



Calcium Amendment and NaCl Salt Pre-treatment Improves Salinity Tolerance in Potato Genotypes

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Abstract

Pre-exposure to low but non-toxic levels of calcium and NaCl salt have been demonstrated to ameliorate the adverse effects of subsequent NaCl stress on plant growth and nutrient uptake. The effect of CaCl₂, NaCl and CaCl₂ + NaCl on subsequent salt stress tolerance, growth, water status and ion accumulation of four contrasting potato genotypes, 9506, 'Norland', ABA-deficient mutant and ABA normal sibling were investigated to provide an additional tool to induce NaCl salt stress tolerance. NaCl pre-treatment was generally the most effective in inducing salt stress resistance reflected by positive response measurements in all genotypes. 'Norland' and '9506' enhanced growth up to 70% via root mass under salt stress and expressed elevated water status under NaCl pre-treatment, possibly via Na⁺ accumulation in the roots. Unlike 'Norland', the 9506 genotype also tolerated the presence of Na⁺ in the shoot. However, NaCl was excluded from the shoot of '9506' after CaCl₂ pre-treatment and may indicate a genotype-dependent Ca⁺² requirement for Na⁺ exclusion. In the ABA normal sibling, salt stress resistance was largely regulated by two mechanisms under all pre-treatments: a) shoot Na⁺ exclusion and root Na⁺ accumulation; b) enhanced water status which was expressed by an elevation of leaf and shoot water content. This response to pre-treatment was facilitated by: a 40% - 100% increase in root mass, enhanced K⁺ uptake into the roots, enhanced K⁺/Na⁺ ratio in the root and shoots and increased leaf osmotic potential. The ABA-deficient mutant expressed only one mechanism of salt stress resistance in response to pre-treatments in which leaf and shoot water content increased. That the pre-treatments of the ABA-deficient mutant were not able to adequately increase shoot K⁺ and exclude Na⁺ from the shoot relative to the ABA normal sibling and other genotypes suggests that ABA is a requirement for this mode of salt stress defence.

Introduction



Over 20% of the global irrigated lands are salt stress affected and this value is rapidly increasing both naturally and due to insufficient soil and water management (FAO, 2006). This threatens:



Agricultural crop production (Parida and Das, 2005).



Agricultural sustainability (Waisel, 2001).



Worldwide, NaCl and Na₂SO₄ are the most common salts associated with saline soils and are largely responsible for this toxicity (Li *et al.*, 2006). This resulted in:



Sodium accumulation in the plants which in turn is the main factor in inhibition of plant growth under salinity (Anil *et al.*, 2005).



Deterioration of uptake of K⁺ is essential for normal function under high salt concentrations and is associated with salt tolerance (Bolat *et al.*, 2006).



Soil salinity levels as low as 2.3 dSm⁻¹ reduce both growth and tuber yield of the relatively salt sensitive potato (Katerji *et al.*, 2003) which globally, is the fourth most important food crop (CIP, 2007).



Acclimation is characterized by the ability to grow at salt concentrations which otherwise would be lethal to non-acclimated plants (Amzallag *et al.*, 1990b).



Plant breeding alone cannot be the sole solution of salinization and has to be seen as part of a total package including management of soil salinity and irrigation.




Calcium has a wide role in plant growth and development and in maintenance and modulation of various cell functions (El-Hamdaoui *et al.*, 2003).

External Ca²⁺ may influence the extent of Na⁺ and Cl⁻ accumulation in salt stressed Plant (Dabuxilatu and Ikeda, 2005), or inhibit Na⁺ uptake (Bolat *et al.*, 2006) via mediating Na⁺-permeable channels (Roberts and Tester, 1997) and maintained membrane permeability (Bolat *et al.*, 2006).



Addition of calcium also increased K⁺ uptake and transport (Bolat *et al.*, 2006) and improved the K⁺/Na⁺ selectivity (Bolat *et al.*, 2006) under salinity.

Interaction between NaCl and CaCl₂ indicated that growth inhibition by NaCl could be counteracted by CaCl₂ (Gehlot *et al.*, 2003).

