



## Root to shoot communication and abscisic acid in calreticulin (*CR*) gene expression and salt-stress tolerance in grafted diploid potato clones

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### Abstract

Potato is an important world crop but its cultivation is relatively limited by its sensitivity to salt-stress. Auto- and hetero-grafting was used to examine the effect of rootstock and abscisic acid (ABA) on expression of the Ca<sup>2+</sup>-storage protein calreticulin (*CR*) and salt-stress tolerance in potato. Sibling-selected diploid clones of potato (*S. tuberosum*) were utilized that are distinguished by differential root Na<sup>+</sup> absorption; including type: late-maturing, LM and excluding type, early-maturing, EM under salt treatment; salt-stress sensitivity (S/T, sensitive or tolerant); and abscisic acid production (AD/AN, ABA-deficient or-normal sibling lines). *CR* expression, osmotic potential (OP) and leaf Ca<sup>2+</sup> were measured at the end of a 5 days NaCl stress treatment applied at tuber initiation. Increased *CR* expression was induced by NaCl stress and associated with salt tolerance in early-maturing tolerant (EMT) and late-maturing tolerant (LMT) clones with higher levels of *CR* in LMT compared to the EMT clone. Early-maturing sensitive (EMS) clone salt tolerance increased when grafted onto LMT but not onto EMT rootstocks. EMS scions maintained less negative leaf OP when grafted onto LMT rootstocks than grafting onto the EMT rootstock. Exogenous ABA application induced a less negative upper leaf OP in the salt-stress sensitive AD clone but not in the AN clone. AD clones were characterized by low *CR* levels, which did not increase after stress. However, grafting the AD clone onto LMT increased *CR* expression in the AD portion of the graft combination. Salt-stress induced *CR* expression and is positively associated with the presence of ABA and the salt-stress tolerant phenotypes. Both, elevation in *CR* expression and salt tolerance in the tolerant rootstocks, were translocated to sensitive scions although highest permeation depended on the LM type. Calreticulin expression appears to be involved in ABA-induced salt tolerance and both salt-stress tolerance and *CR* expression appear to be regulated by the roots.

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**Keywords:** Calreticulin (*CR*); Osmotic potential (OP); Potato; Salt-stress; Abscisic acid (ABA)

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### 1. Introduction

Salinity affects over 25% of the world's irrigated land and is an increasingly serious problem in some of

the most potentially productive regions of the world, such as the Mediterranean basin, California and South East Asia (Serrano and Gaxiola, 1994). Potatoes are a significant source of food worldwide (Poehlman and Sleper, 1995). However, cultivated tetraploid potatoes are considered moderately sensitive to soil salinity, with damage thresholds ranging from 1.5 to 3.0 dS m<sup>-1</sup> (EC) of NaCl (Maas and Hoffman, 1977). In spite of the importance of potato as a world food crop and the increasing salinization of arable lands, there are few reports on salt tolerance mechanisms in potato.

Calcium plays a significant role in salt-stress tolerance. Negative effects of salinity were offset by exogenous application of calcium which increased salt tolerance in *Phaseolus vulgaris* (LaHaye and Epstein, 1969). Recently, Ishitani et al. (2000) reported the *SOS3* (salt overly sensitive3) gene encoding an SOS3 protein required both *N*-myristoylation and calcium binding for its function in plant salt tolerance. *SOS3* appears to be a calcium binding, calcineurin-like protein (Elphick et al., 2001; Zhu, 2002). Intracellular calcium signaling through *SOS3* seems to mediate the positive effect of calcium on plant salt tolerance (Liu and Zhu, 1998). High Na<sup>+</sup> is proposed to induce a calcium signal which then activates the *SOS3*–*SOS2* protein complex and stimulates Na<sup>+</sup>/H<sup>+</sup> exchange (Zhu, 2002).

Calreticulin (CR), an endoplasmic reticulum chaperone protein, regulates cellular Ca<sup>2+</sup> homeostasis through its Ca<sup>2+</sup> binding properties (Michalak et al., 1998). CR is found in the golgi bodies, plasmamembrane as well as the endoplasmic reticulum of plant cells (Coughlan et al., 1997; Borisjuk et al., 1998; Georges et al., 1999) and is considered to be a house-keeping protein involved in Ca<sup>2+</sup> storage in response to environmental stress signal transduction (Burns et al., 1994; Singh et al., 1994; Megidish et al., 1999). In plants, CR is considered to be the major site of calcium storage in the endoplasmic reticulum with its acidic C-domain binding 20–50 moles of Ca<sup>2+</sup> per mole of protein (Wyatt et al., 2002). C-domain transformants increased total calcium by 9–35% compared to controls. CR is a highly conserved protein, with a molecular weight of 46 kDa (Svaerke and Houen, 1998).

