

Scion and Rootstock Effects on ABA-mediated Plant Growth Regulation and Salt Tolerance of Acclimated and Unacclimated Potato Genotypes

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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 (9506) and salt-sensitive ABA(–) mutant and its ABA(+) normal sibling potato genotype. The grafted plants were acclimated with 75 or 100 mM NaCl for 3 weeks and then exposed to 150–180 mM NaCl, depending on the salt tolerance of the rootstock. After 2 weeks of exposure to the salt stress, the acclimated and unacclimated plants were compared for physiologic and morphologic 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(+) sibling. This salt tolerance induced by the rootstock was primarily modulated by salt acclimation and manifested in the scion via increased plant water content, stem diameter, dry matter accumulation, stomatal conductivity, and osmotic potential, and is associated with a reduction in leaf necrosis. There was also a pronounced scion effect on the rootstock. Using 9506 as a scion significantly increased root fresh and dry weights, stem

diameter, and root water content of ABA(–) mutant rootstocks. Specific evidence was found of 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 by more positive stomatal conductivity and upward water flow in ABA-treated grafted and nongrafted plants and the absence of upward water flow in nontreated grafted plants through NMR imaging. Grafting using either salt-tolerant scions or rootstocks with inherently high ABA levels may positively modify subsequent responses of the plant under salt stress.

Keywords NaCl · Salt acclimation · Grafting · ABA · Root · Shoot · NMR imaging

Introduction

The progressive, natural, and anthropogenic salinization of arable lands at the rate of 3 ha/min at global levels (FAO 2006) is a major limiting factor in agricultural crop production (Parida and Das 2005) and agricultural sustainability (Waisel 2001). The consumption of potatoes in Europe and North America (Messer 2000) has been combined with recent cumulative demand for potato production for sustaining food and nutrition security in the developing world (Pandey and others 2005). Potatoes are regarded as the fourth most important food crop globally (CIP 2007). Although halophytic wild types exist in potato species (Shaterian and others 2005b), most cultivated potato genotypes are relatively salt sensitive (Katerji and others 2003), and soil salinity levels as low as 2.3 dS m⁻¹ reduce both growth and tuber yield (Katerji and others 2003). The responses of potato cultivars and wild species vary following exposure to elevated levels of chloride and

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sulfide, 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.

Although salt shock is uncommon in nature (Maas and Grattan 1999), most research on salt stress responses involves a short-term salt exposure on nonacclimated plants. In nature, plants are typically subjected to a gradual buildup of salt due to either fertilizer application for crop growth or increase in salt concentration as water is depleted (Eilers and others 1995). Adapted plants have an inherent level of salinity resistance and can significantly increase that resistance during acclimation (Amzallag and Lerner 1995). Acclimation results from a pre-exposure to low, nonlethal levels of salt stress which increases the plant's subsequent tolerance to higher salt concentrations (Strognov 1964; Conroy and others 1988; Guy 1990; Matthews and Boyer 1984). Acclimated plants will grow at salt concentrations that are lethal to nonacclimated plants (Amzallag and others 1990b). For acclimation to occur, the cell, organ, and organism must be in a proper physiologic state (Amzallag and Lerner 1995). The capacity for acclimation varies considerably among plant species (Baker and others 1986) and also varies among genotypes of the same species (Durrant 1981; Amzallag and others 1993; Azevedo Neto and others 2004). Virtually all plant species can acclimate to salt stress if the stress is imposed gradually (Amzallag and others 1990b; Hasegawa and others 1994). Enhanced salt tolerance following NaCl pretreatment was observed in bell pepper (*Capsicum annuum* L.) (Bethke and Drew 1992), Jojoba (*Simmondsia chinensis*) (Ben Raïs and others 1993), maize seedlings (*Zea mays*) (González-Rodríguez and others 1997), rice (*Oryza sativa*) (Hassanein 2000; Djanaguiraman and others 2006), soybean (*Glycine max*) (Umezawa and others 2000), and cowpea (*Vigna unguiculata*) (Silveira and others 1999, 2001).

ABA is a plant hormone involved in coordinating the growth of roots and shoots of plants (Sharp and LeNoble 2002) and regulating tolerance responses to a number of stresses, including water and salt (Thomas and Eamus 1999; Gómez-Cadenaz and others 2003; Shaterian and others 2005a). For example, the negative effect of NaCl salt on root nodule dry weight of common bean was alleviated by exogenous ABA supply (Khadri and others 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 and others 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 and others 1995). Furthermore, overproduction of ABA is associated with increased transpiration efficiency and root hydraulic conductivity and influences leaf expansion (Thompson and others 2007). Abscisic acid accelerated salt acclimation

(Amzallag and others 1990a), whereas cytokinin (CK) and gibberellic acid (GA) interfered with this process (Amzallag and others 1992). Recently, Shaterian and others (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 and 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 and others 2002; Liu and others 2003; Fricke and others 2004, 2006).

Several practices in agricultural systems are directed toward overcoming salinity. Judicious selection of salt-tolerant genotypes and development of better varieties have shown merit in some situations via traditional potato genetic breeding programs (Elkhatib and others 2004; Shaterian and others 2005b). Gene transformation recently has been used in developing salt-tolerant genotypes. Salt-tolerant transgenic lines expressed substantially more of the transgene that was generated through genetically engineered potato (Hmida-Sayari and others 2005; Behnam and others 2006; Teixeira and others 2006). Transferring desirable properties of the root of salt-resistant rootstocks to the shoot of salt-sensitive scions through gene transcription in the root and action in the shoot is an alternative way of overcoming salinity by grafting (Pardo and others 1998). This manipulation resulted in intensively increasing cultivation of grafted vegetable crops in the past few years (Lee and Oda 2003), particularly in Solanaceae plants subjected to salinity (Fernández-García and others 2002; Santa-Cruz and others 2002; Chen and others 2003). Scion/rootstock interactions can provide useful insights into the mechanisms underlying plant responses to salt stress (Schmutz and Lüdders 1999; Fernández-García and others 2002; Chen and others 2003; Estañ and others 2005). These salt-tolerance mechanisms are usually associated with the plant's ability to restrict the uptake and/or transport of toxic ions from the roots to the shoots (Matsumoto and others 2006).

Grafting is an integrative reciprocal process and therefore both scion and rootstock influence salt tolerance. 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, whereas tolerant scions grafted onto sensitive rootstocks remained tolerant of salinity (Dua 1997). In soybean, both the shoot and the root have been reported to affect salt tolerance. Velagaleti and others (1990) suggest that the root is dominant in determining salinity tolerance of soybean. In addition, the importance of the root system in regulation of salt stress tolerance was also documented in salt-sensitive and salt-tolerant potato genotypes (Shaterian and others 2005a). By

contrast, Abd-Alla (1998) concluded that shoot factors were of primary importance in determining salt tolerance of soybean. Schmutz and Lüdders (1999) showed that scions also play a role in salt tolerance of grafted mango plants. In tomato genotypes, rootstocks generally regulate accumulation of salt ions in leaves (Santa-Cruz and others 2001, 2002), but properties of the rootstock that are important in inducing salt tolerance of the shoot were also dependent on the shoot genotypes (Santa-Cruz and others 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 and others 2003).