Objective:

 To examine salt stress resistance mechanisms by **increasing** of NaCl, CaCl₂ and

combined NaCl + CaCl₂ levels in four contrasting potato genotypes based on:

-  Carbon allocation
-  Acquisition, transport and sequestration of Na⁺, Ca²⁺, K⁺ in plants

Materials and Methods

Physiological responses and acclimation ability of four potato genotypes, vegetatively propagated hydroponically grown (Fig.1), were evaluated under greenhouse conditions:

Diploid clones: 9506-resistant (salt tolerant line); 9120-05 (ABA-deficient mutant, salt sensitive) and 9120-18 (ABA-normal sibling, moderately salt sensitive). Tetraploid cultivar, 'Norland' (moderately salt tolerant), was used as a check.

Pre-treatments were: 0 mM CaCl₂ and 0 mM NaCl; 20 mM CaCl₂ for one week followed by three weeks of NaCl (50 or 75 mM) * salt acclimation; 20 mM CaCl₂ applied for four weeks; and 20 mM CaCl₂ for one week followed by three weeks of NaCl salt acclimation (50 or 75 mM) combined with CaCl₂ (20 mM).

* Based on observed salt tolerance, pre-acclimation treatment of ABA-deficient mutant and its sibling were adjusted to 50 mM NaCl (16.66 mM per week reaching 50 mM in 3 weeks); and 'Norland' and 9506-resistant to 75 mM NaCl (25 mM NaCl per week reaching 75 mM in 3 weeks).

Based on salt tolerance, 150 mM NaCl for the ABA mutant and its sibling and 180 mM NaCl for 'Norland' and '9506' resistant genotypes was used for two weeks of salt stress treatments.



Figure 1. Greenhouse potato hydroponic growing system.

Result

Pre-treatment with NaCl, CaCl₂, and their combination significantly decreased leaf necrosis under salt stress in all potato genotypes (Figure 2) and increased salt tolerance. However, other responses were genotype-dependent:

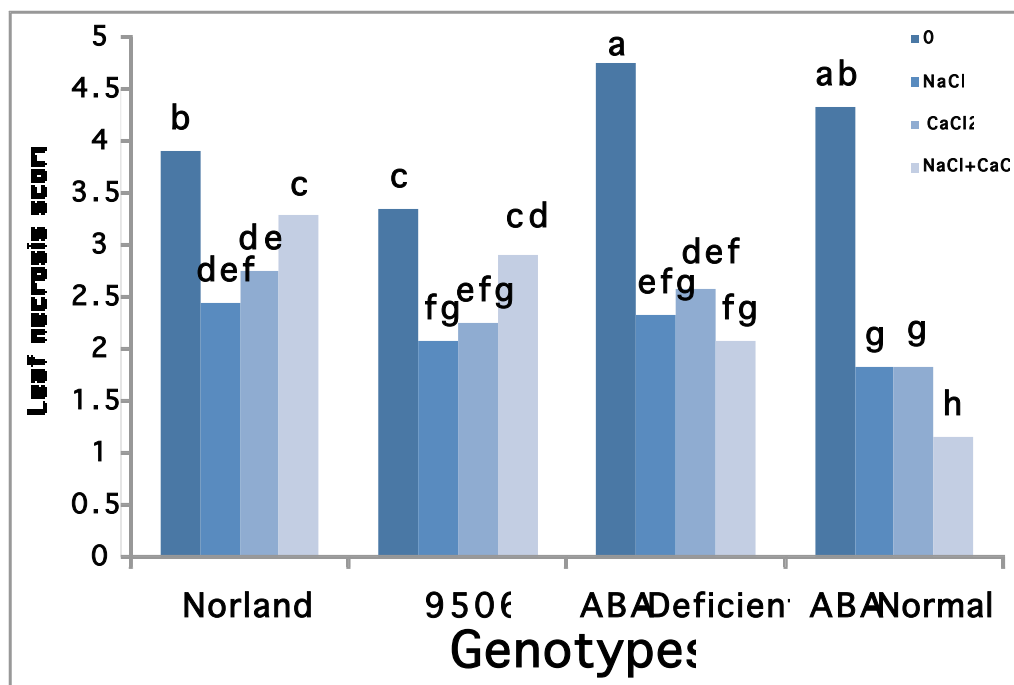


Figure 2. The effects of salt pre-treatments on leaf necrosis rating for four potato genotypes after two weeks of salt stress. Leaf necrosis was scored from: 1-5. 1- (0 % leaf area necrosis), 2- (0-25% leaf area necrosis), 3- (25-50 % leaf area necrosis), 4- (50-75 % leaf area necrosis), 5- (75-100 % leaf area necrosis).