The mRNA for *CR* is most abundant in tissues active in secretion, in vasculature, in floral organs

and in developing and germinating seeds (Coughlan et al., 1997). When maize root apices were challenged with mannitol, cell plasmolysis resulted in CR localization at the plasmodesmata and pit fields (Baluska et al., 1999). Both NaCl and high temperature stress markedly enhanced *CR* mRNA levels in *Brassica napus* seedlings (Georges et al., 1999). A brief exposure to Ca<sup>2+</sup> had only a slight effect on CR content but a 3 h exposure to Ca<sup>2+</sup> induced a 34% increase in CR in maize cells (Williams et al., 1997). Adequate Ca<sup>2+</sup> nutrition helps to maintain the selective permeability and integrity of cell-membranes (Palta, 1996). Exposing plants to NaCl (Allen et al., 1999) causes accumulation of cytosolic Ca<sup>2+</sup>.

The accumulated Ca<sup>2+</sup> acts in part as a secondary messenger to abscisic acid (ABA), transducing adaptive or pathological responses to the stress (Hasegawa et al., 2000). Abscisic acid ameliorates salt-stress injury in a wide range of plants (LaRosa et al., 1985; Amzallag et al., 1990; Gadallah, 1996). Most genes responding to drought and salt-stress are also induced by ABA (Shinozaki and Yamaguchi-Shinozaki, 1996; Bray, 1997). ABA and osmotic stress act synergistically inducing gene expression (Bostock and Quatrano, 1992; Xiong et al., 1999).

Grafting provides an opportunity to determine the role of the root in salt tolerance of the shoots. Grafting is commonly employed in horticultural production to increase yields, to alter growth habit and to improve stress tolerance. Typically, high yielding or high quality scions are grafted onto rootstocks adapted to resist disease, adverse soil or climate conditions (Garner, 1990). Plant growth regulators may be transmitted across the graft union (Murfet, 1985). While osmotic potential (OP) of dehydrated scions of bean (*Phaseolus vulgaris* L.) was determined by the roots, the OP of non-stressed scions was governed by the shoot (Sanders and Markhart, 1990). Drought tolerance provided by either the rootstock or the scion resulted in enhanced nitrogen fixation in soybean (*Glycine max* L.) (Serraj and Sinclair, 1996). Entry of Na<sup>+</sup> or Cl<sup>-</sup> ions into the leaves of lemon (*Citrus limon* L.) can be stopped at the rhizosphere of salt-excluding clones and consequently shoot cytoplasm can be protected from the toxic ions under NaCl or Na<sub>2</sub>SO<sub>4</sub> stresses (Walker et al., 1993).

Our previous results indicated distinct differences in salt-stress tolerance mechanisms based on potato

maturity where the late-maturing (LM) types excluded  $\text{Na}^+$  from the shoots by accumulating  $\text{Na}^+$  in the roots while the early-maturing (EM) types included  $\text{Na}^+$  into the shoots (Shaterian, 2002). Calcium and ABA are considered to play an important role in salt-stress tolerance. However, the response of calcium binding proteins such as calreticulin is less clear. Further, the communication between roots and shoots has not been explored in potatoes. The hypotheses that calreticulin expression is involved in ABA-induced salt tolerance and that both salt-stress tolerance and the trigger for CR expression is regulated by the roots were tested.

## 2. Materials and methods

### 2.1. Genetic background

The diploid potato clones evaluated in this study were developed at the Agriculture and Agri-Food Canada Potato Research Centre in Fredericton, NB. Clone F20.1D is a diploid *S. tuberosum* clone without introgression from other species. Clone 10602-02 is an advanced hybrid between two primitive cultivated diploids (*S. phureja* and *S. stenotomum*). The remaining diploid clones are advanced selections from hybrids between diploid *S. tuberosum* clones and *S. chacoense* (and possibly some other wild Argentine species). *S. chacoense* is known to grow naturally in saline soils in Argentina and Paraguay (Hawkes and Hjerting, 1969). The ABA-deficient/ABA-normal (AD/AN) as well as the tolerant and sensitive clones used in this study were previously selected from 24 potato clones assessed for salt tolerance using clus-

