

# The Method of ABA Application Affects Salt Stress Responses in Resistant and Sensitive Potato Lines

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**Abstract** The phytohormone abscisic acid (ABA) has been proposed to act as a mediator in plant responses to a range of stresses, including salt stress. Most studies of ABA response apply ABA as a single dose. This may not resemble the prolonged increasing endogenous ABA levels that can occur in association with slowly increasing salinity stresses in nature or field situations. Salt stress response based on method of ABA application was examined in four potato genotypes of varying salt stress resistance: the sensitive ABA-deficient mutant and its normal sibling, a resistant genotype line 9506, and commercial cultivar ‘Norland’ of moderate resistance. ABA was applied by root drench at 0, 50, 75, or 100  $\mu\text{M}$  concentrations through a single dose, or by slowly increasing multiple ABA doses in a sand-based growing system under greenhouse conditions. Salt tolerance was then evaluated after 2 weeks of exposure to 150–180 mM NaCl stress. The method of ABA application had a marked effect on the responses to salt stress. Plant responses to the method of ABA application were differentiated according to (1) growth rate, (2) root water content, and (3) apparent shoot growth response. Under a single dose, growth rate increased in all genotypes under salt stress, whereas slowly increasing multiple ABA applications generally maintained stable growth rates except in the ABA-deficient mutant where there was an upward growth trend. Percent root water content was elevated only under slowly increasing multiple ABA doses in two genotypes, whereas none of the single-dose treatments induced any change. The single ABA dose enhanced vertical growth, whereas the slowly increasing multiple ABA

dose applications enhanced lateral shoot growth. Because exogenous application is still an artificial system, endogenous ABA was supplied through grafting of ABA-deficient mutant scions onto rootstocks with known elevated ABA levels. Multiple exogenous ABA applications as low as 50  $\mu\text{M}$  elicited similar shoot water content responses as grafting treatments without ABA application in the mutant genotype but had no effect on the ABA normal sibling. Shoot dry weight was significantly increased through grafting over all exogenous ABA treatments. Our study further indicates that the method of ABA application regime in itself can alter plant responses under salt stress and that certain application regimes may reflect responses to elevated endogenous levels of ABA.

**Keywords** Abscisic acid (ABA) · *Solanum tuberosum* · Application methods · Salinity stress

## Introduction

Plants are frequently subjected to environmental stress such as water deficit, freezing, heat, or salt stress. The phytohormone abscisic acid (ABA) has been suggested to play a role in stress responses and/or adaptation (Chen and Gusta 1983; Thomas and Eamus 1999; Gómez-Cadenaz and others 2003; Sharma and others 2005; Shaterian and others 2005a). *Arabidopsis* *sto1* (salt tolerant) mutant plants were unable to accumulate ABA following a hyperosmotic stress and either complementation of the *sto1* with the native gene from the wild-type genome or supplementation of ABA to the growth medium restored the wild-type phenotype (Ruan and others 2004).

Following a stress event, ABA content increases within a few minutes to several hours, depending on the type and

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severity of the stress (Cramer and Quarrie 2002; Jia and others 2002; Liu and others 2003; Fricke and others 2004, 2006). Under these conditions, expression of specific genes is induced; this can also be triggered in unstressed plants by external ABA (Moons and others 1997; Ma and others 2006; Srivastava and others 2006).

ABA is known to play an important role in enhancing plant water use efficiency under environmental stress. In plants coping with drought, ABA played an important role in root-to-shoot signalling and stomatal regulation, resulting in slowing shoot growth but maintaining primary root elongation (Sharp and LeNoble 2002; Hartung and others 2005). Normally, externally applied ABA coincides with reduced shoot and root growth under nonstress conditions (Griffiths and others 1997; Chen and others 2006b). However, there is detailed evidence of the effect of enhanced accumulation of endogenous ABA on root growth under salinity (Mulholland and others 2003). A recent study demonstrated the importance of applied ABA in root growth, morphology, and regulation of ion accumulation (Chen and others 2006a). The negative effect of NaCl salt on root nodule dry weight of common bean has also been shown to be 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). Furthermore, overproduction of ABA is associated with increased transpiration efficiency and root hydraulic conductivity and influences leaf expansion (Thompson and others 2007).