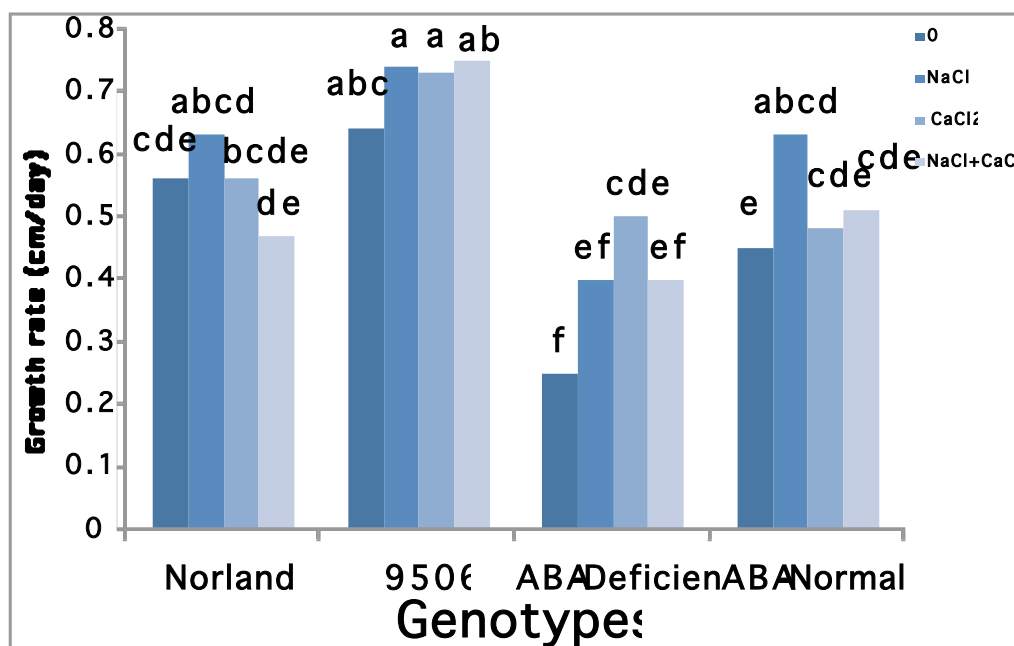


Figure 3. The effects of salt pre-treatments on growth rate (height increase, cm/day) of four potato genotypes after two weeks of salt stress.