ABA-deficient mutants have been found in a number of plant species such as tomato (Tal 1967; Tal and Nevo 1973; Neill and Horgan 1985; Borsani and others 2002), potato (Quarrie 1982; De Jong and others 2001), pea (Wang and others 1984; Kof and others 2006), *Arabidopsis thaliana* (Koorneef and others 1982; Umezawa and others 2006), *Helianthus annuus* (Fambrini and others 1995, 2004), viviparous corn mutants (Smith and others 1978; Robichaud and others 1980; Suzuki and others 2006), and wheat (Holappa and others 2005). Reciprocal grafting of ABA-deficient mutants is a useful method for studying the functions of ABA (Chen and others 2002a). 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 scions more than grafts with the normal roots (Tal 1967). These results also indicated that the shoot genotype was dominant in determining stomatal aperture (closing/opening), although grafting onto ABA-normal rootstock could cause a slight decrease in the stomatal conductance of ABA-deficient scions (Jones and others 1987). Although ABA-mutant scions grafted onto ABA-normal rootstocks reverted to a relatively near-normal phenotype, the ABA-normal scion maintained its own phenotype on the mutant rootstock (Cornish and Zeevaert 1988). Recently, Shaterian and others (2005b) showed ABA(+) rootstocks were important in inducing calreticulin mRNA in the leaves during salt stress.

Nuclear magnetic resonance (NMR) microscopy is a unique and promising tool in plant science (Ishida and others 2000; Köckenberger and others 2004). Holbrook and others (2001) were able to monitor the water status of individual larger xylem vessels of plants like grape (*Vitis vinifera*). Peuke and others (2001), in addition to simultaneous measurement of water flow velocity in xylem and phloem, monitored CO₂ and H₂O concentrations in castor bean seedling by using FLASH imaging capabilities of NMR. Microimaging based on NMR is an experimental technique that can provide a unique view of a variety of plant physiologic processes. 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 flow imaging experiments (Scheenen and others

2002). Water movement in inner and outer xylem in the shoot and leaf using various NMR spectroscopy and imaging techniques was also studied (Schneider and others 2003). Recently, Velikanov and Belova (2005) examined the effect of ABA using NMR imaging. They showed exogenous ABA affects water permeability of the vacuolar symplast in the root cells of maize seedlings by increasing water permeability of the tonoplast.

Despite the global importance of potatoes in developing and developed countries, the growing significance of salt stress, the known involvement of ABA in alleviating stress, and the ability to graft crops like potato, investigations into ABA and salt stress responses in grafted potato have not been widely performed. Thus, the objective of this study is to examine scion and rootstock effects on ABA-mediated plant growth regulation and salt tolerance of acclimated and unacclimated potato genotypes.

Materials and Methods

Genotypes Resources

Three diploid potato lines were obtained from Agriculture and Agri-Food Canada, Fredericton (Dr. H. De Jong). Line 9506 “Resistant” (*S. chacoense* × *S. microdontum* × *S. tuberosum*) is salt stress resistant (Shaterian and others 2005b). Line 9120–05 is an ABA(–) mutant [*Solanum tuberosum* L. group *Phureja* (*phu*)] containing the (*dr*) mutant gene (Simmonds 1965). This line is salt sensitive (Shaterian and others 2005b). Line 9120–18 (*Drdr*) is the ABA(+) normal sibling of 9120–05 and is moderately sensitive to salt (Shaterian and others 2005b). The commercially produced tetraploid potato ‘Norland’ was used as a comparative in the endogenous ABA experiment.

Grafting

Shoot tip cuttings of the three genotypes were rooted in Ottawa sand (1–2 mm diameter, 75.5% very coarse sand; 0.5–1 mm, 24.4% coarse sand; and <0.5 mm, <0.1%). Ottawa sand has minimal ion-binding capacity, which reduces interference by ion absorption in salinity or fertility trials. After 5 weeks the rooted cuttings were transferred to 400-ml pots filled with Ottawa sand. The seedlings were grown in a greenhouse with 25/20°C day/night temperature, 60–85% relative humidity, and 600–800 μM s⁻¹ m⁻² light intensity (PhAR) for 14–16 h (combination of natural light and artificial light provided by high-pressure sodium halogen lamp).

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(–) 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(-) mutant or its ABA(+) sibling [9506/ABA(-)] and [9506/ABA(+)]. A cleft graft was used. The graft unions of the autografts of the ABA(-) mutant and the ABA(+) sibling broke a few days after grafting. However, our other grafting studies on the same plants using autografted material found significant rootstock effects independent of an autografting response (Shaterian and others 2005a). Therefore, nongrafted 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 20–20–20 N–P–K plus micronutrients (Plant Products Co. Ltd., Brampton, Ontario, Canada) three times a day. To prevent foliar diseases, the plants were sprayed with fungicide three times per week.

After 6 weeks the grafted plants were transferred to 1.5-l pots filled with white Ottawa sand. Four pots were used for each scion/rootstock combination. One pot of each scion/rootstock combination was placed in a tray (20 × 40 × 60 cm³). Three to five times each day each tray was flooded for 5 min with a fertilizer solution (1.27 g l⁻¹) (20–20–20 N–P–K plus micronutrients, Plant Products) augmented with differing amounts of NaCl (see below). The EC and pH of the nutrient solution was 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. Sprouts from nodes of the rootstock were continuously removed to maintain only one grafted scion.

Salt Acclimation Treatment

To determine the effects of salt acclimation on subsequent salt stress tolerance, graft combinations (scion/rootstock) were pretreated with low concentrations of NaCl salt for 3 weeks. Based on previous studies of the salt tolerance of the rootstocks, the salt acclimation treatments were as follows: ABA(-), ABA(+), 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.

Unacclimated Control

To compare the effect of salt acclimation with control, unacclimated plants received fertilizer nutrient solution without NaCl salt.

After 3 weeks of salt acclimation, all acclimated and unacclimated plants were exposed to 2 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(-), ABA(+), 9506/ABA(-), 9506/ABA(+) = 150 mM and ABA(-)/9506, ABA(+)/9506, 9506 = 180 mM.

A factorial 7 (graft combinations) × 2 (salt acclimated) randomized complete block design (RCBD) with four replicates and two plants per replicate was used for this study. Fisher's protected LSD ($P = 0.05$) was used for mean comparisons.

