0.01 a	0.04 d	0.03 c	0.02 b	0.02 b	0.03 c
NaCl	0.03 c	0.02 b	0.06 f	0.03 c	0.05 e	0.03 c	0.04 d

Table 6.6. The effect of NaCl and $CaCl_2$ on leaf osmotic potential (Ψs= MPa) of scion/rootstock combinations of different potato genotypes before and after two weeks of salt stress (150-180 mM NaCl). Means with the same letters are not significantly different (LSD *P* = 0.05). 9506 = Salt stress resistant, ABA(-) = ABA-deficient mutant, ABA(+) = ABA normal sibling.

			Before stress				
Pre-treatments	**ABA(-)**	**ABA(-)/9506**	**9506/ABA(-)**	**9506**	**9506/ABA(+)**	**ABA(+)/9506**	**ABA(+)**
$CaCl_2$	-2.13 g	-1.89 f	-1.49 bcd	-1.47 bcd	-1.45 bcd	-1.53 cd	-1.61cde
NaCl	-2.34 g	-1.56 cde	-1.40 abc	-1.66 de	-1.24 a	-1.31 ab	-1.64 de
			After stress				
$CaCl_2$	-2.72 f	-2.22 de	-1.75 ab	-2.04 cd	-2.16 de	-1.64 ab	-1.70 ab
NaCl	-2.32 e	-1.70 ab	-1.52 a	-1.84 bc	-1.74 ab	-1.67 ab	-1.85 bc

Table 6.7. The effect of NaCl and $CaCl_2$ on leaf water content (%) of scion/rootstock combinations of different potato genotypes before and after two weeks of salt stress (150-180 mM NaCl). Means with the same letters are not significantly different (LSD *P* = 0.05).9506 = Salt stress resistant, ABA(-) = ABA-deficient mutant, ABA(+) = ABA normal sibling.

			Before stress				
Pre-treatments	**ABA(-)**	**ABA(-)/9506**	**9506/ABA(-)**	**9506**	**9506/ABA(+)**	**ABA(+)/9506**	**ABA(+)**
$CaCl_2$	56.67 e	62.95 de	82.62 a	83.94 a	77.93 ab	78.36 ab	78.14 ab
NaCl	69.60 cd	70.75 c	79.42 ab	82.19 a	78.70 ab	81.11 a	73.76 bc
				After stress			
$CaCl_2$	42.98 i	51.53 h	68.69 bcdef	69.88 abcde	66.37 cdef	64.60 def	57.03 gh
NaCl	61.31 fg	73.00 abc	77.32 a	76.19 ab	68.66 bcdef	71.85 abcd	63.85 efg

Table 6.8. The effect of NaCl and $CaCl_2$ on shoot dry weight (g) and shoot water content (%) of scion/rootstock combinations of different potato genotypes after two weeks of salt stress (150-180 mM NaCl). Means with the same letters are not significantly different (LSD $P = 0.05$). 9506 = Salt stress resistant, ABA (-) = ABA-deficient mutant, ABA(+) = ABA normal sibling.

Shoot dry weight

Pre-treatments	**ABA(-)**	**ABA(-)/9506**	**9506/ABA(-)**	**9506**	**9506/ABA(+)**	**ABA(+)/9506**	**ABA(+)**
$CaCl_2$	1.72 f	4.19 ef	16.23 b	13.82 b	7.20 cd	7.14 cd	4.01 ef
NaCl	2.75 f	4.25 ef	19.43 a	15.22 b	9.14 c	9.42 c	5.42 de
				Shoot water content			
$CaCl_2$	60.05 f	64.63 f	88.58 a	81.66cd	81.67 bcd	85.53abc	81.48 cd
NaCl	70.55 e	77.48 d	84.68 abc	87.11 ab	78.60 d	87.84 a	85.93abc

Table 6.9. The effect of NaCl and $CaCl_2$ on root dry weight (g) and root water content (%) of scion/rootstock combinations of different potato genotypes after two weeks of salt stress (150-180 mM NaCl). Means with the same letters are not significantly different (LSD $P = 0.05$). 9506 = Salt stress resistant, ABA(-) = ABA-deficient mutant, ABA(+) = ABA normal sibling.

Pre-treatments	**ABA(-)**	**ABA(-)/9506**	**9506/ABA(-)**	**9506**	**9506/ABA(+)**	**ABA(+)/9506**	**ABA(+)**
				Root dry weight			
$CaCl_2$	0.57 e	1.14 bcd	1.33 abc	1.33 abc	0.65 e	1.23 bcd	0.86 de
NaCl	0.60 e	1.42 abc	1.75 a	1.58 ab	1.10 cd	1.20 bcd	1.19 bcd
				Root water content			
$CaCl_2$	42.86 g	69.57 de	84.36 a	70.39 d	72.56 cd	70.07 de	63.46 e
NaCl	54.52 f	70.14 de	88.37 a	70.52 d	78.62 bc	74.44 c	67.87 de

rootstocks containing relatively lower levels of endogenous ABA following salt acclimation (Etehadnia *et al*., 2008a), the stomatal conductivity of the 9506 scion increased both before and after subsequent salt stress. These results are consistent with several reports that elevated ABA levels will induce stomatal closure (Wright, 1978; Montero *et al*., 1998; Sibole *et al*., 2000; Mishra *et al*., 2006) but is in contrast to Jones *et al*. (1987) and Holbrook *et al*. (2002) who found stomatal closure in scions to be independent of the ABA levels in the rootstock.

The osmotic potential of the ABA(-) scion was increased (less negative) when grafted onto the 9506 rootstock. This 9506 rootstock effect was not observed on the ABA(+) scion (Table 6.6). Since Shaterian (unpublished) found that Na^+ was the predominant ion associated with osmotic potential in these genotypes, this supports our finding that ABA may play a role in shoot Na^+ ion management by reducing shoot Na^+ (Table 6.4). The 9506 rootstock also significantly elevated both leaf water content of the ABA(-) and ABA(+) genotypes after salt stress under both pre-treatments (Table 6.7). Chen *et al*. (2003) in a similar work with tomato reported that shoot growth of ABA-deficient mutant tomato (*flacca*) scions grafted onto ABA-normal rootstocks was superior to growth of flacca grafted onto its own rootstock, regardless of the salinity level. In the reciprocal graft, the ABA(-) and ABA(+) as rootstocks also altered the shoot water content of the 9506 scion. The effects of rootstalks in increasing leaf water content (Santa-Cruz *et al*., 2001; 2002; Estañ *et al*., 2005) and increasing leaf osmotic potential of the scion (which was more significant at high salinity levels) (Santa-Cruz *et al*., 2002) indicate that graft unions are not physical barriers to water transport from rootstock to scion (Fernández-García *et al*., 2002). Indeed, graft unions are structurally and chemically very functional in herbaceous plants such as tomato (Fernández-García *et al*., 2004a). Our non-destructive micro-imaging NMR studies on shoot water flow of grafted and non-grafted potato plants grown under normal and salt stress conditions were also consistent with these results (Etehadnia *et al*., 2008a).

While the NaCl pre-treatment in combination with the 9506 as a rootstock increased the shoot water content of ABA(-) after salt stress (Table 6.8), a more dramatic response was observed by the scion impact on root growth (Table 6.9). When the salt stress resistant 9506 scions were grafted onto the ABA(-) rootstocks, by the end of the salt stress treatment, the root dry weight and root water content of the ABA(-) rootstock increased on average 80% with no significant difference between the NaCl and $CaCl_2$ salts. Grafting experiments with salt-

sensitive and salt-tolerant genotypes of mango and chickpea (Dua, 1997; Schmutz and Lüdders, 1999) revealed that scions also play a vital role in salt tolerance of grafted plants. In chickpea, when a salt tolerant scion was grafted onto a sensitive rootstock, the scion remained tolerant to salinity but in the reciprocal grafts, the scion of the sensitive genotype on the tolerant rootstock died a few days after salinization (Dua, 1997). Chen *et al*. (2003), working on tomato, also found that when grafted plants had ABA-normal shoots (Ws), they produced more biomass than did scions that were ABA-deficient mutants (*flacca*). They suggested that this might be due to higher photosynthetic rates in the more vigorous salt-tolerant scions which resulted in greater potential for partitioning of assimilates to the rootstock.

6.5 Conclusion

Overall, the NaCl acclimation pre-treatment was more effective than $CaCl_2$ in protecting plants against subsequent salt stress in both grafted and non-grafted plants. This was associated with a specific Na^+ ion effect rather than a non-specific EC-dependent response. The salt tolerant rootstock with higher endogenous ABA levels (following salt acclimation) positively influenced water content in the more salt sensitive scions after salt stress as reflected through stomatal conductivity, osmotic potential, shoot growth and water content of the scions. Grafting salt stress resistant scions onto the ABA(-) rootstock also led to increased root growth. Grafting of salt-tolerant scions under salt acclimation treatments may be an alternative approach to increasing stress resistance in commercially important potato cultivars. While the $CaCl_2$ acclimation pre-treatment was not as effective as NaCl pre-treatment, the importance of calcium in stress acclimation cannot be excluded, particularly since low levels of Ca^{2+} existed in the background nutrient and NaCl salt stress solutions. Further, both calcium and ABA appear to be involved in increasing the ratio of K^+/ Na^+ in the shoots which was associated with elevated salt stress resistance.

7.0 General discussion

The potato is a key part of the global sustainable food system – representing the most widely grown vegetable crop in Canada and the world (Messer 2000). Potato produces more food energy per unit area than corn, wheat or rice (www.cipotato.org). More than half of the total worldwide production of potato is generated from developing countries, rendering it an important source of income to millions of farmers and good quality food for millions more - often in situations where hunger is a day to day reality. However, potato is moderately susceptible to salinity (Katerji *et al*., 2003), with both growth and yield being adversely affected when soil electrical conductivity (EC) is within the range from 2.16 to 3.38 dS m^{-1} (Katerji *et al*., 2003). Salinization has the potential to significantly negatively impact production in irrigation agriculture, with the most deleterious effects occurring in arid and semiarid regions (Rengasamy *et al*., 2006). NaCl, the most detrimental salt in saline soils, has been shown to negatively impact growth of both cultivated and wild species of potato (Bilski *et al*., 1988a,b; Li *et al*., 2006), and the impact of salinity is anticipated to increase under global climate change due to increase in drought and irrigation.

Salt stress has been recognized as one of the important environmental factors limiting the productivity of crop plants due to negative osmotic effects, and ionic effects on plant growth. The genetic basis of salinity tolerance including heritable variation, selection and breeding are major strategies in developing plants to increase crop tolerance to saline conditions (Ashraf, 1994). However, the mechanisms underlying salinity tolerance remain elusive. The vast majority of studies test the existing salt tolerance levels within the plant and do not assess the ability of the plant to acclimate to salinity. In fact, the vast majority of studies apply salt stress as a salt shock; however, salt shock rarely occurs in the plant habitat. Instead, salt concentrations in the soil solution increase progressively, both within the growing season due to decrease in soil water content, and over years due to build-up of salt. Slow increases in salt concentration over a season may allow plants to sense and adapt or acclimate to this salt stress. However, most previous research studied the impact of sudden and intense salt stress rather than the gradual build-up of salt as occurs under typical growing conditions. This disconnection between real life

and laboratory studies is likely a contributor to the lack of progress towards understanding the mechanism of salt stress tolerance.