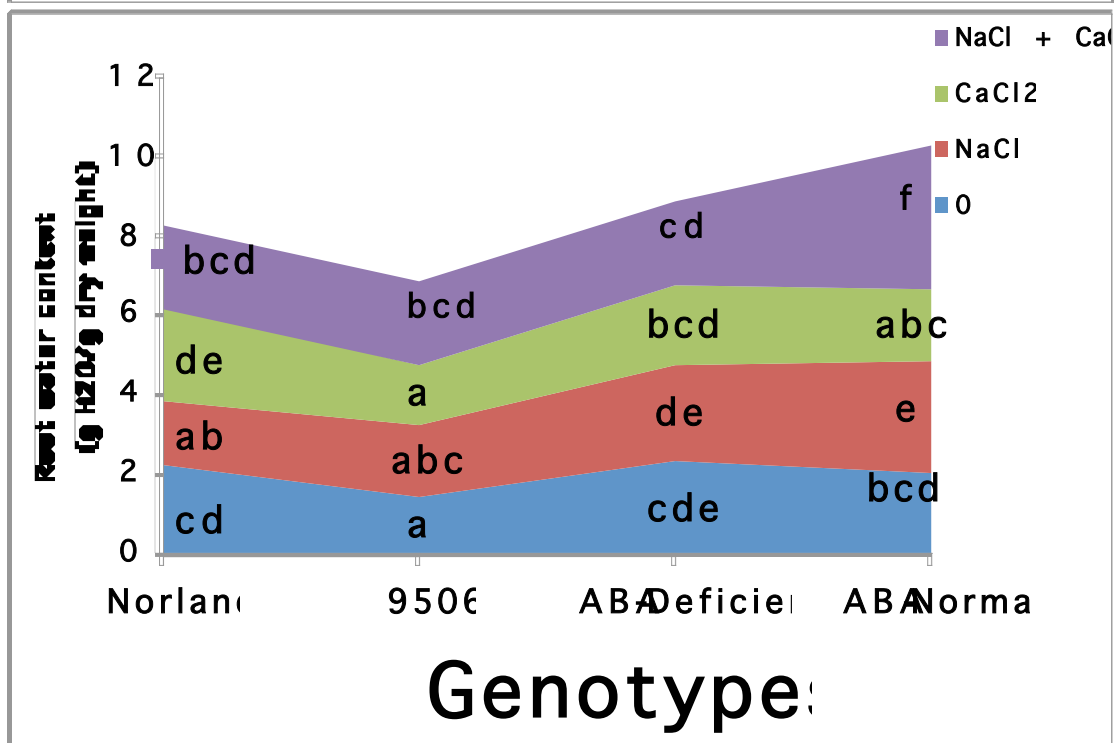
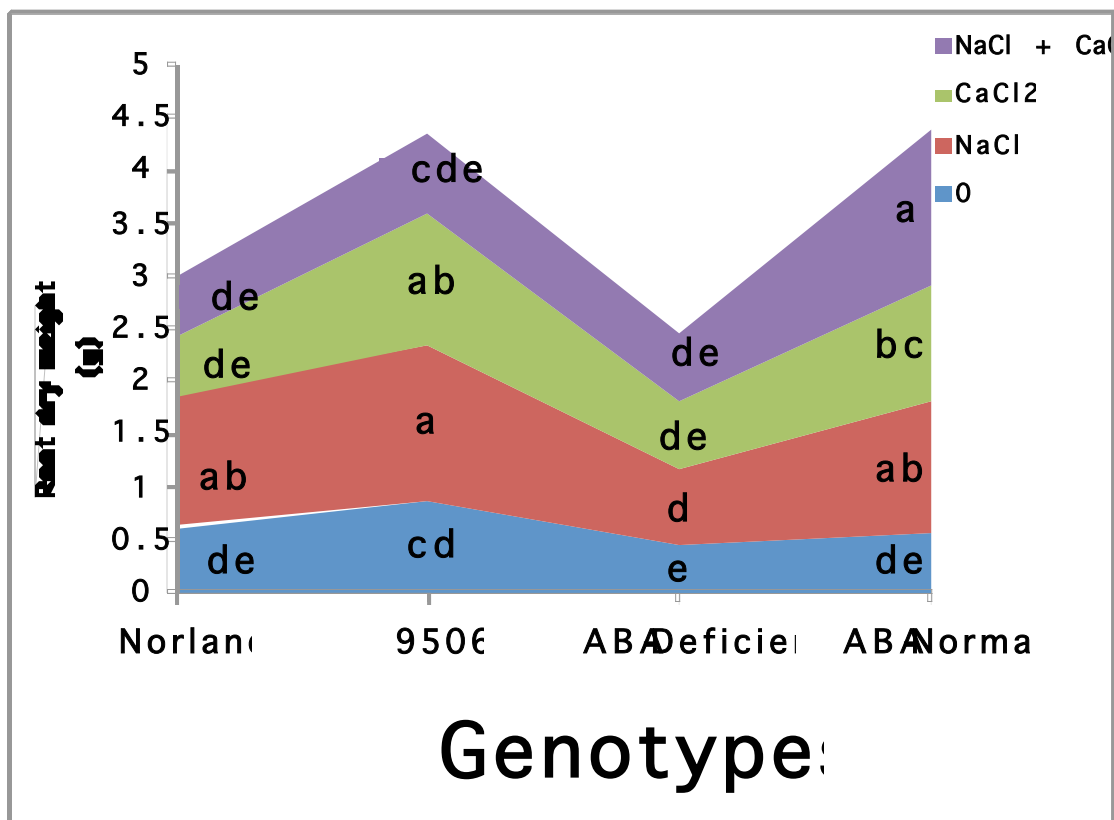


Figure 4. The effects of salt pre-treatments on root dry weight and root water content of four potato genotypes after two weeks salt stress.