Physiologic parameters evaluated after 2 weeks of exposure to salt stress were: leaf injury, leaf stomatal conductivity, 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 to 5 (Shaterian and others 2005b) as follows: 1 = 0% leaf damage, 2 = 1–25% leaf damage, 3 = 26–50% leaf damage, 4 = 51–75% leaf damage, 5 = 76–100% leaf damage. Leaf injury ratings were performed on the most recently fully expanded and the lowest nonwilting leaves. Plant height (stem collar to shoot tip, apex) was monitored on a weekly basis over the 2 weeks of salt stress to allow calculation of the growth rate:

$$\text{Growth rate} = \frac{(\text{height at T2} - \text{height at T1})}{\text{T2} - \text{T1}}$$

where T1 is before stress and T2 is after stress. To determine leaf water content and leaf osmotic potential, tissue samples were taken from the fourth and fifth fully expanded leaves between 10 and 12 p.m. A Wescor Vapour Pressure Osmometer model 5000 (Logan, UT) was used to determine osmotic potential.

A SPAD Meter (model Minolta-502) was used to measure the degree of leaf greenness (approximates chlorophyll content) on the same leaf on which necrosis was measured. 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 1600, LI-COR Biosciences, Lincoln, NE) between 9 and 11 a.m. after 2 weeks of salt stress. At the end of the experiment, root and shoot fresh and dry weights and water content were determined.

ABA Measurements

For ABA measurements, 15–20-g (fresh weight) samples were collected at the completion of salt stress treatments between 10 a.m. and 12 p.m. Leaf samples were immediately frozen in liquid N₂, stored at -20°C, and lyophilized 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 1cc cartridge (Waters Corp., Milford, MA) was preconditioned with methanol and equilibrated with water under vacuum. The extract was dissolved in 200 μ l 99% methanol (v/v) containing 1% acetic acid and mixed with 800 μ l 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 VacufugeTM, Brinkmann Instruments, Inc., Canada). High-performance liquid chromatography (HPLC) and mass spectrometry (MS) were used to quantify the abscisic acid (ABA) (Ross and others 2004).

ABA Application

A racemic mixture of (+/–) ABA (Sigma Chemical, St. Louis, MO) was applied at the prestolon initiation stage (5 weeks of growth of the grafted cuttings) in all genotypes. ABA was applied in increasing dose concentrations every 3 days to reach a final concentration of 75 μ M. Synthetic (+/–) ABA was dissolved in a small amount of NaOH (1 N) diluted to the desired concentration with distilled water. The pH of the 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 6.5. Because solutions were circulated back to individual tanks,

the water of the tanks was changed after 2 days to prevent the accumulation of ABA. The salt stress treatments were started 1 week after the final ABA application. The salt stress was applied as above. Treatments were arranged as a factorial experiment with a RCBD (2×7) with four replications per treatment and three plants per replication. Pooled analysis of variance (ANOVA) for factorial RCBD was performed by the general linear model (GLM) in SAS software (SAS Inc., Chicago, IL) and means of lines were compared using LSD.

NMR Imaging

One sensitive [ABA(–) deficient mutant] and 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 at the end of ABA treatment and after 2 weeks of salt stress. NMR imaging of ABA-treated and nontreated shoots was performed with a Siemens Magnetum Symphony 1.5-Tesla whole-body medical scanner. A flow-sensitive sequence was acquired in a transverse plane.

Results

Leaf Necrosis and Chlorophyll Content

Leaf necrosis of the ABA(+) and 9506 resistant genotype was lower than the ABA(–) in both the unacclimated and

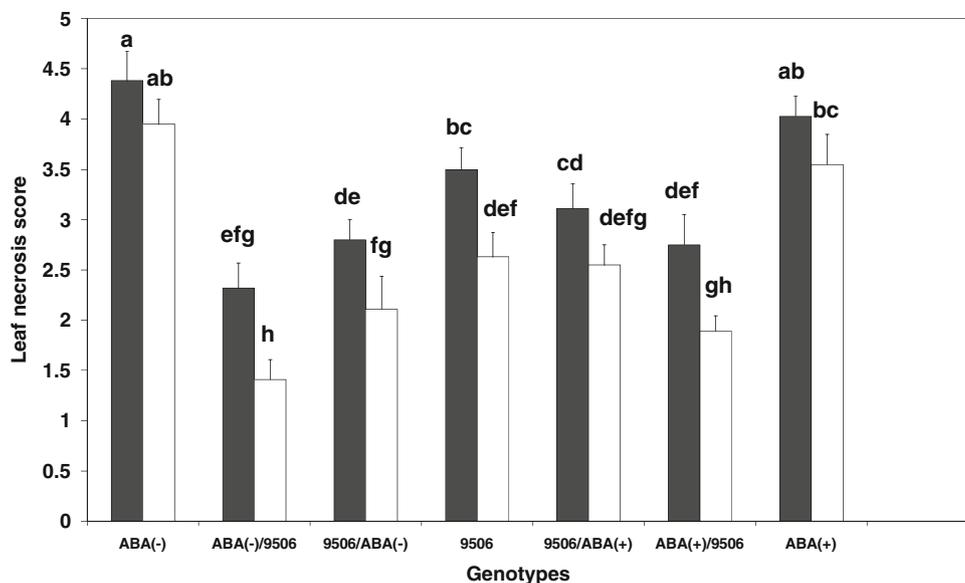


Fig. 1 Salt acclimation (75–100 mM NaCl) effects on leaf necrosis of potato genotypes and their graft combinations (scion/rootstock) measured after 2 weeks of salt stress (150–180 mM NaCl). Leaf necrosis score: 1, 0% leaf area necrosis; 2, 1–25% leaf area necrosis; 3, 26–50% leaf area necrosis; 4, 51–75% leaf area necrosis; 5, 76–

100% leaf area necrosis. Means with the same letters are not significantly different (LSD = 0.05). 9506 = Salt stress resistant, ABA(–) = ABA-deficient mutant, ABA(+) = ABA-normal sibling. (■) Unacclimated; (□) salt acclimated

acclimated plants after salt stress (Figure 1). When acclimated, the 9506 genotype expressed the lowest leaf necrosis compared to the ABA(−) and ABA(+) types after salt stress. However, the impact of the rootstock itself, independent of acclimation, was more significant on inducing salt tolerance in the scion when 9506 was used as a rootstock of the relatively salt-sensitive scions: the ABA(−) mutant and the ABA(+) sibling [ABA(−)/9506 and ABA(+)/9506 graft combinations] (Figure 1). Salt acclimation also reduced the proportion of shoots with injured leaves in these graft combinations. In addition, salt acclimation decreased leaf necrosis in 9506 resistant scions in the reciprocal 9506/ABA(+) and 9506/ABA(−) graft combinations to the same extent as the resistant 9506 plants alone.