To more accurately assess mechanisms of salt adaptation, it is important to reproduce conditions that actually occur in the field. To my knowledge, acclimation to salinity has not been previously studied in potato. Therefore, this thesis included examination of the acclimation response of potato to salt stress. Using four different potato genotypes with varying levels of salinity tolerance, I showed that salt acclimated plants can tolerate higher levels of NaCl compared to non-acclimated plants. Furthermore, the ranking of potato lines for relative tolerance of salt stress changed depending upon whether the plants were acclimated or non-acclimated before exposure to salt stress. This apparent difference in ability to acclimate to salt stress will have a significant impact in selecting appropriate plants for use in breeding programs aimed at increasing salt stress resistance under typical field conditions– i.e; conditions where salinity levels increase gradually. In our study, acclimation in potato genotypes exposed to low levels of salt stress for three weeks was accompanied by increased plant water content and elevated leaf osmotic potential which collectively influenced plant water status. Osmoregulation is a common plant adaptation mechanism under saline conditions (Gupta *et al*., 2000). The more salt-tolerant genotypes, 'Norland' and 9506 had a higher water status and shoot biomass compared to the salt-susceptible group, the ABA-deficient mutant and its ABA-normal sibling. Adaptation level and increase in shoot biomass has also been found to be significantly and positively correlated to each other in sorghum exposed to salt stress (Amzallag and Lerner, 1995). Following gradual acclimation to low levels of salt stress, treated plants had higher elevation of endogenous ABA following salt acclimation than were produced under a salt shock. The increase levels of ABA were associated with increases in stress tolerance in the roots and shoots. Osmotic potential was also generally higher in potato genotypes with higher ABA levels. For example, the highest osmotic potential occurred in the most salt-tolerant genotype, 9506. A modest association between ABA content and osmotic adjustment has been recently found in cassava (Alves and Setter, 2004).

ABA is widely considered to be a stress hormone in plants controlling adaptation of plants to various environmental stresses including cold, drought and salt (Tanino *et al*., 1990, 1991, 1992; Chen and Gusta, 1983; Thomas and Eamus, 1999; Gómez-Codenaz *et al*., 2003; Shaterian *et al*., 2005a; Fricke *et al*., 2006; Khadri *et al.*, 2006). As a plant stress hormone, ABA

mediates the primary responses of plants to environmental stresses such as salinity (Giraudat *et al*., 1994; Chen *et al*., 2006b) by inducing physiological changes that predispose the plant to tolerate salt and water stress (Davies and Mansfield, 1983; Van Steveninck and Van Steveninck, 1983; Lee *et al*., 2003). In our study, the two salt stress sensitive genotypes, particularly the ABA-deficient mutant (relative to its ABA-normal sibling), also showed lower ABA content compared to the two salt-tolerant genotypes after incremental salt stress. This is likely a result of the inability of the ABA mutant to synthesize ABA (De Jong *et al*. (2001). Umezawa *et al*. 2001 concluded that salt tolerance was positively correlated with ABA levels in soybean under salinity stress. Thus, the ABA normal sibling was likely able to survive high salinity stress relative to the ABA-deficient mutant through enhanced synthesis of ABA after salt acclimation. The pattern of salt tolerance paralleled the pattern of endogenous ABA content (after salt acclimation): the most salt-tolerant genotype (9506) had the highest ABA content while the lowest ABA content was found in the salt sensitive ABA-deficient mutant genotype. The strong positive correlation between ABA levels and growth under salt stress, particularly following the salt acclimation treatment, indicates that ABA may be involved in tolerance to salinity stress. The observed differences in salt adaptation abilities between the genotypes is in agreement with previous studies showing that adaptation capacity varies considerably not only among plant species (Baker *et al*., 1986) but also among genotypes within the same species (Amzallag *et al*., 1993; Azevedo Neto *et al*., 2004). The specific and important role of ABA on growth of different crop plants under salinity has previously been shown (Gómez-Cadenas *et al*., 2003; Mulholland *et al*., 2003; Chen *et al*., 2006b; Khadri *et al*., 2007) but had not been widely investigated in potato (Shaterian *et al*., 2005). With the initiation of salt stress, the increase in ABA levels helped the plant to withstand the salt stress, probably through changes in water status and via osmotic adjustment. It is, however, nearly impossible to predict the percentage of endogenous ABA, oxidative degradation of ABA to phaseic acid (PA), and dihydroxyphaseic acid (DPA) can be extremely rapid. Other factors, such as recirculation and exudation may alter the internal ABA concentrations as well (Hartung *et al*., 2005). Since salinity induced simultaneous changes in several hormone groups, dissecting the role of a specific hormone in salinity induced responses will require the use of transgenics, mutants, or other genetic variability to increase or decrease the level of the hormone of interest (Ghanem *et al*., 2008). In the case of tomato, ABA and ethylene content increased linearly with salinization time, while

IAA and total cytokinin contents decreased progressively from the imposition of the stress (Ghanem *et al*., 2008).

Endogenous ABA is involved in regulation of various plant developmental stages e.g. embryo development in seeds, seed dormancy, seed-storage proteins, abscission and juvenility (Zeevaart and Creelman, 1988; Castillo *et al*., 2005). There are reports that elevated ABA concentrations in the leaves and xylem of tomato plants occurred continuously over time in response to salinity stress (Mulholland *et al*., 2003). In spite of the apparent importance of a continuous supply of appropriate levels of ABA, studies examining the impact of exogenous ABA on stress of crop plant have largely focused on a single application (Chen *et al*., 2006b; Khadri *et al*., 2006; 2007). There are no reports on the impact of multiple application of exogenous ABA over time. To our knowledge, this thesis is the first study that analyzed the effect of exogenous ABA applied gradually over time (multiple ABA applications) prior to salt stress application. In general, multiple applications of ABA at low concentration were more effective in inducing salt tolerance in potato genotypes than were higher concentrations of ABA applied as a single application. Moreover, while a single ABA application resulted in vertical shoot growth, plants receiving multiple ABA applications shifted to lateral growth. This increase in lateral growth could be representative of higher dry matter accumulation due to increased stem and leaf growth. The apparent phenotypic plasticity represented by leaf area modulation in Sea hibiscus (*H. tiliaceus*) (Youssef, 2007) and stem growth in *S. maritime* (Small Cordgrass) (Castillo *et al*., 2005) were mechanisms associated with salt stress avoidance. Therefore, gradual application of ABA over time could represent a more effective and "realistic'' method of application compared to a single ABA application which may result in a hormone shock.

The site within the plant regulating salt acclimation responses is unknown. Obvious candidate sites such as the root and shoot can be investigated through the use of reciprocal grafting. Since there were no documented reports of grafting potatoes to study salt stress adaptation, the first step was to develop an approach for reciprocal grafting of different potato genotypes. This was achieved by reciprocal heterographs of salt tolerant genotypes and salt stress sensitive genotypes and autographs of salt tolerant genotypes. The autographs of the two salt sensitive genotypes were unsuccessful, probably due to lack of sufficient hormones (Lee and Oda, 2003). The next step then was to combine the reciprocal grafting and exogenous ABA

as a method to determine the role of roots and shoots. The results of the reciprocal grafting experiments indicated that both scion and rootstock were involved in salt stress tolerance of potato. Scion growth was largely dependent on the rootstock to which it was grafted, i.e., when salt tolerant 9506 was the rootstock, scions of the ABA-deficient mutant and ABA-normal sibling adopted the salt tolerant characteristics of 9506**.** In this regard, the ABA-deficient mutant was the most improved by grafting onto 9506 rootstocks and the second- greatest improvement occurred in the ABA-normal sibling. The salt tolerance observed in the graft combination of ABA(-)/9506, was comparable to 9506 itself. However, the salt-tolerant scion 9506 also improved root growth and thus the salt tolerance of the salt-sensitive ABA-deficient mutant rootstock, 9506/ABA(-). In tomato plants, grafting either scions or rootstocks of the ABA-deficient mutant onto corresponding rootstocks or scions of the ABA normal genotype enhanced growth of the ABA-deficient mutant under salt stress (Chen *et al*., 2002b; Chen *et al*., 2003a). The results obtained with potato suggest that there must be a signal from rootstock to scion and vice versa to control responses to salt stress. ABA has been suggested to play an important role in root-to-shoot (Holbrook *et al.* 2002; Chen *et al*., 2002b; Hartung *et al*., 2005) and shoot-to-root (Chen *et al*., 2002b; Hartung *et al*., 2005) signalling and corresponding regulation of growth under salinity or water stress. Differences in endogenous ABA production of the four potato genotypes following salt acclimation were shown in the first experiment. Therefore, grafting experiments using genotypes with the various levels of ABA could generate evidence for the role of ABA in salt stress tolerance. Grafting scions the ABA-deficient mutant which had the lowest endogenous ABA content onto rootstocks of genotype 9506 which had the higher endogenous levels of ABA induced after salt acclimation appeared to support the role of ABA in enhancing salt tolerance (chapter 5). For tomatoes, Chen *et al.* (2003a) reported a significant positive linear relationship between biomass and ABA supplied from the rootstock to the scion. When ABA-deficient shoots were grafted onto the 9506 rootstock, leaf size and shape as well as stem thickness and profile of the scion shifted toward the profile of 9506. This change was less obvious when the ABA-normal sibling was grafted onto 9506 (chapter 5). Salt stress-induced ABA may improve stomatal control in the ABA-deficient scion (ABA(-)/9506) resulting in a relatively greater plant water status and enhanced growth. These results were similar to those of Chen *et al*. (2003a), who pointed to the importance of ABA in phenotype reversion of the ABA-deficient of tomato *flacca* shoots on ABA-normal wild-type roots. The increased stem diameter

of the ABA-deficient mutant, above and below the graft union in a reciprocal graft with 9506 could also explain the elevated water status of the grafting experiment. The expected opposite effect of the ABA-deficient rootstock decreasing stem diameter of 9506 in the reciprocal graft was not observed; the ABA-deficient scion did have a significant effect on the stem diameter of the 9506 rootstock. It has been reported that a large proportion of the ABA (70%) transported through the xylem to the shoot had originated in the shoot and was subsequently recycled back to the shoot (Chen *et al*., 2002c; Hartung *et al*., 2005). This observation may support our conclusion that 9506 as a scion is providing ABA for the ABA-deficient mutant rootstock and therefore has the potential for elevating salt stress resistance of the meristem for tuber-producing crops. In parallel with the mediating role of internal ABA, which seemed to be provided via grafting, when an additional exogenous ABA treatment was applied, the combined effect on salt stress tolerance of grafted potato genotypes was greater than the effect of grafting alone. A similar response beyond the effect of grafting was observed on the reciprocal ABA(-)/9506 graft combination, when the ABA-deficient mutant was used as a scion on the 9506 resistant rootstock. Salt acclimation also increased the salinity tolerance of the grafted potato genotypes and thus a combination of salt acclimation, exogenous ABA and grafting techniques, may provide the highest level of salt stress tolerance in potato. However, this combination was not directly tested in our study and should be examined in future research.