Near isogenic mutants with altered endogenous ABA levels are important tools for investigation of ABA responses in plants (Fambirini and others 1995). Several mutants with reduced capability to accumulate ABA have been identified: *notabilis* (*not*), *flacca* (*flc*), and *sitiens* (*sit*) mutants of tomato (Neil 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* (nd-1; w-1) (Fambirini and others 1995, 2004), viviparous corn mutants (Robichaud and others 1980; Suzuki and others 2006), and wheat (Holappa and others 2005). A close and connected interrelationship between ABA accumulation and multiple tolerances of osmotic, salt, and chilling stresses has been corroborated (Liu and others 2003). Because ABA production increases with the degree of salt stress (Jia and others 2002), under the slowly increasing salinity stress of natural conditions, ABA elevation will likely be more gradual. ABA treatments also involving slowly increasing concentrations may provide a better simulation of ABA response. Single doses of ABA may actually represent a shock to the plant.

The main objective of this work was to evaluate if the method of ABA application impacts potato plant responses to salt stress.

## Materials and Methods

Four potato genotypes were used: 9120–05, an ABA-deficient mutant (blocked at ABA-aldehyde; De Jong and others 2001) and highly salt stress sensitive; 9120–18 is the ABA normal sibling of 9120–05 and is only moderately sensitive to salt stress; 9506 is highly salt resistant; and a commercial variety, ‘Norland’, is moderately salt resistant (Shaterian and others 2005b). These lines were previously shown to express different levels of ABA after exposure to salt stress, with the mutant and ABA normal sibling at the lowest levels (Etehadnia and others 2008). Potato was clonally propagated using stem cuttings (apices) in Ottawa sand (1–2-mm-diameter, 75.5% very coarse sand; 0.5–1-mm-diameter, 24.4% coarse sand; and <0.5-mm-diameter, <0.1%) 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 each 1.5-L pot filled with white Ottawa sand. The pots were arranged in 20 × 40 × 60-cm-diameter plastic trays, with four pots (one of each line) per tray. The pots were irrigated by flooding the trays 2–3 times a day with a solution containing 10 mg/L of a complete nutrient solution (N-P-K plus micronutrients, Plant Products Co. Inc., Brampton, ON, Canada). The EC and pH of the nutrient solution was checked weekly. The EC and pH of the 0–180-mM NaCl treatment solutions had a range of 1.22–21.50 dS m<sup>-1</sup> and 6.52–6.96, respectively. The tubs were flooded for 3–5 min each time. The trays were drained completely to prevent salt accumulation in the root area. A photoperiod of 14 h was provided by supplementing natural light with high-pressure sodium halogen lamps that provided an average intensity of 700 μmol m<sup>-2</sup> s<sup>-1</sup> with a 25/18°C day/night temperature regime. The main stem and lateral shoots of each line were supported vertically with a bamboo stick.

ABA was applied after 3 weeks of growth just prior to the stolon initiation stage. ABA was applied as either (1) a single dose of 0, 50, 75, or 100 μM or (2) slowly increasing multiple doses (“Multiple application”) where the ABA was applied in five increasing levels to reach a final concentration of 50, 75, or 100 μM (Table 1). For example, in the 50-μM ABA treatment, ABA was increased by 10 μM every 3 days (that is, 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

**Table 1** Multiple application treatment of potato genotypes with different ABA (final concentration) by time intervals (day)

Day					Final [ABA] ( $\mu\text{M}$ )
1	3	6	9	12	
10	20	30	40	50	50
15	30	45	60	75	75
20	40	60	80	100	100

solution was adjusted to 6.5 by adding HCl or NaOH. Control plants were watered with distilled water and NaOH (1 N) with the pH adjusted to the same level as the treated solutions. Because the nutrient solutions were recirculated back to individual tanks, the solutions were changed 1 day after each ABA treatment to prevent the accumulation of ABA in the tank.

Salt stress involved adding NaCl to the nutrient solution until it contained 180 mM of NaCl for ‘Norland’ and 9506-resistant, and 150 mM NaCl for the ABA-mutant and its normal sibling. This salt stress was applied for 2 weeks following the termination of each ABA treatment. The different salt stress levels were applied to induce a similar LD50 based on genotypic variation in salt stress tolerance observed in a preliminary experiment and according to Shaterian and others (2005b).

Treatments were a factorial combination (4 genotypes  $\times$  4 treatments) arranged in a randomized complete block with four replications per treatment and three plants per replication. Pooled analysis of variance (ANOVA) for factorial randomized complete block design (RCBD) was performed by the general linear model (GLM) in SAS (SAS Institute, Cary, NC). Means were compared using least significant difference (LSD) at  $P = 0.05$ .