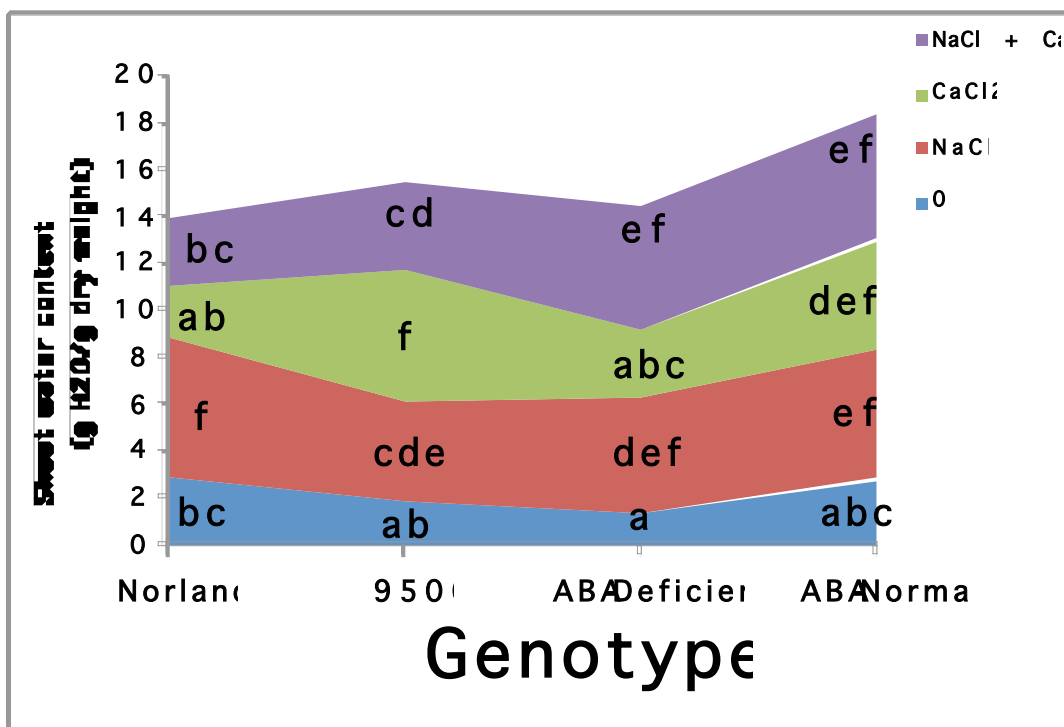
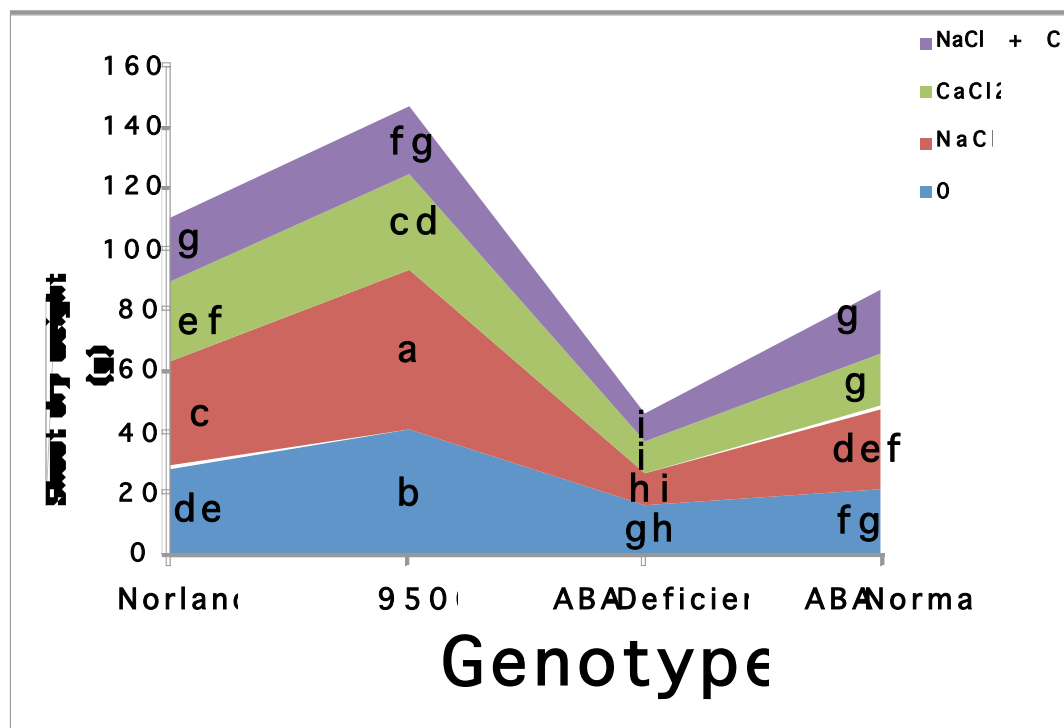


Figure 5. The effects of salt pre-treatments on shoot dry weight and shoot water content of four potato genotypes after two weeks of salt stress.