ABA application significantly reduced leaf necrosis in all genotypes except the 9506 resistant line (Figure 2). Grafting the ABA(−) onto the resistant 9506 rootstock in combination with exogenous ABA application decreased

leaf necrosis beyond grafting alone. This response was not observed for the ABA(+)/9506 grafting combination.

Grafting onto the 9506 resistant rootstock also increased greenness (chlorophyll content) of scions of the ABA(−) mutant after salt stress (Figure 3). Salt acclimation improved this parameter in nongrafted plants of the ABA(−) mutant.

ABA Levels

Concentrations of ABA in acclimated plants were 33% [ABA(−) mutant] to 44% (9506 genotype) higher than the ABA levels in unacclimated plants at the end of salt stress treatment (Figure 4). The ABA(−) mutant had the lowest ABA concentration but was not completely devoid of ABA. Its ABA(+) sibling plants also had a lower ABA concentration compared to the more salt-tolerant ‘Norland’ and the 9506 genotype. In unacclimated plants exposed to salt stress, ‘Norland’ and the 9506 genotype had

Fig. 2 Effects of ABA application on leaf necrosis of potato genotypes and their graft combinations (scion/rootstock) measured after 2 weeks of salt stress (150–180 mM NaCl). Leaf necrosis score: 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 = 0.05). 9506, Salt stress resistant, ABA(−), ABA-deficient mutant, ABA(+), ABA-normal sibling; (■) Control; (□) ABA-treated

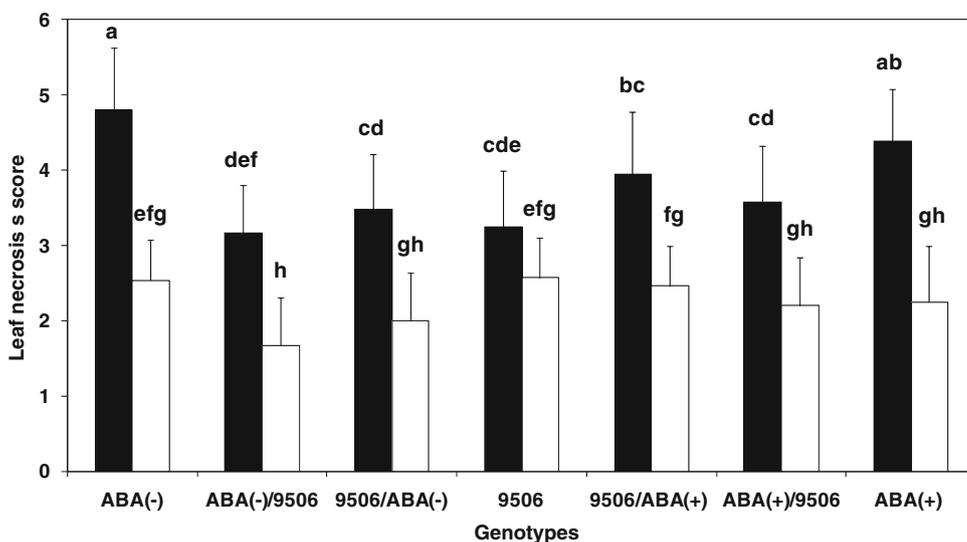


Fig. 3 Effects of salt acclimation (75–100 mM NaCl) on degree of leaf greenness (SPAD values) of potato genotypes and their graft combinations (scion/rootstock) measured after 2 weeks of salt stress (150–180 mM NaCl). Means with the same letters are not significantly different (LSD = 0.05). 9506, Salt stress resistant; ABA(−), ABA-deficient mutant; ABA(+), ABA-normal sibling. (■) Unacclimated; (□) salt acclimated

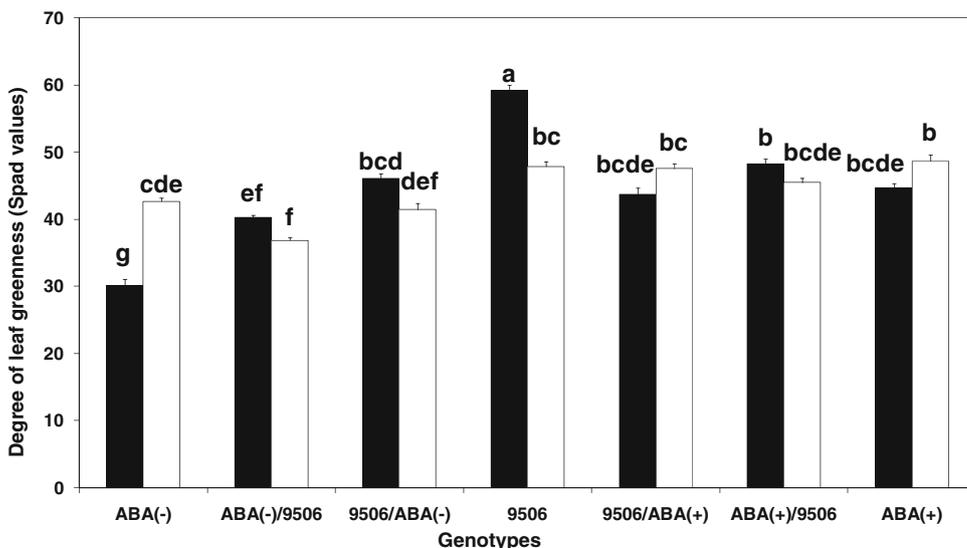


Fig. 4 Effects of salt acclimation (75–100 mM NaCl), on abscisic acid, ABA content (ng/g) of four potato genotypes measured after 2 weeks of salt stress (150–180 mM NaCl). Means with the same letters are not significantly different (LSD $P = 0.05$). 9506, Salt stress resistant, ABA(-), ABA-deficient mutant, ABA(+), ABA-normal sibling. (■) Unacclimated; (□) salt acclimated