The effect of 2 weeks of salt stress on leaf necrosis of the lowest nonwilted leaf and the youngest fully expanded leaves was visually monitored. Leaf necrosis was scored from 1 to 5 with 1 = 0–20% leaf necrosis, 2 = 20–40% leaf necrosis, 3 = 40–60% leaf necrosis, 4 = 60–80% leaf necrosis, and 5 = 80–100% leaf necrosis. Leaf water content, stomatal conductance, and osmotic potential of the fourth and fifth fully expanded leaves were also recorded between 10 a.m. and 12 p.m. at the end of the 2 weeks of salt stress. Water content was determined by measuring leaf fresh and dry weights (one leaf) after drying in a hot-air oven for 48 h at 75°C. Stomatal conductivity was measured using a steady-state porometer (Li 1600, LI-COR Biosciences, Lincoln, NE) between 9 a.m. and 11 a.m. Plant height was measured based on the distance from the plant collar to the tallest point of the main stem. Plant height was monitored every week.

Osmolality was quantified by a vapor pressure osmometer (model 5500, Wescor, Inc., Logan, UT) from samples

obtained by crushing frozen leaf tissue with a handpress. Osmotic potentials were calculated using the formula:  $\Psi_s = -CiRT$  (Salisbury and Ross 1992). After 2 weeks of salt stress, fresh and dry weights of shoots and roots and also shoot and root water content were recorded.

### Grafting

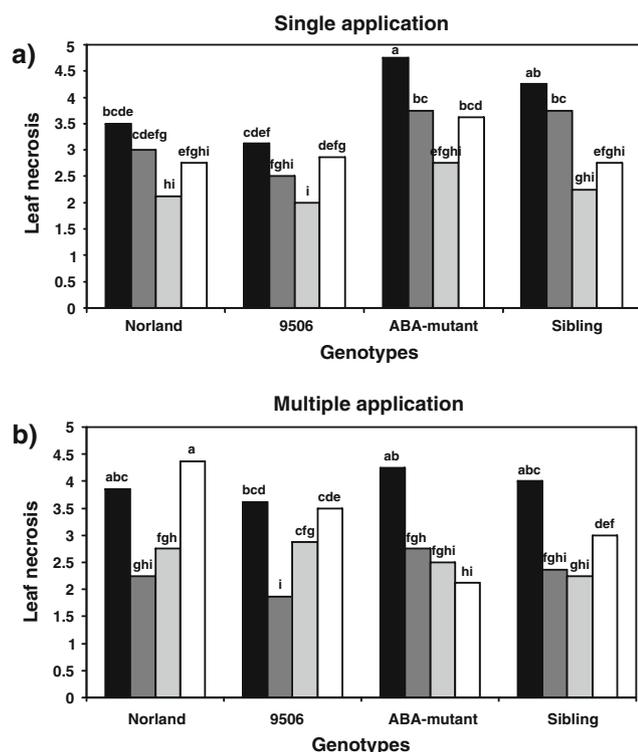
To prepare grafting stock, three diploid potato genotypes [9120–05 (ABA-deficient mutant), 9120–18 (ABA normal sibling), and 9506 (salt tolerant)] were propagated by shoot tip cuttings in soilless mix (Sunshine Mix #4, Sungro Hort. Inc., Bellevue, WA) in the mist chamber and transferred to 1.5-L pots containing white Ottawa sand. Greenhouse conditions were as previously described. Seedlings 15–20 cm long were selected to perform grafting. Healing of the graft union at this stage of growth was very successful compared with other stages of growth. The following scion/rootstock combinations were created: ABA mutant/9506, ABA normal sibling/9506, and reciprocal grafts of 9506/ABA mutant and 9506/ABA normal sibling. A cleft graft was used with the scion being prepared from the shoot tip. Grafting was performed immediately after cutting. The scion and rootstock were held together and protected from desiccation by wrapping with a paraffin-embedded plastic film (Parafilm, American National Can, Menasha, WI). Newly grafted stems were transferred to a mist chamber and covered with a clear plastic bag for a week to provide protection against moisture loss. The bag was then removed and plants were grown for one more week in the mist chamber. Plants were grown as previously described. Sprouts from nodes of the rootstock were continuously removed to maintain only one grafted scion. Grafted plants were then acclimatized for 2 weeks before ABA application. A factorial design arranged in a RCBD (7 genotypes  $\times$  4 ABA treatments) with four replicates and two plants per replicate was used for this study. Shoot dry weight and water content were measured as previously described.

### Results

The method of ABA treatment, either through a single dose or multiple, slowly increasing ABA doses produced significantly different responses in potato genotypes exposed to salt stress. The responses depended on both ABA treatment and potato genotype.