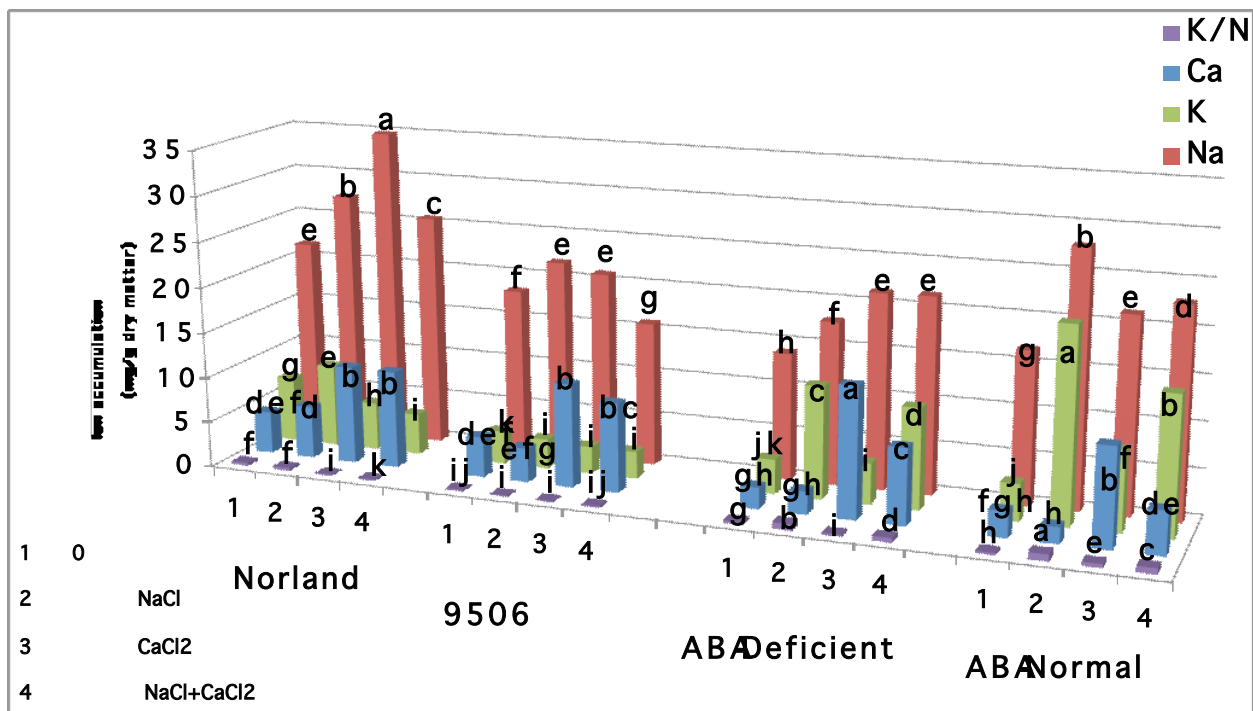


Figure 6. The effects of salt pre-treatments on root Ca^{2+} , Na^+ , K^+ and K^+/Na^+ content (mg/g dry matter) of four potato genotypes after two weeks of salt stress