Table 2

Osmotic potentials in the upper leaves of graft combinations of diploid potato clones after exposure to 150 mM NaCl salt-stress for 5 days

Scion/rootstock <sup>a</sup>	Osmotic potential (MPa)	Ranking leaf necrosis <sup>b</sup>
EMS/EMS	-2.16	5.0
EMS/LMT	-1.81	3.5
EMT/EMS	-1.93	4.0
EMS/EMT	-2.18	4.5
EMT/EMT	-1.86	3.5
LMT/LMT	-1.71	3.0
LSD <sup>c</sup>	0.168	–

<sup>a</sup> EMS, early-maturing sensitive; LMS, late-maturing sensitive; EMT, early-maturing tolerant; LMT, late-maturing tolerant (Shaterian, 2002).

<sup>b</sup> Leaves showing 5–10% necrotic spots ranked as 1; 10–25% necroses ranked as 2; 25–50% ranked as 3; 50–75% ranked as 4 and leaves with 75–90% necroses ranked as 5. Ranking was based on mean response of 24 plants from each graft union from four replicates.

<sup>c</sup> LSD,  $P < 0.05$ . Mean osmotic potential of upper leaf control plants taken at the same time as stressed plants (MPa): EMS/EMS, -0.48; EMT/EMT, -0.55; LMT/LMT, -0.89.

ter analysis of six traits including height, shoot fresh weight (FW), dry weight (DW), water content, growth rate, tuber yield and survival (Shaterian, 2002). Tolerance is defined as the ability to live, grow and yield in the presence of stress. The abbreviations used in this paper (Table 1) and characteristics of the selected clones are outlined in Table 2. Plant material consisted of: early-maturing salt-stress sensitive (EMS) clone; early-maturing salt-stress tolerant (EMT) clone; late-maturing salt-stress sensitive (LMS) clone and late-maturing salt-stress tolerant (LMT) clone. AD is

Table 1  
Characteristics and abbreviations of selected diploid potato clones

Clones	Salinity <sup>a</sup> tolerance	Maturity <sup>b</sup>	Productivity <sup>c</sup>	Abbreviation	Description
10908-05	S	EM	High yielding	EMS	Early-maturing salt sensitive
10909-18	T	EM	High yielding	EMT	Early-maturing salt tolerant
F20-1D	S	LM	Low yielding	LMS	Late-maturing salt sensitive
9506	T	LM	Low yielding	LMT	Late-maturing salt tolerant
9120-05	S	LM	Very low yielding	AD	ABA-deficient, salt sensitive
9120-18	IT	LM	Very low yielding	AN	ABA-normal sibling, intermediate salt tolerant

<sup>a</sup> S, sensitive to NaCl stress; T, tolerant to NaCl stress; IT, intermediate tolerant (Shaterian, 2002).

<sup>b</sup> EM, an early-maturing diploid clone which excludes  $\text{Na}^+$  from the roots; LM, a late-maturing diploid clone which accumulates  $\text{Na}^+$  in the roots (Shaterian, 2002).

<sup>c</sup> Tuber yield under control conditions (Shaterian, 2002).

an ABA-deficient mutant (blocked at ABA-aldehyde, De Jong et al., 2001) and salt-stress sensitive; AN is a sibling of the AD clone with higher ABA levels (Etehadnia and Tanino, unpublished data).

## 2.2. Grafting of potato clones

Vigorously growing scion material from four-selected diploid potato clones (EMS, EMT, LMT and AD clones) were obtained at flower initiation. Wedge type grafts were used in this experiment. After healing, the grafts were cut well below the graft junctions and rooted in SunShine soil-less medium mix #4 (Sungro Hort. Inc., Bellevue, WA).

## 2.3. Experimental method

Grafted plants, 2 weeks of age, were transferred into 1.5 l pots containing white Ottawa sand (75.5%, 1–2 mm; 24.4%, 0.5–1 mm and 0.1%, less than 0.5 mm) that provides an inert and stable medium without salt accumulation. For each scion/rootstock combination, six potted plants grown in one tub constituted a replicate arranged in a randomized complete block design with four replicates (tubs). The tubs were automatically flooded and then drained once or twice a day, depending on the evapotranspiration demand, with 20–20–20 (N–P–K, including micro-nutrients, Plant Products Co. Ltd., Orinda Road, Brampton, ON) nutrient solution at a concentration of 1.28 g l<sup>-1</sup> (osmotic potential of -0.14 MPa, EC of 1.27 dS m<sup>-1</sup> and pH of 6.5–6.6). At the tuber initiation stage, each clone was watered for 5 days with either the control nutrient solution or the control solution supplemented with 150 mM NaCl, treatments previously determined to best separate tolerant and sensitive potato clones (Shaterian, 2002). ABA treatment consisted of 50 µM (+)-S-ABA (Toray Chemical Co. Inc., Japan) applied daily for a period of 7 days prior to stress exposure.