Nuclear Magnetic Resonance (NMR) imaging was used to explore the effect of rootstock on upward water flow in grafted potato genotypes. Under gradually increasing ABA application, the NMR imaging indicated that exogenous ABA applied to the roots significantly increased upward (+) shoot water flow in both grafted and non-grafted potato plants after salt stress. The effect of ABA on grafted plants was also notable before stress when: a) 9506 was used as a rootstock on the ABA-deficient mutant, and where flow changed from a net downward (-) flow to a more upward flow, and b) when the reciprocal graft of 9506/ABA-deficient mutant changed water flow in the 9506 genotype from a positive to a negative condition which was reversed by the addition of an exogenous ABA treatment.

Calcium content has been shown to be positively correlated with increasing levels of ABA in the plant (Staxen *et al*., 1999). Calcium is also a secondary messenger of ABA and is involved in environmental signalling in response to stresses such as salinity (Rengel, 1992; Poovaiah and Reddy, 1993; Lee *et al*., 2004). Furthermore, it has been shown that ABA triggers

repetitive increases (oscillations) in Ca^{2+} (Allen and others 1999a, 1999b; Staxen *et al*., 1999), suggesting that the dynamics of Ca^{2+} may be critical for the transduction of the ABA signal. We studied the tissue levels of Ca^{2+}, Na^{+}, and K^{+} ions during salt stress treatments. Ca^{2+} content was lower in the ABA-deficient mutant and its normal sibling and higher in 'Norland' and the salt-tolerant genotype 9506 under all treatments tested. The ABA-deficient mutant and its normal sibling showed greater positive growth responses to pre-treatments which involved increased levels of $CaCl_2$ than did 'Norland' and the genotype 9506. This positive effect of the $CaCl_2$ treatment occurred in the roots but not in the shoots. Continued root growth has been considered a distinct morphological adaptation to salt stress (Meloni *et al*., 2001; Mulholland *et al*., 2003; Qaderi *et al*., 2006).

In our study, pre-treatment with calcium ($CaCl_2$) induced Na^{+} accumulation in the roots but reduced Na^{+} in the shoots in the majority of genotypes during subsequent exposure to NaCl salt stress. Pre-treatment with $CaCl_2$ resulted in a change of response for 9506, from being a shoot Na^{+} includer to being a shoot Na^{+} excluder. Calcium may play a part in the Na^{+} exclusion mechanism, mainly by preventing Na^{+} transport to the shoot (Melgar *et al*., 2006). Increased external Ca^{2+} concentrations improved K^{+}/Na^{+} selectivity of an *Arabidopsis* mutant, *sos*3 (salt-overly-sensitive 3). This Ca^{2+} effect was twice as large as was seen in the wild type (Liu and Zhu, 1997). In our study, the salt-sensitive ABA-deficient mutant genotype was also the least efficient in excluding shoot Na^{+} under salt stress; the resulting Na^{+} accumulation in the plants might have been a key factor in the inhibition of plant growth under salinity. However, the salt stress resistant 9506 genotype was also a shoot Na^{+} includer, suggesting that 9506 probably was not able to exclude Na^{+} from the ABA-deficient mutant scion. Grafting salt sensitive scions to salt tolerant rootstocks of melon (*Cyrano/P360*; Colla *et al*., 2006b); (*Arava/TZ-148*; Edelstein *et al*., 2005), watermelon (*Tex/Macis* and *Tex/Ercole*; Colla *et al*., 2006a), and of tobacco/tomato (*Sevilla/Jaguar* and *Sevilla/Brillante*; Ruiz *et al*., 2005) has been reported to reduce the concentrations of Na^{+} in the leaves of grafted plants. The increase in growth in the ABA mutant scion when it was grafted onto the 9506 genotype is believed to reflect the vigorous rootstock's greater potential for water and nutrient absorption, as was observed in melon (Ruiz *et al*. 1997), rather than its exclusion of Na^{+}.

In conclusion, we answered the main questions that motivated the start of this work about salt tolerance in potato plants. First, we wondered whether or not potato plants can

acclimate to salt stress. We showed for the first time that salt tolerance in potato plants could be induced by exposing the plants to low levels of salt prior to exposure to more extreme salinity treatments. The level of tolerance achieved depended upon the genotype, the type and concentration of salt applied for salt acclimation. We were also interested in exploring some of the mechanisms underlying salt tolerance. We were able to report for the first time that in potato plants the endogenous levels of ABA were positively correlated with the degree of salt acclimation achieved. Greater shoot growth in the presence of salt stress and higher endogenous ABA content induced after salt acclimation were characteristic of potato genotypes with a high degree of salt tolerance, while lower shoot growth, and lower ABA content elevated after salt acclimation were associated with salt sensitive potato genotypes. From a practical perspective, under field conditions, crops could potentially be salt acclimated by exposure to low levels of saline irrigation water, then allowed to complete their life cycle with natural sources of water (less salty or completely un-salty). Future research should follow this approach in field trials when good quality water is scarce.

Second, we evaluated the impact of methods of ABA application on the salt tolerance of different potato genotypes found that both single and multiple ABA applications were involved in inducing salt stress tolerance of potato genotypes. Osmotic adjustment and the plant's water status played important roles in this process. Multiple exogenous ABA applications with lower doses of ABA had a greater but different effect on plant responses than a single ABA application on inducing salt stress tolerance of potato genotypes. This was a new finding. Growth (dry matter accumulation) was more lateral with multiple applications of ABA compared to the mainly vertical growth under a single application. Third, we explored the role of shoot and root in regulation salt stress tolerance through reciprocal grafting of salt tolerant and sensitive genotypes. Our results showed that roots played an important role in root to shoot communication leading to increased salt stress resistance. Reciprocal grafting indicated that the shoot also had influenced salt tolerance of potato genotypes, confirming the roles of both scion and rootstock in salt stress tolerance. Both root and shoot tolerance to salt was transmitted from the most salt tolerant genotype, 9506, with its inherently higher levels of ABA, to the salt sensitive genotypes, the ABA-deficient mutant and its ABA-normal sibling with their inherently lower levels of ABA. These results indicated that both the root and shoot collaboratively contributed to the development of salt tolerance in potato genotypes. Therefore, the results of

this work gives us the opportunity to increase yield of commercial cultivars substantially and improve quality under salinity stress by grafting with salt tolerant genotypes through the scion. In addition, in tomato, a very closely related species family of *Solanaceae*, there exists a large collection of wild type and cultivated salt tolerant genotypes available for grafting. Practically, this might be achievable at the large scale by improving technology and using robots in the applied science. Fourth, we studied the effects of NaCl salt acclimation, $CaCl_2$ and NaCl + $CaCl_2$ amendment on potato genotypes under salt stress. Although the genotypes must be taken into consideration, the NaCl pre-treatment was the most effective pre-treatment in inducing salt stress tolerance, followed by $CaCl_2$ and NaCl+$CaCl_2$ pre-treatments. Salt-tolerant genotypes ('Norland') were either able to exclude Na^+ from the shoot or were able to tolerate it (9506). Salt-sensitivity, on the other hand, may depend on the degree of Na^+ exclusion from the shoot. Restriction of absorption/translocation of Na^+ from the root, shoot/root K^+ accumulation, maintenance of K^+/Na^+ and shoot and root growth in the most salt-tolerant genotypes are other mechanisms for dealing with salt under stress. These salt tolerance mechanisms may be mediated, at least partly, through leaf ABA and $CaCl_2$ balance, again pointing to the importance of the shoot in regulating salt stress tolerances. Further greenhouse and field studies with other sources of Ca^{2+} such as Ca_2SO_4 are required to deduce the role of Ca^{2+}, especially when NaCl is the dominant resource of salt. Finally, to study the specific ion effects and scion/rootstock responses on physiological parameters of potato genotypes, different concentrations of NaCl and $CaCl_2$ with equal EC were compared. The observed specific effect of Na^+ on growth enhancement of potato genotypes following NaCl application also indicates the effectiveness of this pre-treatment in inducing salt stress tolerance.

This thesis has provided a foundation for future salt stress work on potato and has indicated the importance of ABA, salt acclimation and grafting for increasing potato salinity tolerance.

8.0 Future work

1- Detailed study of the yield of potatoes following salt acclimation and salt shock with emphasis on examination of stolon and tuber growth.

2-Investigate multiple tolerance: salt acclimation, drought and freezing tolerance and focusing on their relationship to ABA levels in the root and shoot.

3-Quantitate salt shock proteins and proline under salt acclimation and salt shock to get a better understanding of their effect on salt stress tolerance.

4-Measure other hormones like cytokinins and GA.

5-Evaluate the effects exogenous ABA on yields and quality of potato tubers.

6-Examine the role of endogenous and exogenous ABA on size, number and distribution of stomata under salinity stress and the consequential impact on photosynthesis.

7-Grafting scions of wild type or salt tolerant genotypes of potato or tomato onto rootstocks of commercial potato cultivars with emphasis on yield as well as alkaloid compounds in leaves and tubers under salinity stress.

8-Anatomical studies of the roots in grafted and ABA treated plants using NMR imaging technique with particular attention to water flow dynamics.

9- Precise monitoring of ion translocation, re-translocation and sequestration in grafted and ABA treated plants using NMR micro-imaging

10- Research the compartmentalization of toxic ions between vacuole and cytoplasm.

11- Explore the effects of ABA and Ca^{2+} on cation channels in the roots.

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Appendix I

The percentage of micro- and macro-nutrients of 20-20-20 N-P-K fertilizer.

Micro/Macro	**(%)**
Total Nitrogen (N)	20
Phosphoric acid (P_2O_5)	20
Soluble potash (K_2O)	20
Boron (actual) (B)	0.02
Chelated copper (Cu)	0.05
Chelated iron (Fe)	0.1
Chelated manganese	0.05
Molybdenum (actual) (Mo)	0.0005
Chelated zinc (Zn)	0.05
EDTA (Ethylenediamine tetraacetic acid) (Chelating agent)	1.0

Appendix II

Chapters 3, 4, 5, 6. The EC and pH values for different solutions.