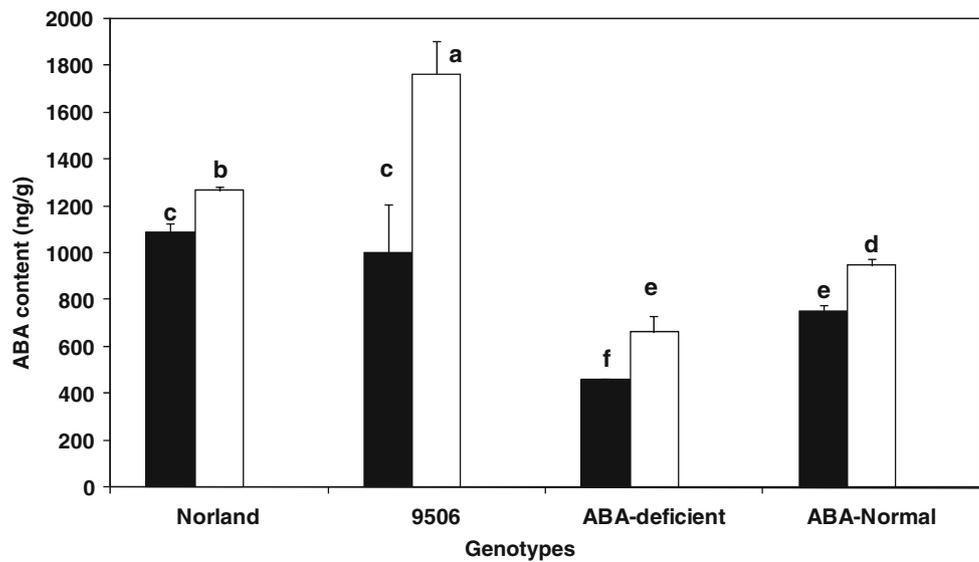


Table 1 Mean root fresh and dry weights and water content of salt-acclimated (75–100 mM NaCl) and unacclimated potato genotypes and their graft combinations (scion/rootstock) measured after 2 weeks of salt stress (150–180 mM NaCl)

Treatments	ABA(-)	ABA(-)/9506	9506/ABA(-)	9506	9506/ABA(+)	ABA(+)/9506	ABA(+)
Root fresh weight (g)	Unacclimated 1.11 f	5.66 e	10.87 bc	14.07 a	9.76 c	14.53 a	8.65 cd
Salt 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 (g)	Unacclimated 0.22 g	0.93 f	1.87 d	2.59 c	1.62 de	4.74 a	1.42 e
Salt 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 (%)	Unacclimated 80.00 b	83.10 ab	82.12 ab	81.40 b	83.21 ab	83.44 ab	67.24 c
Salt acclimated	70.88 c	81.44 b	89.43 a	83.76 ab	83.27 ab	84.58 ab	70.49 c

Means for each parameter with the same letters are not significantly different (LSD = 0.05). ABA(-), ABA-deficient mutant; ABA(+), ABA-normal sibling; 9506, salt stress resistant

comparable leaf ABA concentrations, but with acclimation the 9506 genotype had higher levels of ABA than ‘Norland.’

Plant Biomass

Under both unacclimated and acclimated conditions, using 9506 resistant as a scion increased root fresh and dry weights of the ABA(-) mutant rootstock compared to nongrafted ABA(-) mutants (Table 1). Correspondingly, using the ABA(-) mutant as a scion significantly reduced the root fresh and dry weights of 9506 resistant rootstocks in both the salt-acclimated and unacclimated treatments. The ABA(+) sibling as a scion increased root dry weight of the 9506 resistant rootstock. Although the salt acclimation treatment had no effect on root fresh weights of the ABA(-) mutant, ABA(-)/9506, and 9506 resistant genotypes, 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 unacclimated conditions.

Grafting salt-sensitive genotypes onto the 9506 salt-tolerant rootstock resulted in increased shoot fresh and dry weights of ABA(-)/9506 and ABA(+)/9506 graft combinations relative to nongrafted plants (Table 2). Correspondingly, the reciprocal grafts of the 9506/ABA(-) and 9506/ABA(+) sibling reduced shoot fresh weight of the 9506 resistant scions. The salt-acclimation treatments reduced shoot fresh weight in all genotypes and graft combinations tested except in the ABA(-), ABA(-)/9506 and the ABA(+)/9506 combination in which there were no change (Table 2).

Stem Diameter

Grafting had a significant impact on stem diameter under salt stress (Table 3). Stem diameter of the ABA(-) mutant scions grafted onto 9506 resistant rootstocks increased relative to nongrafted plants under both the unacclimated and salt-acclimation treatments (Table 3A). This enhanced grafting response was observed only following salt acclimation in the ABA(+) normal scions on 9506 rootstocks. The diameter of 9506 resistant scions did not change

Table 2 Mean shoot fresh and dry weights and water content of salt-acclimated (75–100 mM NaCl) and unacclimated potato genotypes and their graft combinations (scion/rootstock) measured after 2 weeks of salt stress (150–180 mM NaCl)

	Treatments	ABA(–)	ABA(–)/ 9506	9506/ ABA(–)	9506	9506/ ABA(+)	ABA(+)/ 9506	ABA(+)
Shoot fresh weight (g)	Unacclimated	18.76 i	42.83 h	109.70 d	177.75 a	123.08 c	136.12 b	91.53 e
	Salt 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 (g)	Unacclimated	3.53 e	17.58 c	18.28 c	28.02 b	17.61 c	38.73 a	15.80 c
	Salt 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 (%)	Unacclimated	59.26 f	81.18 bcd	83.25 abcd	84.22 abcd	85.70 ab	82.42 abcd	71.53 e
	Salt acclimated	74.20 e	81.26 bcd	86.55 a	84.70 abc	80.87 cd	82.92 abcd	79.51 d

Means for each parameter with the same letters are not significantly different (LSD = 0.05). ABA(–), ABA-deficient mutant; ABA(+), ABA-normal sibling; 9506, salt stress resistant

Table 3 Mean stem diameter of salt-acclimated (75–100 mM NaCl) and unacclimated potato genotypes and their graft combinations (scion/rootstock) measured after 2 weeks of salt stress (150–180 mM NaCl)

	Treatments	ABA(–)	ABA(–)/ 9506	9506/ ABA(–)	9506	9506/ ABA(+)	ABA(+)/ 9506	ABA(+)
(A) Above graft union (mm)	Unacclimated	1.75 e	4.16 d	5.83 abc	6.83 ab	5.08 cd	5.33 bcd	5.16 cd
	Salt 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 (mm)	Unacclimated	1.66 g	4.00 def	4.00 def	5.16 abc	3.50 ef	5.83 a	4.66 bcd
	Salt acclimated	1.08 g	3.33 f	3.83 def	5.33 ab	4.33 cde	5.50 ab	4.33 cde

Means for each parameter with the same letters are not significantly different (LSD = 0.05). 9506, salt stress resistant; ABA(–), ABA-deficient mutant; ABA(+), ABA-normal sibling

except when grafted onto the ABA(+) sibling rootstock, in which case the diameter of 9506 scions was significantly reduced under unacclimated conditions.

As a rootstock, the 9506 stem diameter was not affected by grafting with scions of the ABA(+) sibling; however, the ABA(–) mutant scion reduced 9506 rootstock diameter under both unacclimated and acclimated conditions (Table 3B).

Water Status

When the 9506 resistant genotype was used as a scion onto either the ABA(–) mutant or ABA(+) sibling, root water content significantly increased under salt acclimation (Table 1). Salt acclimation alone increased shoot water content of the ABA(–) and ABA(+) sibling after salt stress (Table 2). Under both unacclimated and salt-acclimation conditions, the 9506 resistant rootstock increased shoot water content in the ABA(–)/9506 graft combination compared to nongrafted ABA(–) mutants (Table 2). In the ABA(+)/9506 graft combination, this 9506 rootstock effect was observed only under unacclimated conditions.