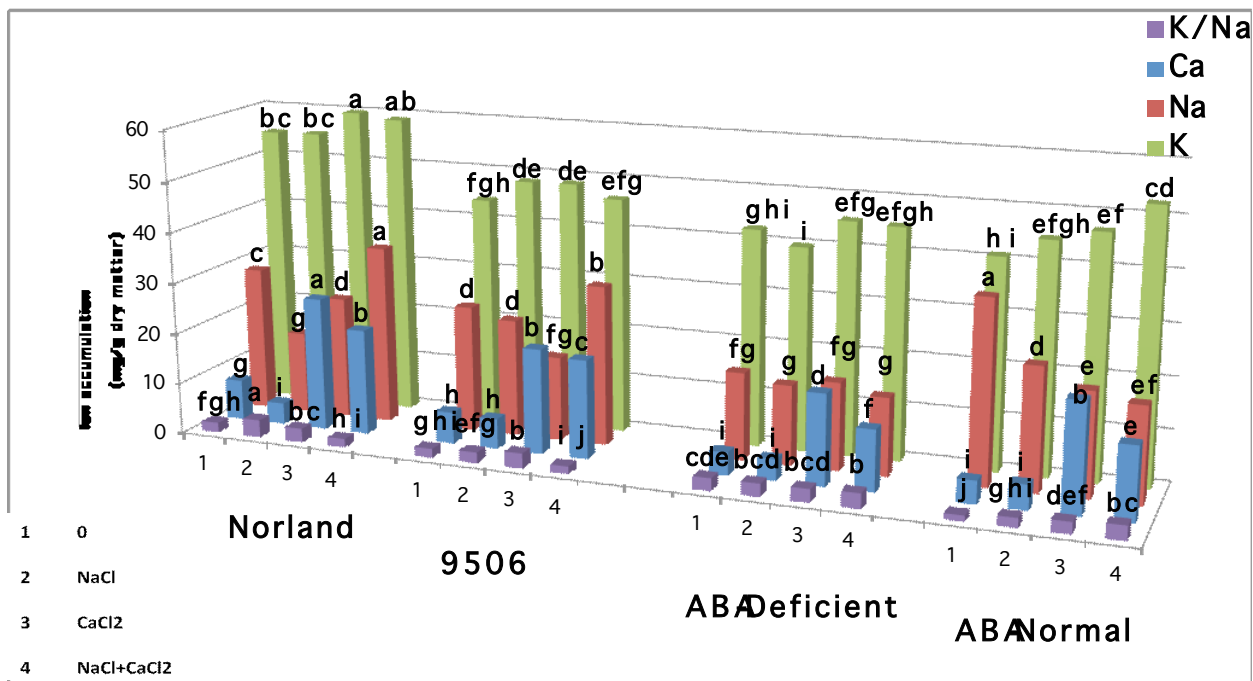


Figure 7. The effects of salt pre-treatments shoot Ca^{2+} , Na^+ , K^+ and K^+/Na^+ content (mg/g dry matter) of four potato genotypes after two weeks of salt stress

Table 1. The Effects of Salt Pre-treatments on Stomatal Conductivity ($\mu\text{M m}^{-2} \text{s}^{-1}$), Leaf Osmotic Potential ($\Psi_s = \text{M pa}$), and Leaf water Content ($\text{g H}_2\text{O/g dry weight}$) of Four Potato Genotypes After two Weeks of Salt Stress.

Leaf stomatal conductivity				
	‘Norland’	9506	ABA-mutant	Normal sibling
0	0.048 b	0.074 a	0.014 fg	0.016 fg
NaCl	0.036 c	0.029 cd	0.011 g	0.017 fg
CaCl ₂	0.036 c	0.047 b	0.011 g	0.020 ef
NaCl + CaCl ₂	0.027 de	0.025 de	0.010 g	0.017 fg
Leaf osmotic potential				
0	-1.94 efgh	-1.31 a	-2.42 i	-2.04 gh
NaCl	-1.27 a	-1.30 a	-2.02 fgh	-1.65 bcd
CaCl ₂	-1.74 cdef	-1.39 ab	-1.73 cde	-1.49 abc
NaCl + CaCl ₂	-1.68 bcde	-1.81 defg	-2.14 hi	-1.68 bcde
Leaf water content				
0	5.37 abc	4.10 abc	1.72 ab	2.73 abc
NaCl	6.29 bc	4.42 abc	4.11 abc	6.18 bc
CaCl ₂	7.47 c	5.52 abc	5.92 bc	7.18 c
NaCl + CaCl ₂	7.88 c	6.32 c	7.55 c	7.27 c

Conclusion

- ▮ Responses to salt stress were dependent on both genotype and pre-treatment.
- ▮ Salt tolerance was largely the result of root growth which likely facilitated improved plant water status and favourable ion accumulation.
- ▮ Genotype responses to pre-treatment relied on either avoidance (‘Norland’, ABA normal sibling) or tolerance (9506) mechanisms.
- ▮ ABA seems to be a requirement for Na⁺ exclusion.
- ▮ Avoidance mechanisms involved restricting the entry of Na⁺ to the shoot and maintenance of higher shoot concentrations of K⁺, Ca²⁺ and K⁺/Na⁺ ratios.

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