Solution (mM NaCl)	EC (dS m^{-1}) (average)	pH
0	1.22	6.52
25	3.02	6.57
33.3	4.80	6.91
50	6.39	6.97
66.6	8.28	7.08
75	8.41	7.13
100	11.40	7.12
125	14.08	6.98
150	16.18	6.96
175	20.90	6.97
180	22.16	6.96

Appendix III

The Analysis of variances of experiments:

Chapter 3-Salt acclimation
3.4. Leaf necrosis

Source	DF	SS	MS	F	Pr > F
Rep	7	3.68	0.52	1.32	0.2613
Conc	1	49.00	49.00	122.73	< .0001
Line	3	11.31	3.77	9.45	< .0001
Conc * Line	3	1.87	0.62	1.57	0.2096
Error	49	19.56	0.39		

C.V. = 18.54

3.4. ABA content

Source	DF	SS	MS	F	Pr > F
Rep	3	21062	7020.64	0.78	0.5196
Conc	2	1048344	524172	58.06	< .0001
Line	3	1275202	425067	47.08	< .0001
Conc * Line	2	335950	167975	18.61	< .0001
Error	21	189586	9027.90		

C.V. = 9.57

3.4. Growth rate

Source	DF	SS	MS	F	Pr > F
Rep	7	0.90	0.12	5.12	0. .0001
Conc	1	2.00	2.00	79.36	< .0001
Line	3	1.77	0.59	23.42	< .0001
Conc * Line	3	0.13	0.04	1.80	0.1505
Rep*Conc*Line	49	2.44	0.04		

C.V. 37.47

3.4. Shoot fresh weight

Source	DF	SS	MS	F	Pr > F
Rep	7	1265.78	180.82	0.85	0.5483
Conc	1	1552.95	1552.95	7.34	< .0093
Line	3	159112.49	53037.49	250.74	< .0001
Conc * Line	3	681.28	227.09	1.07	0.3690
Error	49	10364.74	211.52		

C.V. 21.39

3.4. Shoot dry weight

Source	DF	SS	MS	F	Pr > F
Rep	7	144.44	20.63	1.68	0.1371
Conc	1	1148.87	1148.87	93.29	< .0001
Line	3	7749.40	2583.13	209.76	< .0001
Conc * Line	3	411.43	137.14	11.14	< .0001
Error	49	603.42	12.31		

C.V. = 21.64

3.4. Shoot water content

Source	DF	SS	MS	F	Pr > F
Rep	7	1920.06	274.29	2.64	0.0215
Conc	1	1996.63	1996.63	19.18	< .0001
Line	3	10986.48	3662.16	35.18	< .0001
Conc * Line	3	400.86	133.62	1.28	0.2904
Error	49	5100.62	104.09		

C.V. =15.68

3.4. Leaf water content:

Source	DF	SS	MS	F	Pr > F
Rep	7	1650.35	235.76	1.84	0.0997
Conc	1	295.17	295.17	2.31	0. 135
Line	3	11584.48	3861.49	30.21	< .0001
Conc * Line	3	898.74	299.58	2.34	0.0844
Error	49	6262.94	127.81		

C.V = 15.87

3.4. Leaf osmotic potential

Source	DF	SS	MS	F	Pr > F
Rep	7	0.16	0.02	0.94	0.4849
Conc	1	4.74	4.74	186.15	< .0001
Line	3	1.91	0.63	25.06	< .0001
Conc * Line	3	0.89	0.29	11.70	< .0001
Error	49	1.24	0.02		

C.V. = 12.49

Chapter-4
4.4. A-Single application

Leaf necrosis

Source	DF	SS	MS	F	Pr > F
Rep	3	4.59	1.53	2.30	0.0897
Treat	3	43.28	14.42	21.71	< .0001
Line	3	22.28	7.42	11.18	< .0001
Treat * Line	9	6.40	0.71	1.07	0 .4021
Rep* Treat *Line	45	29.90	0.66		

C.V. = 12.71

B-Multiple applications
4.4. Leaf necrosis

Source	DF	SS	MS	F	Pr > F
Rep	3	0.71	0.23	0.58	0.6317
Treat	3	50.31	16.77	40.99	< .0001
Line	3	3.57	1.19	2.91	0 .0448
Treat * Line	9	24.29	2.69	6.60	0 .0001
Rep* Treat *Line	45	18.41	0.40		

C.V. = 19.17

4.4. A-Single application
Root/shoot

Source	DF	SS	MS	F	Pr > F
Rep	3	0.0051	0.0017	0.78	0.5095
Treat	3	0.1429	0.0476	21.76	< .0001
Line	3	0.3564	0.1188	54.28	< .0001
Treat * Line	9	0.0850	0.0094	4.32	< .0004
Rep* Treat *Line	45	0.0985	0.0021		

C.V. = 25.34

4.4. B-Multiple applications
Root/Shoot

Source	DF	SS	MS	F	Pr > F
Rep	3	0.003	0.0012	0.87	0.4656
Treat	3	0.004	0.0013	0.91	0.4461
Line	3	0.057	0.0191	12.76	0.0001
Treat * Line	9	0.129	0.0143	9.60	0.0001
Rep* Treat *Line	45	0.067	0.0014		

C.V. = 25.13

4.4. A-Single application
Growth rate (after stress)

Source	DF	SS	, MS	F	Pr > F
Rep	3	0.010	0.003	0.37	0.7773
Treat	3	0.836	0.278	27.99	< .0001
Line	3	1.934	0.644	64.72	< .0001
Treat * Line	9	0.333	0.037	3.72	0 .0015
Rep* Treat *Line	45	0.448	0.009		

C. V. = 17.50

4.4. B-Multiple applications
Growth rate

a) Before stress

Source	DF	SS	MS	F	Pr > F
Rep	3	3.23	1.07	3.36	0.0269
Treat	3	4.20	1.40	4.36	< .0088
Line	3	13.07	4.35	13.58	< .0001
Treat * Line	9	6.16	0.68	2.13	< .0461
Rep* Treat *Line	45	14.44	0.32		

C.V. = 24.17

b) After stress

Source	DF	SS	MS	F	Pr > F
Rep	3	0.77	0.25	1.74	0.1725
Treat	3	1.29	0.43	2.91	0 .0445
Line	3	2.83	0.94	6.39	0 .0011
Treat * Line	9	1.35	0.15	1.02	0 .4410
Rep* Treat *Line	45	6.65	0.14		

C.V. = 30.51

4.4. A-Single application
Root fresh weight

Source	DF	SS	MS	F	Pr > F
Rep	3	32.09	10.69	1.69	0.1835
Treat	3	365.46	121.82	19.20	< .0001
Line	3	10855	3618.34	570.16	< .0001
Treat * Line	9	144.18	16.02	2.52	0.0195
Rep* Treat *Line	45	285.57	6.34		

C.V. = 12.73

4.4. B-Multiple applications
Root fresh weight

Source	DF	SS	MS	F	Pr > F
Rep	3	13.37	4.45	0.87	0.4630
Treat	3	1498.99	499.66	97.63	< .0001
Line	3	7464.56	2488.18	486.15	< .0001
Treat * Line	9	1556.14	172.90	33.78	< .0001
Rep* Treat *Line	45	230.31	5.11		

C.V. = 18.01

4.4. A-Single application
Root dry weight

Source	DF	SS	MS	F	Pr > F
Rep	3	3.07	1.02	1.42	0.2483
Treat	3	25.86	8.62	11.97	< .0001
Line	3	509.87	169.95	236.00	< .0001
Treat * Line	9	19.89	2.21	3.07	0 .0059
Rep* Treat *Line	45	32.40	0.72		

C.V. = 21.95

4.4. B-Multiple applications
Root dry weight

Source	DF	SS	MS	F	Pr > F
Rep	3	1.41	0.47	1.09	0.3647
Treat	3	13.89	4.63	10.70	< .0001
Line	3	260.86	86.95	200.91	< .0001
Treat * Line	9	11	1.22	2.83	< .0101
Rep* Treat *Line	45	19.47	0.43		

C.V. = 25.00

4.4. A-Single application
Root water content

Source	DF	SS	MS	F	Pr > F
Rep	3	76.35	25.45	0.41	0.7485
Treat	3	0.78	0.26	0.00	.9996
Line	3	2255.77	751.92	12.03	< .0001
Treat * Line	9	488.65	54.29	0.87	< .5594
Rep* Treat *Line	45	2812.56	62.50		

C.V. = 8.97

4.4. B-Multiple applications
Root water content

Source	DF	SS	MS	F	Pr > F
Rep	3	42.18	14.06	0.34	0.7952
Treat	3	3034.31	1011.43	24.59	< .0001
Line	3	946.15	315.38	7.67	0.0003
Treat * Line	9	6274.01	697.11	16.95	< .0001
Rep* Treat *Line	45	1851.04	41.13		

C.V. = 8.64

4.4. A-Single application
Shoot fresh weight

Source	DF	SS	MS	F	Pr > F
Rep	3	270.87	90.29	0.58	0.6314
Conc	3	48736	16245	104.30	< .0001
Treat	3	195328	65109	418.02	< .0001
Treat * Line	9	22964	2551.50	16.38	< .0001
Rep* Treat *Line	45	7009.10			

C.V. = 11.49

4.4. B-Multiple applications
Shoot fresh weight

Source	DF	SS	MS	F	Pr > F
Rep	3	1266.04	422.01	2.37	0.0832
Treat	3	136879	45626	256.14	< .0001
Line	3	675132	225044	1263.34	< .0001
Treat * Line	9	117080	13009	73.03	< .0001
Rep* Treat *Line	45	8016.02			

C.V. = 10.20

4.4. A-Single application
Shoot dry weight

Source	DF	SS	MS	F	Pr > F
Rep	3	3.99	1.33	0.27	0.8472
Treat	3	101.67	33.89	6.86	< .0007
Line	3	6684.95	2228.31	451.15	< .0001
Treat * Line	9	119.72	13.30	2.69	0 .0135
Rep* Treat *Line	45	222.26	4.93		

C.V. = 10.54

4.4. B-Multiple applications
Shoot dry weight

Source	DF	SS	MS	F	Pr > F
Rep	3	8.73	2.91	0.26	0.8558
Treat	3	1894.21	631.40	55.78	< .0001
Line	3	20.77	6925.52	611.80	< .0001
Treat * Line	9	2346.08	260.67	23.03	< .0001
Rep* Treat *Line	45	509.40	11.32		

C.V. = 11.91

4.4. A-Single application
Shoot water content

Source	DF	SS	MS	F	Pr > F
Rep	3	4.21	1.40	0.13	0.943
Treat	3	2367.02	789.00	71.66	< .0001
Line	3	307.37	102.45	9.31	< .0001
Treat * Line	9	728.03	80.89	7.35	< 0.001
Rep* Treat *Line	45	495.46	11.01		

C.V. = 5.45

4.4. B-Multiple applications
Shoot water content

Source	DF	SS	MS	F	Pr > F
Rep	3	29.59	9.86	0.90	0.4505
Treat	3	3339.04	1113.01	101.12	< .0001
Line	3	137.53	45.84	4.17	< .0110
Treat * Line	9	608.72	67.63	6.15	< .0001
Rep* Treat *Line	45	495.28	11.00		