In a similar response to shoot water content, salt acclimation alone also significantly increased stem water content of the ABA(–) and ABA(+) (Table 4). Even in the absence of acclimation, having 9506 as a rootstock increased stem water content in scions of the ABA(–) and ABA(+) compared to nongrafted plants.

The effect of the 9506 rootstock on increasing leaf water content of the ABA(–) mutant scion under both acclimation and unacclimated treatments was significant (Table 4). Exogenous ABA application did not influence leaf water content in the absence of stress (Table 5). However, after stress, leaf water content was significantly increased in ABA-treated plants of ABA(–), ABA(–)/9506, ABA(+)/9506, and ABA(+) grafted plants.

Leaf Osmotic Potential

Without acclimation, the 9506 resistant genotype as a rootstock in itself induced a more negative leaf osmotic potential of ABA(–) mutant scions but had no effect on scions of the ABA(+) sibling (Table 4). Subsequent acclimation (Table 4) and exogenous ABA application (Table 5) of the ABA(–), ABA(+), ABA(–)/9506, and ABA(+)/9506 induced a less negative leaf osmotic potential under salt stress.

Leaf Stomatal Conductivity and Water Flow

Salt acclimation increased leaf stomatal conductivity of all genotypes and graft combinations during subsequent salt stress except for nongrafted plants of the ABA(–) mutant

Table 4 Mean leaf water content, leaf osmotic potential, stem water content, and leaf stomatal conductivity of salt-acclimated (75–100 mM NaCl) and unacclimated potato genotypes and their graft combinations (scion/rootstock) measured after 2 weeks of salt stress (150–180 mM NaCl)

	Treatments	ABA(–)	ABA(–)/9506	9506/ABA(–)	9506	9506/ABA(+)	ABA(+)/9506	ABA(+)
Leaf water content (%)	Unacclimated	75.13 cd	81.75 ab	78.77 abc	83.26 ab	81.57 abc	79.72 abc	83.63 a
	Salt 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 ($\Psi_s = \text{MPa}$)	Unacclimated	–2.06 f	–2.38 g	–1.29 abc	–1.36 abc	–1.51 cd	–1.63 de	–1.74 e
	Salt 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 (%)	Unacclimated	56.92 e	70.11 d	76.54 abcd	76.96 abc	76.26 abcd	79.81 ab	61.35 e
	Salt 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 ($\mu\text{m}^{-2} \text{s}^{-1}$)	Unacclimated	0.011 ef	0.010 f	0.115 b	0.115 b	0.118 b	0.021 e	0.018 ef
	Salt acclimated	0.015 ef	0.058 c	0.135 a	0.130 a	0.125 ab	0.051 c	0.035 d

Means for each parameter with the same letters are not significantly different (LSD = 0.05). 9506, salt stress resistant; ABA(–), ABA-deficient mutant; ABA(+), ABA-normal sibling

Table 5 Mean leaf water content and leaf osmotic potential of ABA-treated and nontreated potato genotypes before and after 2 weeks of salt stress (150–180 mM NaCl)

	Time	ABA (μM)	ABA(–)	ABA(–)/9506	9506/ABA(–)	9506	9506/ABA(+)	ABA(+)/9506	ABA(+)
Leaf water content (%)	Before stress	0	74.12 a	76.67 a	80.22 a	83.61 a	74.01 a	77.24 a	83.09 a
		75	79.90 a	81.07 a	79.32 a	78.56 a	78.46 a	83.73 a	82.55 a
	After stress	0	34.41 f	42.41 f	72.25 bcde	76.42 abc	70.07 cde	70.39 cde	70.27 cde
		75	65.92 e	79.75 ab	74.95 abcd	76.68 abc	66.58 de	81.75 a	82.57 a
Leaf osmotic potential ($\Psi_s = \text{MPa}$)	Before stress	0	–1.50 d	–1.16 c	–1.05 abc	–0.94 abc	–1.09 bc	–1.01 abc	–1.00 abc
		75	–0.99 abc	–0.82 a	–0.89 ab	–0.95 abc	–0.98 abc	–0.88 ab	–0.97 abc
	After stress	0	–2.95 g	–2.42 f	–1.13 ab	–1.47 cd	–1.22 bc	–2.56 f	–1.97 e
		75	–1.80 e	–1.51 d	–0.95 a	–1.48 cd	–1.31 bcd	–1.51 d	–1.48 cd

Means for each parameter with the same letters are not significantly different (LSD = 0.05). 9506, salt stress resistant; ABA(–), ABA-deficient mutant; ABA(+), ABA-normal sibling

and the 9506/ABA(+) sibling combination (Table 4). Grafts using 9506 resistant as a rootstock increased leaf stomatal conductivity in ABA(–) and ABA(+) scions, however, only under salt acclimation. Similarly, ABA application increased leaf stomatal conductivity before stress in the ABA(–), ABA(–)/9506, 9506/ABA(+), and ABA(+)/9506 grafted plants (Table 6). Moreover, after stress, all ABA treatments induced higher stomatal conductivity compared to the control in all plants.

Exogenous ABA application on ABA(–) and ABA(–)/9506 grafted plants significantly altered water flow from a net downward flow to a more positive upward flow (Table 7). This response was not observed through grafting of ABA(–) onto the 9506 rootstock alone.

Discussion

Both scions and rootstocks of various crops were shown to influence salinity tolerance with the majority of research focused on rootstocks (Schmutz and Lüdders 1999;

Santa-Cruz 2001; Fernández-García and others 2002; Chen and others 2003). This response was most pronounced using halophytic plant rootstocks (Chen and others 2003; Estañ and others 2005; Shaterian and others 2005a). In our present study on potato, the beneficial influence of the ABA-accumulating salt-resistant 9506 line as both a rootstock and a scion on 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.