#### ‘Norland’

In ‘Norland’ a single dose of ABA produced plants with less leaf necrosis compared with control plants after 2 weeks of salt stress (Figure 1); however, a single ABA



**Fig. 1** The effect of single (a) and multiple (b) ABA applications ( $\mu\text{M}$ ) on leaf necrosis of four potato genotypes after 2 weeks of salt stress. Leaf necrosis was scored from 1 to 5, 1, 0% leaf area necrosis; 2, 0–25% leaf area necrosis; 3, 25–50% leaf area necrosis; 4, 50–75% leaf area necrosis; 5, 75–100% leaf area necrosis. Means followed by the same letters are not significantly different at 5% using LSD. (■) Control; (■) 50  $\mu\text{M}$  ABA; (■) 75  $\mu\text{M}$  ABA; (□) 100  $\mu\text{M}$  ABA

treatment was not as effective as multiple ABA applications. Only the 75- $\mu\text{M}$  single dose was effective in significantly reducing leaf necrosis. By contrast, slowly increasing multiple ABA applications to 50- and 75- $\mu\text{M}$  final concentrations significantly reduced leaf necrosis after exposure to salt stress. The highest dose of ABA tested (100  $\mu\text{M}$ ) was ineffective in reducing leaf necrosis under either single or multiple ABA applications.

A single ABA dose did not increase root dry weight until the 100- $\mu\text{M}$  level (Table 2). Water content was not changed under most doses (Table 2). However, slowly increasing multiple applications from as little as the 50- $\mu\text{M}$  final ABA concentration elevated root dry weight with no change in root water content. Only the 100- $\mu\text{M}$  multiple application treatment resulted in a significant reduction of root water content (Table 2).

A single ABA dose at all concentrations stimulated increased shoot water content but reduced dry weight at the 75- and 100- $\mu\text{M}$  ABA levels (Table 2). Slowly increasing multiple ABA applications also induced elevated shoot water content, with as little as 50- $\mu\text{M}$  final level of multiple ABA applications. Multiple ABA applications at the 50- and 75- $\mu\text{M}$  final concentrations also induced a twofold

higher shoot fresh weight than the corresponding single ABA application (data not shown).

Growth rates were distinctly different for single versus multiple doses of ABA (Figures 2 and 3). After salt stress, a single ABA dose significantly increased growth rates, with the highest growth rate occurring at 75  $\mu\text{M}$ . By contrast, multiple ABA applications did not induce any change in growth rate at any of the treatment concentrations. Stomatal conductivity also responded differently depending on the method of ABA treatment. When a single ABA dose was used, leaf stomatal conductance after 2 weeks of salt stress markedly increased relative to the control under all ABA concentrations (Table 3). This increase in conductance may have contributed to the observed increase in growth rate (Figure 2). Under multiple ABA applications, stomatal conductance, both before or after salt stress, showed only a marginal elevation at the 75- and 100- $\mu\text{M}$  ABA treatments (Table 3). Leaf water content after salt stress was not changed by the single ABA dose (Table 4) and maintained stable leaf osmotic potential (Table 4). Under multiple ABA applications, both leaf water content and osmotic potential before stress were not changed (data not shown). However after salt stress, leaf water content increased for all multiple ABA concentrations, whereas leaf osmotic potential still remained constant except at the 100- $\mu\text{M}$  level (Table 4).

#### 9506

For the single dose of ABA, only the 75- $\mu\text{M}$  treatment had a significant and positive effect on reducing leaf necrosis in the 9506 genotype under salt stress compared with non-treated plants (Figure 1). By contrast, in the slowly increasing multiple ABA applications, only the 50- $\mu\text{M}$  ABA final concentration treatment was effective in reducing leaf necrosis in this genotype.

A single ABA dose at 75 and 100  $\mu\text{M}$  increased root dry weight but not water content (Table 2). In contrast to the single ABA treatment, multiple ABA applications with as low as 50- $\mu\text{M}$  final concentration increased root water content but not dry weight.

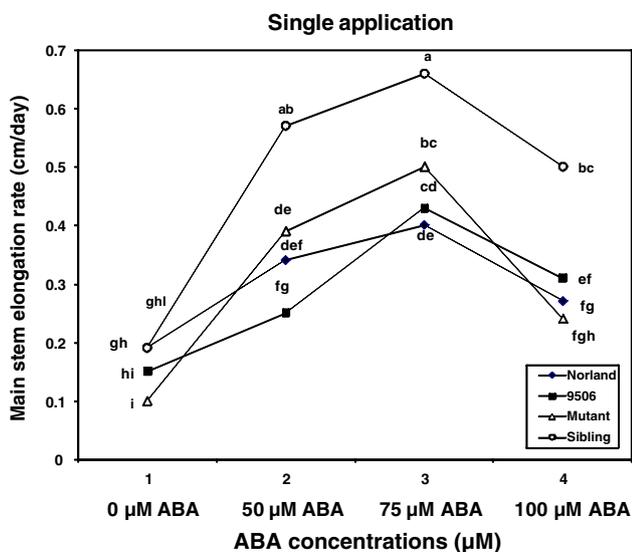
All concentrations of ABA applied as either a single dose or multiple doses enhanced shoot water content relative to the control (Table 2). However, multiple ABA applications also decreased shoot dry weight.