C.V. = 3.24

4.4. A-Single application
Leaf stomatal conductivity

Source	DF	SS	MS	F	Pr > F
Rep	3	0.020	0.006	3.67	0.0190
Treat	3	0.421	0.140	75.00	< .0001
Line	3	0.020	0.006	3.67	< .0190
Treat * Line	9	0.111	0.012	6.59	< .0001
Rep* Treat *Line	45	0.084	0.001		

C.V. = 23.75

4.4. B-Multiple applications
Leaf stomatal conductivity

Source	DF	SS	MS	F	Pr > F
Rep	3	0.0002	0.0000	0.53	0.6615
Treat	3	0.0084	0.0028	18.34	< .0001
Line	3	0.0000	0.0000	0.18	< .9085
Treat * Line	9	0.0066	0.0007	4.77	< .0002
Rep* Treat *Line	45	0.0066	0.0001		

C.V. =16.84

4.4. A-Single application
Leaf water content

Source	DF	SS	MS	F	Pr > F
Rep	3	784.24	261.41	9.18	0.0001
Treat	3	3546.94	1182.31	41.53	< .0001
Line	3	8828.08	2942.69	103.37	< .0001
Treat * Line	9	5032.06	559.118	19.64	< .0001
Rep* Treat *Line	45	1281.04	28.46		

C.V. = 4.37

4.4. B-Multiple applications
Leaf water content

Source	DF	SS	MS	F	Pr > F
Rep	3	406.39	135.46	1.62	0.1988
Treat	3	8568.04	2856.01	34.08	< .0001
Line	3	1191.22	397.07	4.74	0.0059
Treat * Line	9	2683.88	298.20	3.56	0 .0021
Rep* Treat *Line	45	3771.48	83.81		

C.V. = 4.12

4.4. A-Single application
Leaf osmotic potential

Source	DF	SS	MS	F	Pr > F
Rep	3	0.30	0.10	1.23	0.3071
Treat	3	20.72	6.90	83.57	< .0001
Line	3	9.22	3.07	37.20	< .0001
Treat * Line	9	5.40	0.60	7.26	< .0001
Rep* Treat *Line	45	13.90	0.30		

C.V. = 13.42

4.4. B-Multiple applications
Leaf osmotic potential

Source	DF	SS	MS	F	Pr > F
Rep	3	1.01	0.33	3.66	0.0191
Treat	3	4.55	1.51	16.35	< .0001
Line	3	11.60	3.86	41.68	< .0001
Treat * Line	9	9.37	1.04	11.22	< .0001
Rep* Treat *Line	45	4.17	0.09		

C.V. = 11.07

4.4. B-Multiple applications
Stem water content

Source	DF	SS	MS	F	Pr > F
Rep	3	250.53	83.51	1.34	0.2746
Treat	3	3076.08	1025.36	16.40	< .0001
Line	3	706.09	235.36	3.77	0.0170
Treat * Line	9	519.39	57.71	0.92	0.5143
Rep* Treat *Line	45	2813.08	62.51		

C.V. = 11.07

Chapter 5-

Salt acclimation and grafting
5.4.1. Leaf necrosis

Source	DF	SS	MS	F	Pr > F
Rep	2	0.16	0.08	0.25	0.7843
Conc	1	9.86	9.86	29.02	< .0001
Line	6	48.26	8.04	23.68	< .0001
Conc * Line	6	0.70	0.11	0.34	0.9070
Rep*Conc*Line	26	8.83	0.33		

C.V.= 22.64

ABA and grafting
5.4.1. Leaf necrosis

Source	DF	SS	MS	F	Pr > F
Rep	3	2.21	0.73	1.49	0.2316
Treat	1	67.89	67.89	137.29	< .0001
Line	6	16.23	2.70	5.47	< .0001
Treat * Line	6	6.57	1.09	2.22	0.0618
Rep* Treat *Line	39	19.28	0.49		

C.V.= 22.10

Salt acclimation and grafting
5.4.1. Chlorophyll content

Source	DF	SS	MS	F	Pr > F
Rep	2	76.49	38.24	1.77	0.1905
Conc	1	1.33	1.33	0.06	0.8061
Line	6	2340.89	390.14	18.04	< .0001
Conc * Line	6	1068.47	178.07	8.23	< .0001
Rep*Conc*Line	26	562.24	21.62		

C.V.= 9.70

Salt acclimation and grafting
5.4.2. Root fresh weight

Source	DF	SS	MS	F	Pr > F
Rep	2	0.12	0.061	0.02	0.9836
Conc	1	143.70	143.70	38.68	< .0001
Line	6	1297.04	216.17	58.18	< .0001
Conc * Line	6	45.02	7.50	2.02	0.0991
Rep*Conc*Line	26	96.60	3.71		

C.V.= 14.81

Salt acclimation and grafting
5.4.2. Root dry weight

Source	DF	SS	MS	F	Pr > F
Rep	2	0.05	0.028	0.28	0.7550
Conc	1	10.11	10.11	99.12	< .0001
Line	6	105.37	17.56	172.18	< .0001
Conc * Line	6	5.67	0.94	9.28	< .0001
Rep*Conc*Line	26	2.65	0.10		

C.V.= 25.34

Salt acclimation and grafting
5.4.4. Root water content

Source	DF	SS	MS	F	Pr > F
Rep	2	57.14	28.57	0.75	0.4829
Conc	1	4.78	4.78	0.13	0.7261
Line	6	2589.54	431.59	11.31	< .0001
Conc * Line	6	464.95	77.49	2.03	0.0975
Rep*Conc*Line	26	992.26	38.16		

C.V.= 5.71

Salt acclimation and grafting
5.4.2. Shoot fresh weight

Source	DF	SS	MS	F	Pr > F
Rep	2	158.04	79.02	1.18	0.3222
Conc	1	13036	13036	195.19	< .0001
Line	6	165141	27524	412.10	< .0001
Conc * Line	6	13431	2238.47	33.52	< .0001
Rep*Conc*Line	26	1736.48	66.78		

C.V.= 10.02

Salt acclimation and grafting
5.4.2. Shoot dry weight

Source	DF	SS	MS	F	Pr > F
Rep	2	4.49	2.24	0.19	0.8245
Conc	1	866.82	866.82	75.07	< .0001
Line	6	6604.67	1100.77	95.33	< .0001
Conc * Line	6	162.37	27.06	2.34	0.0608
Rep*Conc*Line	26	300.23	11.54		

C.V.= 16.92

Salt acclimation and grafting
5.4.4. Shoot water content

Source	DF	SS	MS	F	Pr > F
Rep	2	5.40	2.70	0.17	0.8479
Conc	1	215.61	215.61	13.23	< .0012
Line	6	3110.93	518.48	31.82	< .0001
Conc * Line	6	748.53	124.75	7.66	< .0001
Rep*Conc*Line	26	423.59	16.29		

C.V.= 4.23

Salt acclimation and grafting

5.4.3. Stem diameter
a) Upper part of graft union

Source	DF	SS	MS	F	Pr > F
Rep	2	9.97	4.98	2.92	0.0717
Conc	1	0.19	0.19	0.11	0.7410
Line	6	202.79	33.79	19.81	< .0001
Conc * Line	6	16.60	2.76	1.62	0.1811
Rep*Conc*Line	26	44.36	1.70		

C.V.= 13.25

5.4.3. Stem diameter
b) Lower part of graft union

Source	DF	SS	MS	F	Pr > F
Rep	2	0.59	0.29	0.49	0.617
Conc	1	0.50	0.50	0.83	0.370
Line	6	139.12	23.18	38.31	< .0001
Conc * Line	6	4.76	0.79	1.31	0.2867
Rep*Conc*Line	26	15.73	0.60		

C.V.= 14.22

ABA and grafting
5.4.3. Stem diameter

a) Upper part of graft union

Source	DF	SS	MS	F	Pr > F
Rep	3	5.43	1.81	1.46	0.2391
Treat	1	0.02	0.02	0.02	0.8859
Line	6	117.32	19.55	15.81	< .0001
Treat * Line	6	10.18	1.69	1.37	0.2500
Rep* Treat *Line	39	48.23	1.23		

C.V.= 14.61

5.4.3. b) Lower part of graft union

Source	DF	SS	MS	F	Pr > F
Rep	3	2.91	0.97	0.83	0.4881
Treat	1	0.14	0.14	0.12	0.7313
Line	6	114.59	19.09	16.24	< .0001
Treat * Line	6	11.16	1.86	1.58	0.1781
Rep* Treat *Line	39	45.86	1.17		

C.V.= 13.35

5.4.4. ABA and grafting
Stem water content

a) Below graft union

Source	DF	SS	MS	F	Pr > F
Rep	3	289.62	96.54	1.03	0. 3917
Treat	1	2062.63	2062.63	21.92	< .0001
Line	6	3182.80	530.46	5.64	0.0005
Treat * Line	6	2921.95	486.99	5.18	0.0005
Rep* Treat *Line	39	3669.84	94.09		

C.V.= 10.17

5.4.4. b) Above graft union

Source	DF	SS	MS	F	Pr > F
Rep	3	238.26	79.42	1.12	0.3511
Treat	1	1673.85	1673.85	23.69	< .0001
Line	6	2231.33	371.88	5.26	< .0001
Treat * Line	6	994.22	165.70	2.35	0.0498
Rep* Treat *Line	39	2755.70	70.65		

C.V.= 11.13

Salt acclimation and grafting
5.4.4. Leaf water content

Source	DF	SS	MS	F	Pr > F
Rep	2	434.19	217.09	7.09	0.0035
Conc	1	173.31	173.31	5.66	0.0249
Line	6	799.57	133.26	4.35	0.0036
Conc * Line	6	44.44	7.40	0.24	0.9582
Rep*Conc*Line	26	795.68	30.60		

C.V.= 4.13

Salt acclimation and grafting
5.4.5. Osmotic potential

Source	DF	SS	MS	F	Pr > F
Rep	2	0.04	0.020	0.58	0.5670
Conc	1	2.08	2.080	58.23	< .0001
Line	6	5.26	0.877	24.55	< .0001
Conc * Line	6	1.70	0.284	7.98	< .0001
Rep*Conc*Line	26	0.92	0.035		

C.V.= 7.43

Salt acclimation and grafting
5.4.4. Stem water content

Source	DF	SS	MS	F	Pr > F
Rep	2	517.23	258.61	8.32	0.0016
Conc	1	397.63	397.63	12.79	0.0014
Line	6	2186.99	364.49	11.72	< .0001
Conc * Line	6	1095.38	182.56	5.87	0.0006
Rep*Conc*Line	26	808.42	31.09		

C.V.= 7.68

Salt acclimation and grafting
5.4.6. Stomatal conductivity

Source	DF	SS	MS	F	Pr > F
Rep	2	0.00002	0.000010	0.12	0.8899
Conc	1	0.00840	0.008400	91.82	< .0001
Line	6	0.19104	0.031841	348.05	< .0001
Conc * Line	6	0.00418	0.000697	7.62	< .0001
Rep*Conc*Line	26	0.00237	0.000091		