Greenhouse conditions included 490–550 µM m<sup>-2</sup> s<sup>-1</sup> (PAR) light intensity, a 15 h photoperiod and 25/15 °C day/night temperatures. After 5 days of exposure to salt-stress, all potato clones developed significant salt-stress symptoms on the lower leaves. Samples of the young developing leaves were then collected in Eppendorf test tubes and kept frozen at -20 °C until required. The OP of leaf sap was measured with a vapor pressure osmometer (Wescor

5500) and adjusted for full turgor. Plant injury was visually ranked according to leaf necrosis after 5 days of the salt-stress. Ranking of leaf necrosis was positively correlated with leaf osmotic potential at  $P < 0.05$  (data not shown), and therefore, OP was used as an indicator of relative salt-stress tolerance. At the end of the stress period, 10–15 g from the young upper expanding leaves and lower expanded mature leaves were collected to measure Ca<sup>2+</sup> composition (ASOC method, Thomas et al., 1967). Each sample was run through a calibrated atomic absorption spectrometer/flame photometer (Perkin-Elmer 3100). This study was repeated in 1997 and 1998. Analysis of variance was determined by GLM, using SAS statistical package (SAS, 1997) separately on the data for each year. Fisher's protected LSD<sub>5%</sub> compared the means of graft combinations separately for each year, to show possible variation between years. Bartlett test showed standard variances of the experiments from both years were similar. Only the 1997 trials for Table 3 are presented due to the smaller coefficient of variation (CV) values.

## 2.4. Analysis of CR expression

At the end of the 5 days salt treatment, a 100 mg pooled sample of developing leaves from three replicates of each graft combination was taken in both the stressed and control treatments. Northern blot analyses were conducted on three different samples pooled from different plant material. Visual comparison of the blots indicated results from the samples were consistent.

Leaf samples were frozen in (liquid nitrogen) LN2 and stored at -18 °C. Before extraction, samples were ground in LN2 and the total RNA was isolated by the RNeasy Mini Protocol (RNeasy Mini (1997), Qiagen Inc., Argentina Road, 23 Mississauga, Ontario). Northern blot analysis of isolated RNA was performed as described by Sambrook et al. (1989) using ethidium bromide staining. The 5 µg RNA samples were loaded onto a gel and blotted onto a positively charged membrane using a filter bridge technique with a carrier of 20% SSC for over 10 h. The RNA was fixed onto the membrane by exposure to UV light (U.V. Strata Linker™ 1800, Strata-Gene).

Expression of CR from EMS, EMT, LMS, LMT, AD, AN and AD scion grafted onto the LMT root-

Table 3  
Shoot growth (cm) measured at the end of the test period in diploid potato lines

Treatment	Clones	Non-stressed	Salt-stress	Change (%)
Control	AD	16.1	6.9	−57 <sup>a,*</sup>
	AN	22.4	13.6	−39*
ABA	AD	24.8	18.6	−25*
	AN	26.7	14.9	−44*
Control vs. ABA				
Non-stress conditions	AD			+35*
	AN			+16*
Salt-stress conditions	AD			+63*

Plants were treated with water (control) or daily with 50  $\mu$ M ABA for 7 days. Plants were exposed to non-stress or 150 mM NaCl salt-stress conditions for 14 days.

<sup>a</sup> Plus and minus signs indicate an increase and decrease in the comparisons, respectively. Percent change was measured as a function of  $(1 - (\text{salt-stress}/\text{non-stressed})) \times 100$ . Orthogonal contrasts for AD and AN clones were performed between control and ABA treatments under non-stressed and stressed conditions. Only significant contrasts are presented. The results were based on mean response of 24 plants per treatment from four replicates.

\* Significant differences at  $P \leq 0.05$  for means within a row.

stock under both salt-stress and control conditions was evaluated. The AD scion onto the AN rootstock graft combination was not successful, and therefore, was not included.