Significant increases in stomatal conductance were observed for all single doses of ABA (Table 3). Under multiple ABA applications, stomatal conductivity increased at 100  $\mu\text{M}$  (after stress) (Table 3). All concentrations of the single-dose ABA treatment also significantly enhanced the growth rate under salt stress (Figure 2). Similar to 'Norland', growth rates after salt stress were distinctly different for single versus multiple doses of ABA

**Table 2** The effect of single and multiple ABA applications on root and shoot dry weights (g) and water contents (%) of four potato genotypes after two weeks of salt stress (150 mM NaCl for ABA-deficient mutant and its normal sibling and 180 for ‘Norland’ and 9506)

	Single application				Multiple applications			
	‘Norland’	9506	ABA mutant	Normal sibling	‘Norland’	9506	ABA mutant	Normal sibling
<i>Root dry weight</i>								
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	2.03 de
100	5.52 b	6.47 a	0.66 h	2.41 g	3.58 c	2.48 d	0.39 f	1.47 e
<i>Root water content</i>								
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
<i>Shoot dry weight</i>								
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
<i>Shoot water content</i>								
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

Means within columns and rows followed by the same letters are not significantly different at 5% using LSD



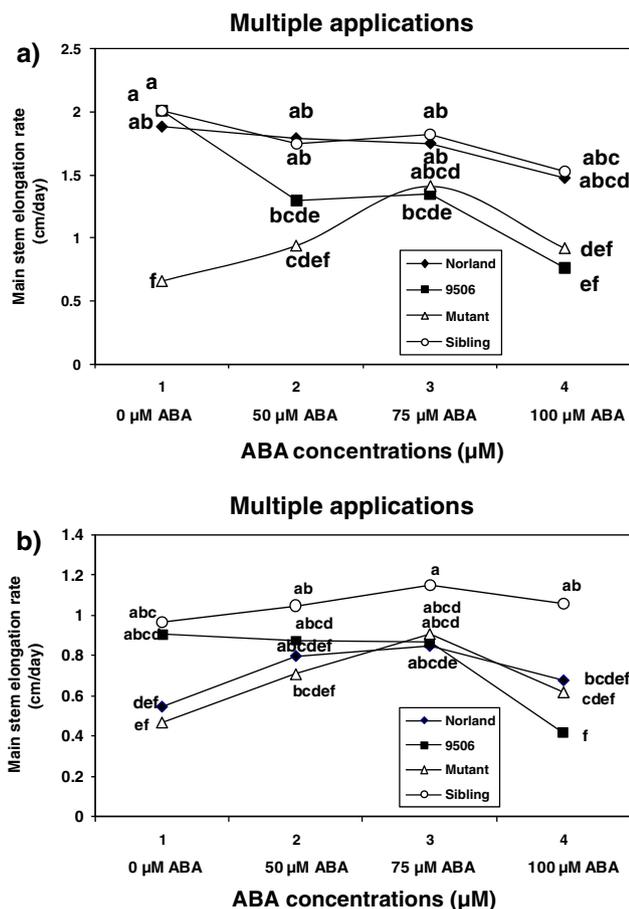
**Fig. 2** The effect of a single ABA application on main stem elongation rate of four potato genotypes after 2 weeks of salt stress (150 mM NaCl for ABA-deficient mutant and its normal sibling and 180 for ‘Norland’ and 9506). Means followed by the same letters are not significantly different at 5% using LSD. (◆) ‘Norland’; (■) 9506; (△) mutant; (○) normal sibling

for the 9506 genotype (Figures 2 and 3). A single ABA dose significantly increased growth rates, with the highest growth rate occurring at 75 µM. By contrast, under multiple ABA applications, growth rate did not change with the 50- and 75-µM multiple ABA applications and diminished under the 100-µM ABA treatment (Figure 3). Although ABA had no effect on leaf water content under a single dose (Table 4), all single ABA applications increased leaf osmotic potential. However, all multiple ABA applications raised both the leaf water content and leaf osmotic potential after salt stress (Table 4) but had no effect prior to salt stress treatment (data not shown).