C.V.= 7.79

5.4.4. ABA and grafting
Leaf water content

a) Before stress

Source	DF	SS	MS	F	Pr > F
Rep	3	1102.19	367.39	3.31	0.0300
Treat	1	122.47	122.47	1.10	0.3002
Line	6	509.26	84.87	0.76	0.6025
Treat * Line	6	442.20	73.70	0.66	0.6794
Rep* Treat *Line	39	4332.37	111.06		

C.V.= 12.05

5.4.4. b) After stress

Source	DF	SS	MS	F	Pr > F
Rep	3	198.50	66.16	0.93	0.4347
Treat	1	4834.99	4834.99	68.06	< .0001
Line	6	9616.93	1602.82	22.56	< .0001
Treat * Line	6	5916.34	986.05	13.88	< .0001
Rep* Treat *Line	39	2770.44	71.03		

C.V.= 11.58

ABA and grafting
5.4.5. Leaf osmotic potential
a) Before stress

Source	DF	SS	MS	F	Pr > F
Rep	3	0.28	0.09	1.42	0.2509
Treat	1	0.91	0.91	13.61	< .0007
Line	6	1.08	0.18	2.69	0.0278
Treat * Line	6	0.81	0.13	2.03	0. 0851
Rep* Treat *Line	39	2.60	0.06		

C.V.= 20.27

5.4.5 b) After stress

Source	DF	SS	MS	F	Pr > F
Rep	3	0.03	0.01	0.17	0.9156
Treat	1	7.79	7.79	113.06	< .0001
Line	6	19.86	3.31	48.03	< .0001
Treat * Line	6	7. 62	1.27	18.44	< .0001
Rep* Treat *Line	39	2. 68	0.06		

C.V.= 11.92

ABA and grafting
5.4.6. Stomatal conductivity
a) Before stress

Source	DF	SS	MS	F	Pr > F
Rep	3	0.007	0.002	1.20	0.3210
Treat	1	0.129	0.129	66.11	< .0001
Line	6	0.033	0.005	2.84	0.0215
Treat * Line	6	0.031	0.005	2.66	0.0294
Rep* Treat *Line	39	0.076	0.001		

C.V.= 22.15

5.4.6. b) After stress

Source	DF	SS	MS	F	Pr > F
Rep	3	0.0007	0.0002	3.27	0.0311
Treat	1	0.0255	0.0255	326.55	< .0001
Line	6	0.0109	0.0018	23.42	< .0001
Treat * Line	6	0.0059	0.0009	12.71	< .0001
Rep* Treat *Line	39	0.0030	0.0000		

C.V.= 20.34

ABA and grafting
5.4.6. NMR
Water flow:

a) Before stress

Source	DF	SS	MS	F	Pr > F
Rep	3	0.031	0.01	0.36	0.78
Treat	1	1.56	1.56	53.89	0.0001
Line	3	0.94	0.31	10.82	< .0002
Treat * Line	3	o.45	0.15	5.23	0.0074

C.V.= 524.57

5.4.6. b) After stress

Source	DF	SS	MS	F	Pr > F
Rep	3	0.052	0.017	0.68	0.5727
Treat	1	0.19	0.196	7.73	0.0112
Line	3	0.28	0.095	3.37	< 0.0270
Treat * Line	3	0.09	0.033	1.30	0.2993

C.V.= 147.19

Chapter 6- Calcium and grafting
6.4.1. Leaf necrosis, Calcium

Source	DF	SS	MS	F	Pr > F
Rep	3	2.76	0.92	2.47	0.0743
Conc	3	116.12	38.70	103.57	< .0001
Line	3	18	6.00	16.06	< .0001
Conc * Line	9	35.37	3.93	10.52	< .0001
Rep*Conc*Line	45	16.81	0.37		

C.V. = 25.08

6.4.1. Leaf necrosis, grafting

Source	DF	SS	MS	F	Pr > F
Rep	4	2.94	0.73	2.01	0.1071
Conc	1	0.59	0.59	1.62	0 .2085
Line	6	5.08	0.84	2.31	0 .0470
Conc * Line	6	3. 56	0.59	1.62	0.16 01
Error	52	19.05	0.36		

C.V. = 22.94

6.4.2. Leaf greenness, grafting

Source	DF	SS	MS	F	Pr > F
Rep	4	90.70	22.67	1.92	0.1205
Conc	1	341.88	341.88	28.99	< .0001
Line	6	1509.82	251.63	21.34	< .0001
Conc * Line	6	752. 42	125.40	10.63	< .0001
Error	52	613.27	11.79		

C.V. = 7.61

6.4.3. Shoot fresh weight, Calcium

Source	DF	SS	MS	F	Pr > F
Rep	3	2594.11	864.70	1.89	0.1450
Conc	3	337731	112577	245.94	< .0001
Line	3	294759	98253	214.65	< .0001
Conc * Line	9	148067	16452	35.94	< .0001
Rep*Conc*Line	45	20598	457.73		

C.V. = 15.02

6.4.3. Shoot dry weight, Calcium

Source	DF	SS	MS	F	Pr > F
Rep	3	90.04	30.01	0.64	0.5937
Conc	3	4616.88	1538.96	32.78	< .0001
Line	3	15717.00	5238.99	111.59	< .0001
Conc * Line	9	3249.55	361.06	7.69	< .0001
Rep*Conc*Line	45	2112.74			

C.V. = 19.32

6.4.3. Shoot water content, Calcium

Source	DF	SS	MS	F	Pr > F
Rep	3	47.53	15.84	0.31	0.8148
Conc	3	6586.28	2195.42	43.58	< .0001
Line	3	2356.08	785.36	15.59	< .0001
Conc * Line	9	2844.35	316.03	6.27	< .0001
Rep*Conc*Line	45	2266.74	50.37		

C.V. = 8.81

6.4.3. Root fresh weight, Calcium

Source	DF	SS	MS	F	Pr > F
Rep	3	0.45	0.15	0.28	0.8390
Conc	3	36.96	12.32	22.63	< .0001
Line	3	48.57	16.19	29.74	< .0001
Conc * Line	9	49.54	5.50	10.11	< .0001
Rep*Conc*Line	45	24.50	0.54		

C.V. = 21.00

6.4.3. Root dry weight, Calcium

Source	DF	SS	MS	F	Pr > F
Rep	3	0.60	0.20	1.17	0.3330
Conc	3	7.06	2.35	13.70	< .0001
Line	3	8.40	2.80	16.30	< .0001
Conc * Line	9	6.39	0.71	4.13	0.0006
Rep*Conc*Line	45	7.73	0.17		

C.V. = 31.42

6.4.3. Root water content, Calcium

Source	DF	SS	MS	F	Pr > F
Rep	3	153.16	51.05	0.35	0.7918
Conc	3	981.86	327.28	2.22	0.0986
Line	3	820.77	273.59	1.86	0.1505
Conc * Line	9	1597.31	177.47	1.20	0.3159
Rep*Conc*Line	45	6629.44	147.32		

C.V. = 13.73

6.4.4. Leaf stomatal conductivity, Calcium

Source	DF	SS	MS	F	Pr > F
Rep	3	0.0001	0.00004	0.47	0.7012
Conc	3	0.0087	0.00293	31.95	< .0001
Line	3	0.0337	0.01124	122.56	< .0001
Conc * Line	9	0.0116	0.00129	14.10	< .0001
Rep*Conc*Line	45	0.0041	0.00009		

C.V. = 32.02

6.4.4. Leaf osmotic potential, Calcium

Source	DF	SS	MS	F	Pr > F
Rep	3	0.50	0.16	1.38	0.2601
Conc	3	4.67	1.55	12.79	< .0001
Line	3	9.65	3.21	26.42	< .0001
Conc * Line	9	5.16	0.57	4.70	< .0002
Rep*Conc*Line	45	5.48	0.12		

C.v. = 7.45

6.4.3. Leaf water content

Source	DF	SS	MS	F	Pr > F
Rep	3	191.02	63.67	1.24	0.3070
Conc	3	2439.57	813.19	15.81	< .0001
Line	3	106o.21	353.40	6.87	< .0007
Conc * Line	9	3033.46	337.05	6.55	< .0001
Rep*Conc*Line	45	2314.20	51.42		

C.V. = 6.02

Ion concentration

6.4.5. Shoot

6.4.5. a) Calcium content

Source	DF	SS	MS	F	Pr > F
Rep	3	0.89	0.29	0.68	0.5663
Conc	3	239.44	79.81	182.54	< .0001
Line	3	3208.29	1069.43	2445.79	< .0001
Conc * Line	9	154.61	17.17	39.29	< .0001
Error	45	19.67	0.43		

C.V.= 5.44

6.4.5. b) Sodium content

Source	DF	SS	MS	F	Pr > F
Rep	3	11.18	3.72	1.20	0.3191
Conc	3	909.66	303.22	97.95	< .0001
Line	3	634.00	211.33	68.27	< .0001
Conc * Line	9	1292.06	143.56	46.38	< .0001
Error	45	139.30	3.09		

C.V.= 7.77

6.4.5. c) Potassium content

Source	DF	SS	MS	F	Pr > F
Rep	3	17.16	5.72	0.62	0.6041
Conc	3	1772.47	590.82	64.27	< .0001
Line	3	291.14	97.04	10.56	< .0001
Conc * Line	9	203.22	22.58	2.46	0.0227
Error	45	413.69	9.19		

C.V.= 6.26

6.4.5. d) Potassium / sodium

Source	DF	SS	MS	F	Pr > F
Rep	3	0.30	0.10	1.23	0.3099
Conc	3	4.35	1.45	17.72	< .0001
Line	3	6.21	2.07	25.31	< .0001
Conc * Line	9	12.80	1.42	17.37	< .0001
Error	45	3.68	0.08		

C.V.= 12.39

6.4.5. Root

6.4.5. a) Calcium

Source	DF	SS	MS	F	Pr > F
Rep	3	5.01	1.67	0.96	0.4222
Conc	3	76.88	25.62	14.65	< .0001
Line	3	782.39	260.79	149.10	< .0001
Conc * Line	9	91.44	10.16	5.81	< .0001
Error	45	78.71	1.74		

C.V.= 19.46

6.4.5. b) Sodium

Source	DF	SS	MS	F	Pr > F
Rep	3	0.98	0.32	0.66	0.5791
Conc	3	758.85	252.95	512.17	< .0001
Line	3	470.19	156.73	317.35	< .0001
Conc * Line	9	395.19	43.91	88.91	< .0001
Error	45	22.24	0.49		

C.V.= 3.21

6.4.5. c) Potassium

Source	DF	SS	MS	F	Pr > F
Rep	3	0.017	0.0059	0.10	0.9581
Conc	3	676.27	225.42	3917.89	< .0001
Line	3	462.04	154.01	2676.80	< .0001
Conc * Line	9	485.54	53.94	937.64	< .0001
Error	45	2.58	0.057		