Rootstock Effects

Fresh and dry matter accumulation in both the ABA(–) line and its normal sibling scions were positively affected by grafting onto rootstocks of the highly vigorous 9506 resistant line. This effect of grafting was consistent with the work of Chen and others (2003) 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

Table 6 Mean leaf stomatal conductivity (SC) ($\mu\text{m}^{-2} \text{s}^{-1}$) of ABA-treated and nontreated potato genotypes before and after 2 weeks of salt stress (150–180 mM NaCl)

	ABA (μM)	ABA(-)	ABA(-)/9506	9506/ABA(-)	9506	9506/ABA(+)	ABA(+)/9506	ABA(+)
Before stress	0	0.09 def	0.03 g	0.13 bcd	0.11 cdef	0.09 def	0.08 f	0.11 cdef
	75	0.19 a	0.15 abc	0.17 ab	0.13 bcd	0.16 ab	0.16 ab	0.14 abc
After stress	0	0.01 f	0.01 f	0.04 c	0.03 d	0.03 d	0.01 f	0.02 e
	75	0.04 c	0.04 c	0.05 b	0.08 a	0.04 c	0.05 b	0.05 b

Means for each parameter with the same letters are not significantly different (LSD = 0.05). 9506, salt stress resistant, ABA(-), ABA-deficient mutant, ABA(+), ABA-normal sibling

Table 7 Mean shoot water flow ($\mu\text{l h}^{-1}$) of ABA-treated and nontreated grafted and nongrafted potato genotypes before and after 3 weeks of salt stress (150–180 mM NaCl)

	ABA(-)	ABA(-)/9506	9506	9506/ABA(-)
<i>Before salt stress</i>				
0 μM ABA	-0.35 d	-0.41 d	+0.07 b	-0.32 cd
75 μM ABA	-0.07 bc	+0.44 a	+0.38 a	+0.01 b
<i>After salt stress</i>				
0 μM ABA	-0.27 cd	-0.34 d	+0.05 a	-0.19 bcd
75 μM ABA	-0.03 ab	-0.09 abc	+0.02 ab	-0.02 ab

+ = \uparrow , - = \downarrow

Means for each parameter with the same letters are not significantly different (LSD = 0.05). 9506, salt stress resistant; ABA(-), ABA-deficient mutant

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 water status and enhanced growth (Chen and others 2003). Phenotype reversion of ABA-deficient shoots on ABA-normal wild-type roots was reported with the graft of tomato (Cornish and Zeevaert 1988) and sunflower (Fambrini and others 1995). Shoot dry weight was also significantly affected by root genotype in grafted faba bean (*Vicia faba*) (Barbera and others 1998). Furthermore, grafting onto the salt-tolerant 9506 rootstock increased stomatal conductivity of scions of both sensitive potato lines in this trial. The increase in dry mass accumulation in these two sensitive genotypes might be due to this increase in stomatal conductivity. The distinct consequence of grafting and rootstock effect on increasing stomatal conductivity has also been evidenced in tomato (Fernández-García and others 2004b). Holbrook and others (2002) also indicated that root signals controlled stomatal conductance in tomato through changes in apoplastic ABA levels in leaves.

In addition, the higher leaf and shoot water content characteristic of the 9506 resistant rootstock was transmissible to scions of the ABA(-) mutant. This change was

accompanied by increased leaf osmotic potential of the ABA(-) mutant scion in the ABA(-)/9506 graft combination. Graft unions are not a physical barrier to water transport from rootstock to scion (Fernández-García and others 2002) and, in fact, they are structurally and chemically greatly functional in herbaceous plants like tomato (Fernández-García and others 2004a). A nondestructive 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 rootstock on increased leaf water content (Santa-Cruz and others 2001, 2002; Estañ and others 2005) and increased leaf osmotic potential of the scion was more significant, particularly at high-salinity levels (Santa-Cruz and others 2002). Shoot water content was enhanced when scions of the ABA(-) mutant of tomato (*flacca*) were grafted onto the wild-type rootstocks relative to grafting *flacca* scions on its own rootstock (Chen and others 2002b). The increase in water content (Fricke and others 2004, 2006) and leaf osmotic potential (Wilkinson and Davies 1997; Zhu and others 2005) appeared to be related to ABA accumulation in the leaf. The increase in water content will increase tissue succulence and, under saline conditions, may facilitate the dilution of salt within the tissue (Ottow and others 2005). Cherian and Reddy (2000) reported the large increase in fresh weight was mainly due to plant water content. Shoot dry weight, total dry weight, and plant water content were also positively correlated (Basal and others 2006). Plant biomass is the most widely used graft index in defining salt stress tolerance. Higher fresh matter accumulation (Santa-Cruz and others 2002; Chen and others 2003) and dry matter accumulation in grafted relative to nongrafted plants was reported in tomato/tomato (Fernández-García and others 2004a) and tobacco/tomato (Ruiz and others 2005) watermelon (Colla and others 2006).

Chlorophyll content, a readily measured objective indicator of leaf health and growth potential (Percival 2005) as indicated by leaf greenness, was also explored in this study. Chlorophyll content showed varying responses to the salt stress and grafting treatments. Grafting of ABA(-) scions

onto salt-resistant rootstock increased chlorophyll content of the scion following 2 weeks of exposure to salt stress. These results are in agreement with those of Romero and others (1997) and Fernández-García and others (2002) who demonstrated that under saline conditions in grafted plants, leaf pigments and chlorophyll content were determined by the genotype used as the rootstock. Using the salt-sensitive ABA(−) line as a rootstock reduced chlorophyll concentrations of the stress-resistant 9506 scion. This might be due to low vigor of root systems in the uptake and translocation of ions, photosynthates, and plant hormones to the scions (Lee and Oda 2003) or to the loss of chlorophyll content of salt-susceptible genotypes by increasing salt stress (Mandal and Singh 2001).

Scion Effects

Although much work has been done on rootstock-to-scion response, fewer reports are available on scion-to-rootstock effects. Because potato is a tuberous crop, the influence of scions on the rootstock is of obvious importance to this economic commodity. Our study in potato indicates a significant influence of the scion on the rootstock.

The resistant 9506 genotype as a scion had a positive impact on increasing biomass of the root. Relative to nongrafted plants, the biomass of the ABA(−) mutant and ABA(+) sibling roots were elevated by grafting onto the 9506 resistant scions. This finding might be due to a higher photosynthesis rate by the more vigorous, salt-tolerant 9506 scions, leading to greater potential for partitioning of assimilates to the rootstock (Chen and others 2003). Chen and others (2003) 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(−) mutant was grafted onto the 9506 resistant rootstock, the reduced root growth of 9506 resistant rootstock might also be due to a low photosynthetic rate of the scion and reduced production and distribution of photosynthate. This might also explain the increased stem diameter of ABA(−) rootstocks grafted with 9506 resistant scions.

The scion may be sending a hormonal signal to the root system (Holbrook and others 2002). Holbrook and others (2002) showed that the control of shoot physiology under water stress resides in the shoots rather than the roots in reciprocal grafts of wild-type and ABA(−) mutants of tomato. Dunlap and Binzel (1996) reported that ABA levels were higher in leaves than roots. This may be particularly significant in light of our findings of greater endogenous ABA levels in the 9506 genotype and, in particular, the subsequent induction of higher ABA levels in the 9506 resistant line compared to all other genotypes after 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 and others 2002b). Chen and others (2002b) believed that an unknown “phenotype reversion factor” (PRF) produced in wild-type shoots rather than roots determines phenotype reversion of *flacca* in the Ws/Fr combination. They also found a significant linear relationship between biomass production and ABA levels in the scion.