#### ABA-Deficient Mutant

In the absence of applied ABA, compared to the ABA normal genotype the ABA-deficient mutant consistently had the lowest root dry weight and water content, shoot dry weight, and growth rate following exposure to salt stress (Table 2, Figures 2 and 3).

Leaf necrosis of the ABA-deficient mutant was reduced by all concentrations of a single ABA dose, with 75 µM



**Fig. 3** The effect of multiple ABA applications on main stem elongation rate of four potato genotypes before (a) and after (b) 2 weeks of salt stress. Means followed by the same letters are not significantly different at 5% using LSD. (◆) 'Norland'; (■) 9506; (△) mutant; (○) normal sibling

ABA inducing the greatest effect (Figure 1). Similarly, all multiple ABA applications also significantly reduced leaf necrosis (Figure 1).

The distinction between responses to a single dose and multiple ABA doses was observed in the enhanced root water content under all slowly increasing multiple doses but not under a single dose (Table 2). Slowly increasing multiple doses elevated both shoot dry weight and water content (Table 2) whereas single doses only increased water content.

All concentrations of a single dose and multiple ABA doses significantly increased leaf stomatal conductance (Table 3). However, growth rates differed between the two methods of ABA applications. With a single ABA dose, the growth rate was increased by all ABA concentrations, peaking at the 75-μM level (Figure 2). Growth rates did not change after salt stress under multiple ABA applications (Figure 3). With the single ABA treatment, and particularly at 75 μM, leaf water content increased appreciably

**Table 3** The effects of single and multiple ABA applications on leaf stomatal conductivity ( $\text{cm s}^{-1}$ ) after two weeks of salt stress (150 mM NaCl for ABA-deficient mutant and its normal sibling and 180 for 'Norland' and 9506)

ABA (μM)	'Norland'	9506	ABA mutant	Normal sibling
<i>Single dose</i>				
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
<i>Multiple applications: before salt stress</i>				
0	0.12 ef	0.10 g	0.11 fg	0.11 fg
50	0.12 ef	0.10 g	0.11 fg	0.12 ef
75	0.14 d	0.12 ef	0.14 d	0.13 de
100	0.23 a	0.21 b	0.19 c	0.18 c
<i>Multiple applications: after salt stress</i>				
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

Means within columns and rows followed by the same letters are not significantly different at 5% using LSD

relative to control (Table 4). Leaf osmotic potentials were also less negative under these ABA treatments (Table 4). Only the multiple ABA application of 75 μM enhanced leaf water content (Table 4). Leaf osmotic potentials were unaffected by multiple ABA treatments (Table 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 1). However, with slowly increasing multiple ABA applications, there was a marked reduction in leaf necrosis at all concentrations tested.

Neither a single ABA dose or multiple doses markedly affected root dry weight or water content (Table 2). In contrast to the ABA mutant, multiple ABA doses had no effect on root water content in the ABA normal sibling (Table 2).

Although only the 75- and 100-μM single-dose ABA treatment enhanced shoot water content, all concentrations of ABA applied as slowly increasing multiple doses elevated this response (Table 2).

For both single and multiple ABA applications, leaf stomatal conductance after salt stress only increased under the 75-μM ABA level (Table 3). As observed in all other genotypes, growth rates were increased by all single ABA doses but not by the multiple ABA applications (Figures 2, 3). Leaf water content increased under all

**Table 4** The effect of single and multiple ABA applications on leaf water content (%) and leaf osmotic potential ( $\Psi_s$  = MPa) of four potato genotypes after 2 weeks of salt stress (150 mM NaCl for ABA-deficient mutant and its normal sibling and 180 for ‘Norland’ and 9506)

	Leaf water content				Leaf osmotic potential			
	‘Norland’	9506	ABA mutant	Normal sibling	‘Norland’	9506	ABA mutant	Normal sibling
<i>Single application</i>								
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
<i>Multiple applications</i>								
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

Means within columns and rows followed by the same letters are not significantly different at 5% using LSD

**Table 5** Mean shoot dry weight (g) and water content (%) of ABA-treated and nontreated potato genotypes after 2 weeks of salt stress (150 mM NaCl for ABA-deficient mutant, sibling, 9506/mutant, 9506/sibling, and 180 mM for 9506, mutant/9506, and sibling/9506)