C.V.= 3.20

6.4.5. d) Potassium / sodium

Source	DF	SS	MS	F	Pr > F
Rep	3	0.0003	0.0001	0.88	0.4574
Conc	3	1.2058	0.4019	3039.86	< .0001
Line	3	0.6464	0.2154	1629.61	< .0001
Conc * Line	9	0.7126	0.0791	598.87	< .0001
Error	45	0.0059	0.0001		

C.V.= 3.38

6.4.6. Stomatal conductivity before stress, grafting

Source	DF	SS	MS	F	Pr > F
Rep	4	0.0021	0.0005	2.91	0.0302
Conc	1	0.0010	0.0010	5.75	0.0201
Line	6	0.0048	0.0081	4.48	0 .0010
Conc * Line	6	0.0066	0.0011	6.14	< .0001
Error	52	0.0094	0.0001		

C.V. = 29.34

6.4.6. Stomatal conductivity after stress, grafting

Source	DF	SS	MS	F	Pr > F
Rep	4	0.0005	0.0001	1.32	0.2752
Conc	1	0.0002	0.0002	2.77	0.1024
Line	6	0.0083	0.0013	13.72	< .0001
Conc * Line	6	0.0025	0.0004	4.21	0 .0016
Error	52	0.0052	0.0001		

C.V. = 30.10

6.4.6. Osmotic potential before stress, grafting

Source	DF	SS	MS	F	Pr > F
Rep	4	0.26	0.06	2.34	0.0672
Conc	1	0.89	0.89	31.87	< .0001
Line	6	4.61	0.76	27.43	< .0001
Conc * Line	6	0.55	0.09	3.30	0 .0079
Error	52	1.45	0.02		

C.V. = 10.15

6.4.6. Osmotic potential after stress, grafting

Source	DF	SS	MS	F	Pr > F
Rep	4	0.37	0.09	1.98	0.1110
Conc	1	0.43	0.43	9.11	0.0039
Line	6	5.34	0.89	18.63	< .0001
Conc * Line	6	1.36	0.22	4.77	0 .0006
Error	52	2.48	0.04		

C.V. = 11.36

6.4.6. Leaf water content before stress, grafting

Source	DF	SS	MS	F	Pr > F
Rep	4	161.07	40.26	1.35	0.2629
Conc	1	79.67	79.67	2.68	0.1078
Line	6	3405.85	567.64	19.08	< .0001
Conc * Line	6	600.21	100.03	3.36	0 .0071
Error	52	1547.21	29.75		

C.V. = 7.23

6.4.6. Leaf water content after stress, grafting

Source	DF	SS	MS	F	Pr > F
Rep	4	254.17	63.54	1.79	0.1453
Conc	1	61.10	61.10	1.72	0.1955
Line	6	3384.92	564.15	15.87	< .0001
Conc * Line	6	2478.36	413.06	11.62	< .0001
Error	52	1848.07	35.53		

C.V. = 9.13

6.4.6. Shoot dry weight, grafting

Source	DF	SS	MS	F	Pr > F
Rep	4	6.62	1.65	0.39	0.8151
Conc	1	0.44	0.44	0.10	0.7488
Line	6	1771.37	295.22	69.50	< .0001
Conc * Line	6	170. 74	28.45	6.70	< .0001
Error	52	220.88	4.24		

C.V. = 24.24

6.4.6. Shoot water content, grafting

Source	DF	SS	MS	F	Pr > F
Rep	4	67.41	16.85	0.92	0.4620
Conc	1	0.17	0.17	0.01	0.9221
Line	6	3974.41	662.40	35.98	< .0001
Conc * Line	6	1083. 60	180.60	9.81	< .0001
Error	52	957.41	18.41		

C.V. = 5.38

6.4.6. Root dry weight, grafting

Source	DF	SS	MS	F	Pr > F
Rep	4	0.75	0.18	1.54	0.2030
Conc	1	0.05	0.05	0.44	0.5077
Line	6	6.77	1.12	9.24	< .0001
Conc * Line	6	1. 52	0.25	2.08	0 .0719
Error	52	6.34	0.12		

C.V. = 30.57

6.4.6. Root water content, grafting

Source	DF	SS	MS	F	Pr > F
Rep	4	85.27	21.31	0.75	0.5620
Conc	1	370.25	370.25	13.04	0.0007
Line	6	7232.83	1205.47	42.45	< .0001
Conc * Line	6	417. 52	69.58	2.45	0 .0367
Error	52	1476.52	28.39		

C.V. = 7.62

Appendix IV

Chapter 3.0 ABA measurement: Leaf samples were harvested after three weeks of salt pre-treatment, immediately frozen in liquid N_2, stored at –20 ^{o}C and lyophilised prior to extraction. Approximately 100 mg of dried samples were placed in a mortar. This sample was ground with 3 mL of aqueous 80 % acetone (v/v) containing 1% acetic acid (v/v). The internal standard D3-ABA was added to each sample at 10 µL (2 ng mL $^{-1}$). The samples were mixed and transferred to 1.5 mL microfuge tube and centrifuged (Beckman Microfuge E^{TM} U.S.A.) for 1 min (13,000 rpm). The residue was re-extracted with 2 mL of 99% acetone (v/v) with 1% acetic acid (v/v) and centrifuged at 13,000 rpm for one min. The combined supernatant was transferred into a 1x10 cm glass tube and dried by stream on nitrogen evaporator (Organomation Associates Inc. N-Evap^{TM} 112, U.S.A.). Then an Oasis HLB 1cc cartridge (Waters U.S.A.) was conditioned with 1 mL methanol and equilibrated with 1 mL water under vacuum. The extract was dissolved in 200 mL 99% methanol (v/v) containing 1% acetic acid and mixed with 800 mL 1% acetic acid (v/v). To make the solution clear, the extract was transferred to a 1.5 mL micro-centrifuge tube and centrifuged for 1 min at 13,000 rpm. The supernatant was loaded onto the cartridge and washed with 1 mL water under vacuum. ABA was eluted from the column with 1 mL of 80% methanol containing 1% acetic acid. After drying by speed vacuum (Eppendorf Vacufuge ^{TM}, Brinkmann Instruments, Inc. Canada), the ABA was ready for LC/MS analysis.

Liquid chromatograph (LC) and mass spectrometry (MS) was used to quantitate abscisic acid (ABA). A 2.1x100 mm reverse phase Genesis C18 column (Jones Chromatography Hengoed, U.K.) was used with Agilent Technologies (Palo Alto, CA, USA) HP1100 series HPLC system to separate abscisic acid from other components of the plant extracts. A binary gradient was used for this chromatographic separation, where solvent A=0.07% acetic acid and B=90:10 acetonitrile:water with 0.07% acetic acid giving a constant pH=3.3. The gradient used was started at a solvent ratio of A:B of 85:15. This ratio changed to 67:33 over 10 min then was ramped to an A:B ratio of 0:100 by 16.7 min and remained at this ratio until 18.0 min, the column is then stabilized to its starting ratio value of 85:15, elution time of ABA is 12.3 min. Detection of ABA was performed by negative ion electrospray tandem mass spectrometry with a Micromass Quattro LC (Manchester,UK), quantification is done against an internal standard a deutro-analogue of ABA; d_3-abscisic acid (d_3-ABA supplied by NRC-PBI Saskatoon, SK

Canada). Multiple reaction monitoring (MRM) was used to detect and quantify ABA, in particular negative ion transitions of ABA 263>153m/z and 266>156m/z for d_3-ABA. Amounts of ABA were calculated from a calibration curve of integrated chromatogram area response ratios of ABA/d_3-ABA against concentrations of standard ABA.

Appendix V

Chapter 6.0 The EC and pH values for different solutions.

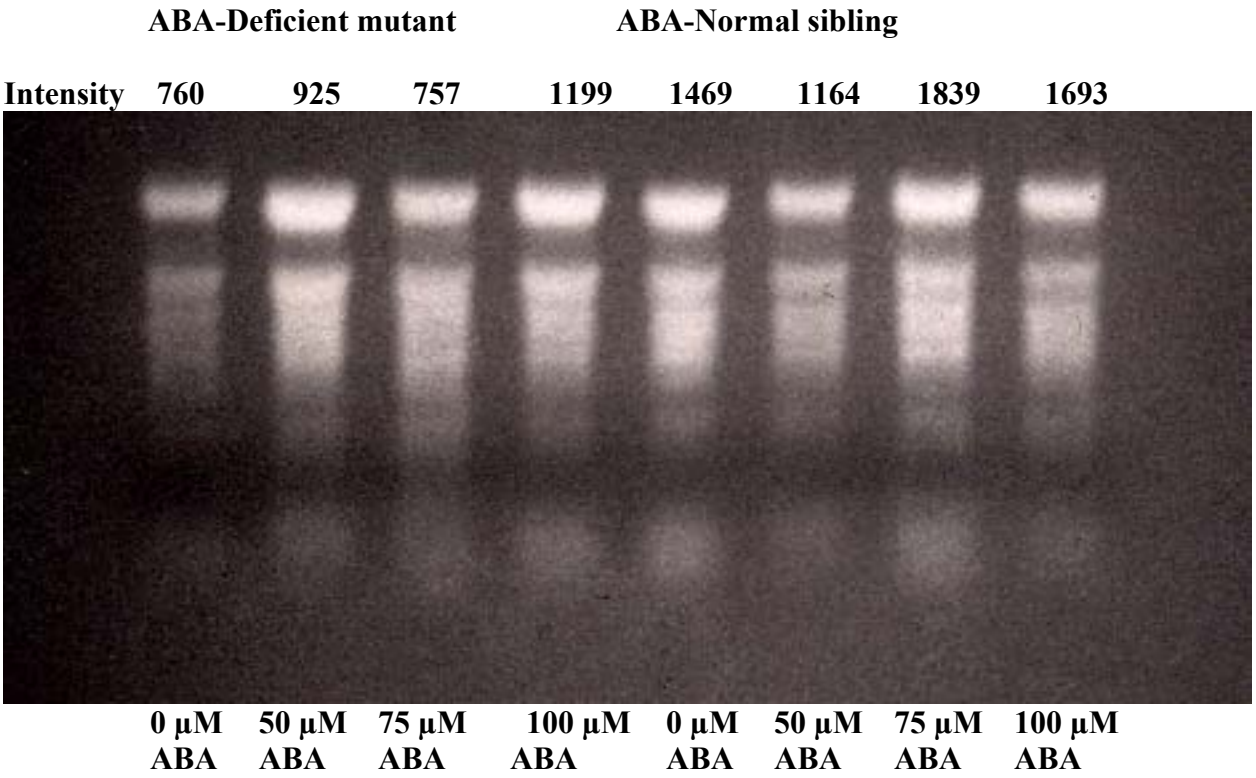
0 Solution (mM)	**EC dS m^{-1}(average)**	**pH**
0	1.22	7.6
25 NaCl	4.00	7.9
20 $CaCl_2$	5.34	7.3
25 NaCl + 20 $CaCl_2$	7.57	7.3
50 NaCl	6.57	6.97
50 NaCl +20 $CaCl_2$	9.95	6.65
75 NaCl	8.93	6.94
75 NaCl + 20 $CaCl_2$	13.44	6.63

Appendix VI

The calcium content in the tap water used to prepare nutrient solutions (mg L^{-1}).