Acclimation

Differential plant responses like dry matter production, stem diameter, water status, hormonal balance, and other physiologic and biochemical reactions are prime indicators of salinity endurance. Our study showed that alterations were induced in the rootstock by acclimation and in some cases transferred to the scion. Reduction of leaf necrosis, a sensitive indicator of salt tolerance in grafted plants (Wahome and others 2000, 2001), was transmitted to the scions grafted onto salt-tolerant rootstocks by salt-acclimation treatment during a subsequent salt stress event.

Uniquely, our study further indicates that acclimation of resistant scions can transfer responses to the rootstock. Acclimated 9506 resistant scions increased both root biomass and stem diameter of its ABA(−) rootstock. Biomass of shoots and roots of plants grafted with the 9506 resistant genotype increased in comparison with nongrafted plants, and the enhanced salt tolerance might be attributed to our observed higher ABA levels in this genotype both before and after acclimation.

Acclimation itself plays a role, and when nongrafted ABA(−) mutant plants were salt-acclimated, leaf greenness increased after subsequent salt stress. This is consistent with Djanaguiraman and others (2006) who found that rice plants that had been gradually salt-treated had a higher chlorophyll content than control plants. The salt-acclimation treatment on potato genotypes reduced shoot dry weight accumulation compared to nontreated plants. Similarly, Silveira and others (2001) showed that treatment of cowpea with 50 mM NaCl for 8 days resulted in a 25% reduction in shoot dry mass compared with control plants. Compared with the control plants, NaCl-adapted *Sorghum* plants also had reduced shoot dry weight (Amzallag and others 1990b; Amzallag 1996; de Lacerda and others 2001). In soybean, leaf dry weights were reduced slightly to severely by salt pretreatment, depending on the salt concentration used during the pretreatment (Umezawa and others 2000). Shoot dry weight in bean (*Phaseolus vulgaris*) (Bayuelo-Jiménez and others 2003) was also reduced.

Salt acclimation increased stem water content in the nongrafted ABA(−) mutant and its normal sibling. Ramoliya and Pandey (2003) found that stems in *Cordia*

rothii were the most salt-tolerant tissues, followed by leaves. This might be due to the high volume of water stored in the stem enabling a dilution effect of salt ions. Accumulation of salts in the shoots and prevention of absorption by photosynthetic and actively growing leaves is another salt-tolerance mechanism used by many plants (Reddy and others 1992).

Salt acclimated potato genotypes had higher leaf stomatal conductivity and leaf water content after 2 weeks of salt stress relative to unacclimated potato genotypes. Higher stomatal conductance in salt-acclimated plants has also been reported in rice (Djanaguiraman and others 2006). The increase in leaf osmotic potential in the non-grafted ABA(−) mutant, ABA(−)/9506, and ABA(+)/9506 under salt acclimation was also similar to sunflower (Steduto and others 2000).

ABA-mediated Responses

ABA accumulated to varying extents in all genotypes in our study and also varied with acclimation treatment. Acclimation induced significantly higher ABA levels than unacclimated treatments after exposure to salt stress. Salt acclimation-induced accumulation of endogenous ABA has not been previously reported in potato, however it has been examined in salt stressed potato (Pruvot and others 1996) and other crops including rice (Asch and others 1995) (60 mM NaCl), tomato (Dunlap and Binzel 1996; Mulholland and others 2003) (50 mM NaCl), *Brassica* (He and Cramer 1996; Verslues and others 2006) (85 mM NaCl), bean (Sibole and others 1998) (75 mM NaCl), soybean (Umezawa and others 2001) (50 mM NaCl), barley (Jia and others 2002) (100 mM NaCl), and *Arabidopsis* ABA mutants (Cramer 2002) (80 mM NaCl). The ranking of salt stress resistance after acclimation followed the ranking for endogenous ABA: ABA(−) mutant < ABA(+) sibling < ‘Norland’ ≤ 9506. Salt-tolerant genotypes maintained higher concentrations of ABA than salt-sensitive genotypes. ‘Norland’ and the 9506 genotype had similar ABA profiles in unacclimated plants after salt stress; however, acclimation induced higher levels of ABA in the more salt-tolerant 9506 genotype (76% increase) than ‘Norland’ (17% increase).

As expected, exogenously applied ABA significantly alleviated leaf necrosis after salt stress exposure in the genotypes that accumulated less ABA, in particular the ABA(−) mutant. However, additional exogenous ABA did not improve 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 stress. As a rootstock, the 9506 line also had the most significant impact on enhancing scion salt stress resistance in ABA(−) and ABA(+) grafted plants.

Although ABA was not measured in grafted plants after salt stress, other work has shown a distinct translocation of signal from the root to the shoot inducing ABA biosynthesis in the leaves. Even though *flacca* roots had some endogenous ABA, the root had difficulty transporting ABA to the shoots (Sagi and others 1999). Under stress conditions, the shoots and roots of the mutant were unable to accumulate ABA (Grillo and others 1995; Bray and others 1999). In research with the ABA-deficient mutant of tomato, *Flacca* (*Fs*), Chen and others (2002b) showed that when it was grafted on the ABA-normal rootstock (*Wr*) to yield *Fs/Wr*, the scions exhibited higher ABA content, lower transpiration rate, and higher water content than when the mutant was grafted on its own rootstock (*Fs/Fr*). Using the 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 and others (2002) concluded that a chemical signal in the root led to a change in apoplastic ABA levels within the scions to cause stomatal closure. To study the effect of rootstock on salt stress tolerance, a grafting experiment with three potato genotypes—an early-maturing salt-sensitive (EMS), an early-maturing salt-tolerant (EMT), and a late-maturing salt-tolerant (LMT) was performed (Shaterian and others 2005a). Salt tolerance of the EMS scion increased when grafted on the LMT rootstock as opposed to what happened 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 under our ABA treatment. This response is consistent with the observed increase in stomatal conductance. NMR studies have demonstrated an interrelationship between transpiration rate and calculated water rates (Kuchenbrod and others 1996; Köckenberger and others 1997). Magnetic resonance spectrometry (Peuke and others 2001) confirmed that greater water loss from leaves was compensated for by a greater water supply via the xylem. In 2005, using NMR imaging Velikanov and Belova (2005), showed that exogenous ABA (100 μM) also increased water permeability of the vacuolar symplast in the root cells of maize.

Based on our grafting studies and ABA treatments, salt tolerance was 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(−) mutant and ABA(+) sibling suggested that root parameters may represent a good

criterion for evaluating plant responses to salt stress. The 9506 resistant 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 positive upward water flow, 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 distinct scion-to-rootstock effect was also shown by our studies and has a positive impact for increasing commercial potato crop tolerance under available mechanized grafting systems. Further observations on performance of grafted material under field conditions are required to verify these results.

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