Responses	ABA treatments	Mutant	Mutant/9506	9506/Mutant	9506	9506/Sibling	Sibling/9506	Sibling
Shoot dry weight	0 $\mu$ M	3.37 n	9.16 hijk	21.38 a	19.53 ab	7.27 klm	11.27 ghij	10.12 ghijk
	50 $\mu$ M	4.35 mn	12.07 fg	19.52 ab	15.02 cdef	8.89 ijk	15.40 cde	13.10 defg
	75 $\mu$ M	5.12 lmn	9.12 hijk	19.33 ab	15.88 cd	10.66 ghij	10.63 ghij	12.48 efg
	100 $\mu$ M	3.78 n	8.40 jkl	17.91 bc	17.21 bc	12.20 efgh	8.97 hijk	11.48 ghij
Shoot water content	0 $\mu$ M	73.10 k	78.34 ij	85.98 abcdef	79.11 hij	88.62 a	84.02 bcdefg	79.96 ghij
	50 $\mu$ M	75.90 jk	84.93 abcdef	87.68 abc	86.86 abcde	87.25 abc	83.35 cdefgh	82.49 efghi
	75 $\mu$ M	81.45 cdefgh	85.48 abcdef	88.03 ab	84.89 abcdef	87.57 abc	86.62 abcde	84.07 bcdefg
	100 $\mu$ M	84.57 abcdef	88.37 ab	87.11 abcd	81.77 fg	85.58 abcdef	82.57 defghi	84.52 abcdef

Means within columns and rows followed by the same letters are not significantly different at 5% using LSD

single- and multiple-dose treatments after salt stress (Table 4). A parallel increase in leaf osmotic potential was observed (Table 4).

**Grafting**

In the absence of ABA, using 9506 as rootstock with known elevated levels of ABA, grafting itself induced responses similar to the nongrafted mutant and sibling shoot dry weight and water content responses as low as the 50- $\mu$ M ABA level (Table 5).

**Discussion**

The method of ABA treatment, either through a single dose or slowly increasing multiple ABA doses, produced significantly different responses in potato genotypes exposed to salt stress. The responses depended on both ABA treatment and potato genotype; however, several consistent trends emerged.

Both single and slowly increasing multiple ABA applications reduced leaf necrosis. Wahome and others (2001) reported that leaf tip necrosis was the first sign of NaCl salt injury. 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 determine the stress resistance of the potato clone (Shaterian and others 2005b). In our study, growth response to ABA application differentiated these treatments. Only ABA as a single dose enhanced plant growth rate (height increase) under salt stress. Slowly increasing multiple ABA doses maintained growth rates. Growth enhancement by ABA treatment is widely reported in the literature (Amzallag and others 1990; Sharp and others 2000; Sansberro and others 2004; Khadri and others 2006, 2007). However, growth rate in our study was measured as a factor of vertical height, not lateral growth. Although multiple ABA applications did not induce an increase in height, because total shoot dry weight more than doubled under multiple ABA application regimes in the ABA-deficient mutant, ABA appears to be inducing

greater lateral growth under this treatment. ABA has been shown to increase branching in *Cucurbita pepo* (Tanino and others 2002) via long-distance signaling produced in roots and shoots (Foo and others 2001). Thus, shoot growth response varied between the two methods of ABA application in that a single ABA dose enhanced vertical shoot growth rate, whereas multiple ABA applications partitioned the growth into lateral rather than vertical growth.

The ABA treatment increased shoot water content under both application regimes. ABA has been reported to be involved in plant water uptake thereby resulting in increased xylem water potential in the presence of salt (Fricke and others 2004). However, the apparent mechanism of continued water uptake in the presence of NaCl salt was different between the two methods of ABA application. Water uptake of 9506 under a single dose of ABA appears to be via the transpiration stream because stomatal conductivity was higher than controls at the end of the salt stress. Although ABA is known to induce stomatal closure (Wright 1978; Montero and others 1998; Sibole and others 2000; Mishra and others 2006), there are also reports on higher stomatal conductance in ABA-treated plants than nontreated plants under single (Tenhunen and others 1994; Ruan and others 2005) and multiple ABA treatment (Gómez-Gadenas and others 2003).

Water uptake with multiple ABA applications may have occurred through the symplastic route because plants continued to exhibit the low stomatal conductivity of the nontreated plants. Water uptake could be facilitated through aquaporins as well as osmotic adjustment (Wang and others 2003), the latter potentially achieved through sodium accumulation under salt stress. In another study, Etehadnia and others (2008) showed that ABA was associated with a 48% elevation of Na<sup>+</sup> in the roots which was accompanied by Na<sup>+</sup> exclusion from the shoots when plants were exposed to salt stress. Plant water improvement is a known outcome of ABA treatment (Hose and others 2000; Chen and others 2002; Sansberro and others 2004; Sharma and others 2005). ABA applied in multiple doses facilitated water uptake into roots as soil drying started, especially under nontranspiring conditions when the apoplastic path of water transport was largely excluded (Hose and others 2000). ABA treatment also resulted in increased relative water content (Sansberro and others 2004). A rapid increase in ABA led to a rise in xylem water potential through reduced transpiration or increased root hydraulic conductance (Fricke and others 2004; Vysotskaya and others 2004a, b).