Year	**Average**	**Range**
1998	25.2	23-27
1999	23.5	21-25
2000	26.0	24-28
2001	25.5	22-32
2002	26.3	22-29

Appendix VII



Northern blot analysis of calreticulin mRNA extracted from roots of the ABA-deficient mutant and its ABA normal sibling after two weeks of salt stress.

Appendix VIII

Pictures from chapters 4-5, are representitive of different levels of single and multiple ABA applications on grafted and non-grafted plants.

Picture 1- Greenhouse hydroponic sand base growing system.

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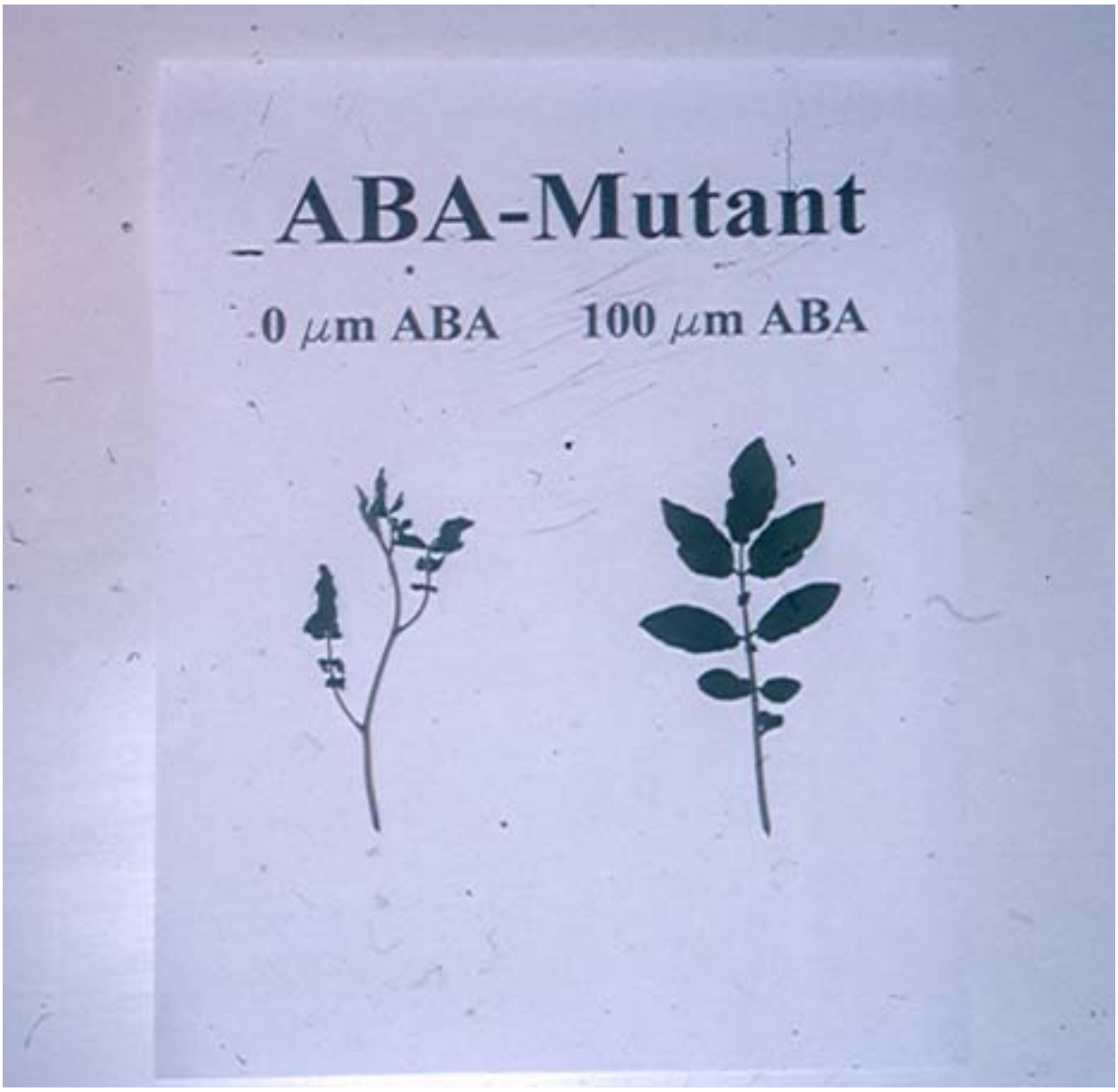


Picture 2- The effect of single ABA application on leaf growth of the ABA-Deficient mutant over 2 weeks of salt stress.

Picture 3- The effect of single ABA application on growth and leaf necrosis of the ABA-Deficient mutant over 2 weeks of salt stress. M= ABA-Deficient Mutant, from right to left: 0, 50, 75 and 100 µM ABA.

Picture 4- The effect of single ABA application on growth and leaf necrosis of the ABA-Normal sibling over 2 weeks of salt stress. S= Sibling, from left to right: 0, 50, 75 and 100 µM ABA.

Picture 5- The effect of single ABA application on growth and leaf necrosis of 9506 over 2 weeks of salt stress. R= 9506 Resistant, from left to right: 0, 50, 75 and 100 µM ABA.

Picture 6- The effect of single ABA application on growth and leaf necrosis of ABA(-)/9506 over 2 weeks of salt stress. M= ABA-Deficient Mutant, R= 9506 Resistant from left to right: 0, 50, 75 and 100 µM ABA.

Picture 7- The effect of single ABA application on growth and leaf necrosis of 9506/ABA(-) over 2 weeks of salt stress. R= 9506 Resistant, M= ABA-Deficient Mutant, from left to right: 0, 50, 75 and 100 µM ABA.

Picture 8- The effect of single ABA application on growth and leaf necrosis 'Norland' over 2 weeks of salt stress. 'Norland', from left to right: 0, 50, 75 and 100 μM ABA.

Picture 9- The effect grafting on stem diameter (Below graft union) of ABA-Deficient Mutant (ABA-) over 2 weeks of salt stress, M= ABA-Deficient Mutant, R= 9506 Resistant, Treatment: 0 mM NaCl salt acclimation.

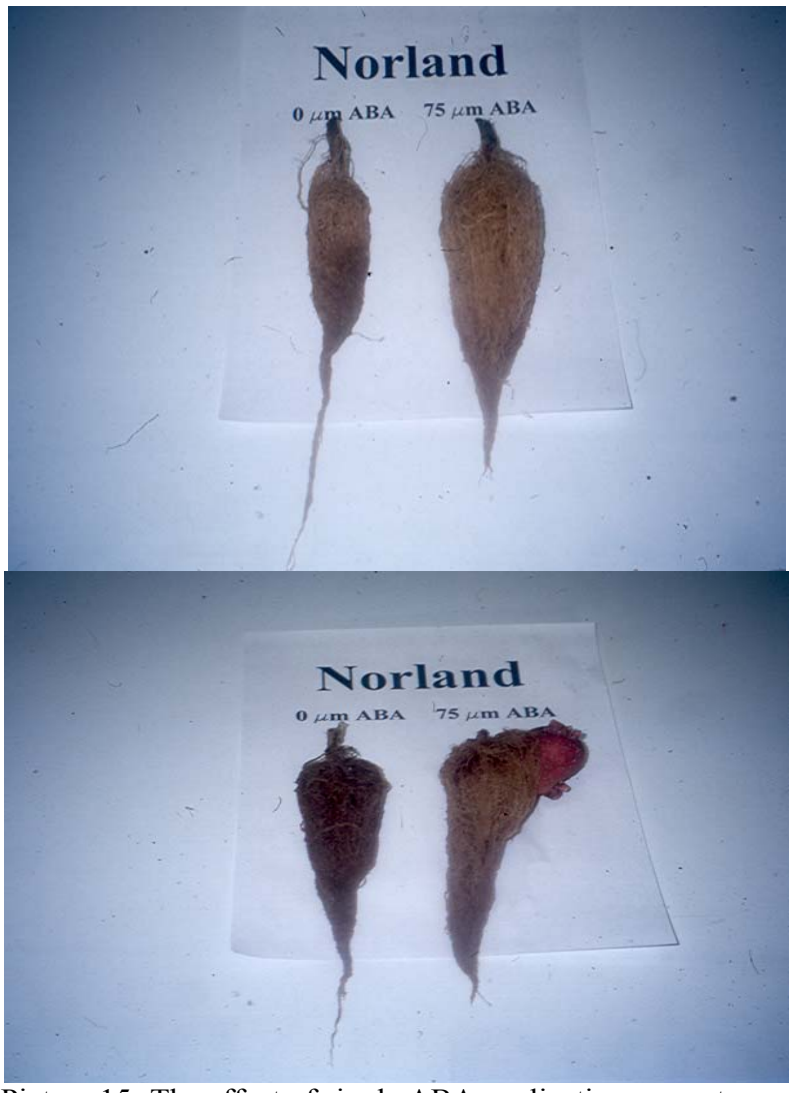
Picture 10- The effect grafting on root growth of ABA-Deficient mutant (ABA-) in the graft combination of 9506/ABA(-) over 2 weeks of salt stress. M= ABA-Deficient Mutant, R= 9506 Resistant, Treatment: 50 µM ABA.

Picture 11- The effect of grafting on root growth of ABA-Normal sibling ABA(+) in the graft combination of 9506/ABA(+) over 2 weeks of salt stress. S= ABA-Normal Sibling, R= 9506 Resistant. Treatment: 75 μM ABA.

Picture 12- The effect of single ABA application on root growth of ABA-Normal sibling [9506/ABA(+)] over 2 weeks of salt stress. R= 9506 Resistant, S= ABA-Normal Sibling, Treatment: 0, 50, 75 and 100 µM ABA.

Picture 13- The effect of multiple ABA applications on root growth of ABA-Deficient mutant over 2 weeks of salt stress. Treatments: 0, 50, 75 and 100 µM ABA.

Picture 14- The effect of multiple ABA applications on root growth of ABA-Normal sibling over 2 weeks of salt stress. 9120-18-Sibling= ABA-Normal Sibling, Treatments: 0, 50, 75 and 100 µM ABA.



Picture 15- The effect of single ABA application on root growth of 'Norland'over 2 weeks of salt stress. Treatments: 0 and 75 μM ABA.

Picture 16- The effect of multiple ABA applications on root growth of 'Norland'over 2 weeks of salt stress. Treatments: 0, 50, 75 and 100 µM ABA.