ABA enhanced salt tolerance by also altering root growth. However, the response differed depending on 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 and others 2005a, b). Under a single dose, both genotypes

responded similarly in that root dry weight was enhanced but not water content (Table 2). By contrast, the slowly increasing multiple ABA applications distinguished ‘Norland’ from 9506 in that root water content was primarily increased in the most salt-tolerant 9506 clone (Table 2). Whether through single or multiple ABA doses, ‘Norland’ root fresh weight was largely attributable to root dry weight (data not shown). A novel function of ABA in root growth, development, formation of root hair, and lateral roots has recently been reported (Chen and others 2006a). Root length, root number, and root cortex thickness of salt-stressed rice seedlings were increased as a result of pretreatment with ABA before exposure to soil salinity (Cham and others 2007).

#### ABA-deficient Mutant and ABA Normal Sibling

Because the ABA-deficient mutant consistently expressed the lowest root dry weight, water content, shoot dry weight, and growth rate therefore, the presence of ABA appears to be important for these parameters. ABA is widely known to be important in root and shoot growth (for example, Sharp and Le Noble 2002). Application of external ABA, particularly in multiple applications, affected the phenotype and growth habit of the ABA-deficient mutant, but not to the level of its normal sibling. The responsiveness of the ABA-deficient mutant to multiple ABA concentrations is consistent with the result of Lenzi and others (1995) who reported that in the ABA-deficient mutant of sunflower (w-1), higher amounts of exogenous ABA were required to supplement the endogenous hormonal level to reach the same level of the wild type (W-1).

Sharp and others (2000) demonstrated that normal levels of endogenous ABA are required to maintain shoot development. They found shoot and root growth were greatly reduced in *flacca* and *notabilis* grown under controlled-humidity conditions, although 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, the leaf water contents in our ABA-deficient mutant and normal sibling genotypes were generally equivalent throughout salt stress, and yet root and shoot growth were reduced in the mutant genotype. Conversely, leaf osmotic potential was largely more negative in the ABA-deficient mutant. This is consistent with the finding of Etehadnia and others (2008) in which ABA was associated with avoidance of Na<sup>+</sup> accumulation in the shoots under salt stress. Shoot growth was also increased in the ABA-deficient mutant potato (*droopy*) with ABA application under salt stress (Shaterian and others 2005a). Additional experiments with *flacca* showed that shoot growth substantially recovered when wild-type levels of ABA were restored by exogenous

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 and others 2003). In our earlier study (Etehadnia and others 2008), endogenous ABA levels were lower in the ABA-deficient mutant and salt stress did not elevate internal ABA to sibling levels.

Before ABA application, the degree of leaf necrosis in the ABA-deficient mutant and its normal sibling was not different. Although endogenous ABA levels were lower in the mutant compared to the sibling (Etehadnia and others 2008), those differences were not large and are consistent with those of Coleman and Schneider (1996). 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 which both single and multiple ABA applications consistently increased leaf water content in both genotypes and stem water content in the ABA-deficient mutant (data not shown). Because leaf stomatal conductivity also increased under both ABA application regimes, particularly in the mutant, enhanced water uptake may be occurring through the transpiration stream. Although ABA is known to induce stomatal closure (Wright 1978; Montero and others 1998; Sibole and others 2000; Mishra and others 2006) as previously mentioned, there are also reports on higher stomatal conductance in ABA-treated plants than in nontreated plants (Tenhunen and others 1994; Gómez-Gadenas and others 2003; Ruan and others 2005). The discrepancy might be further explained in a potential signaling factor from the root to the shoot. Studies indicate that ABA is produced in the leaves after signal induction from the roots after stress (Ali and others 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 (bypassing the ABA source from the leaf). Conceivably, this may produce more salt stress-resistant roots and, thus, less signal generated to the leaves. ABA, either in a single- or multiple-dose application, induced pronounced increases to both root water content and dry weight which may have assisted in stress resistance.

Exogenous ABA application can produce spurious results (Freundl and others 1998, 2000). Our study further indicates that the method of ABA application in itself can alter plant responses under salt stress.

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