A 25 ng sample of the *CR* cDNA template was brought to 11  $\mu$ l volume using RNA-free water and added to 4  $\mu$ l of High Prime solution (Boehringer Mannheim Inc.). The *CR* probe was a cloned gene from *B. napus* L., created through release of the *CR* inset from the plasmid using a restriction enzyme and nick-translation (Georges, pers. comm). A 200  $\mu$ l random primer mixture containing 1 U/ $\mu$ l Klenow polymerase, 0.125 mM dATP, 0.125 mM dGTP, 0.125 mM dTTP and radioactive dCTP ( $P^{32}$ ) were added into a stabilizing 5 M ( $5\times$ ) Tris buffer containing 50% (v/v) glycerol. The *CR* probe was denatured in a boiling water bath and hybridized with membranes while bathed in High Prime solution at high stringency (65 °C). A film was exposed to the labelled membrane at −80 °C overnight. The *CR* probe of *Brassica* and potato was found to have >81% homology with the known *CR* sequence (Crookshanks et al., 2001).

### 3. Results

#### 3.1. Grafting, ABA treatment and salt-stress tolerance

When scion OPs of the sensitive and tolerant lines were compared, the EMT/EMT combination

(scion/rootstock) maintained a high (less negative) OP in the upper leaves compared to EMS/EMS after exposure to salt treatment (Table 2). Grafting the EMS scion onto the EMT rootstock did not improve OP of the EMS leaves ( $P \leq 0.05$ ). The reciprocal graft of the EMT scion onto the EMS rootstock also did not change the OP of the EMT scion after exposure to salt treatment. However, the LMT rootstock had a significant effect on the OP of the EMS scion, which became less negative under salt treatment compared to the EMS/EMS graft union (Table 2). The LMT rootstock also reduced both EMS and AD scion leaf necrosis (data not shown). Exogenous ABA application increased shoot growth in both the ABA-deficient and-normal sibling clones (Table 3). Under salt treatment, ABA application increased AD shoot growth by 63% compared to plants without ABA treatment. ABA application also increased upper leaf osmotic potential by 19% in the AD but not AN clone under salt treatment compared to non-treated plants (Table 4).

#### 3.2. *CR* expression

Grafting also altered *CR* expression. Under control conditions, *CR* expression was lowest in the LMS clone (32 pixels), moderate in the EMT clone and highest in the LMT clone (78 pixels) (Fig. 1). Under control conditions, *CR* expression in the leaves

Table 4

Upper leaf osmotic potential measured at the end of the test period in the abscisic acid-deficient (AD) and abscisic acid-normal sibling (AN)

Treatment	Lines	Non-stressed (MPa)	Salt-stress	Change (%)
Control	AD	−1.00	−2.07	−52 <sup>a,*</sup>
	AN	−0.60	−1.63	−63*
ABA	AD	−0.62	−1.68	−63*
Control vs. ABA				
Non-stress conditions	AD			+38*
Salt-stress conditions	AD			+19*

Plants were treated with water (control) or daily with 50  $\mu$ M ABA for a 7 days. Plants were subsequently exposed to non-stress or 150 mM NaCl salt-stress conditions for 14 days.

<sup>a</sup> Plus and minus signs indicate an increase and decrease in the comparisons, respectively. Orthogonal contrasts for AD and AN clones were performed between control and ABA treatments under non-stressed and stressed conditions. Only significant contrasts are presented. The results were based on mean response of 24 plants per treatment from four replicates.

\* Significant differences at  $P \leq 0.05$  for means within a row.

of the AD clone was low (28 pixels), moderate in the AD/LMT heterograft (64 pixels) and high in the AN (93 pixels) clone (Fig. 2). Levels of *CR* expression in the AD scion increased under both control and salt-stress conditions when AD scions were grafted onto LMT rootstocks. *CR* expression in AN was comparable to the AD/LMT graft union, especially under stress conditions (Fig. 2). The AD clone was very sensitive to salt treatment and had very low OP (Table 4), however, grafting AD scions onto the salt tolerant LMT rootstock increased OP levels in the AD scion.

Both the EMS and LMS clones had lower *CR* expression than the tolerant clones under control and

stress conditions. The EMS clone exhibited reduced *CR* expression under salt treatment compared to control conditions. Conversely under salt treatment, *CR* expression was higher in the EMT and LMT clones compared to control conditions and higher than both sensitive clones (Fig. 1).

Salt treatment did not alter the  $\text{Ca}^{2+}$  levels in either upper or lower leaf tissue of the tested clones (Table 5). However compared to salt sensitive types, salt tolerant early and late potato clones had higher concentrations of  $\text{Ca}^{2+}$  in the upper and lower leaves, respectively, under both control and NaCl stress conditions.

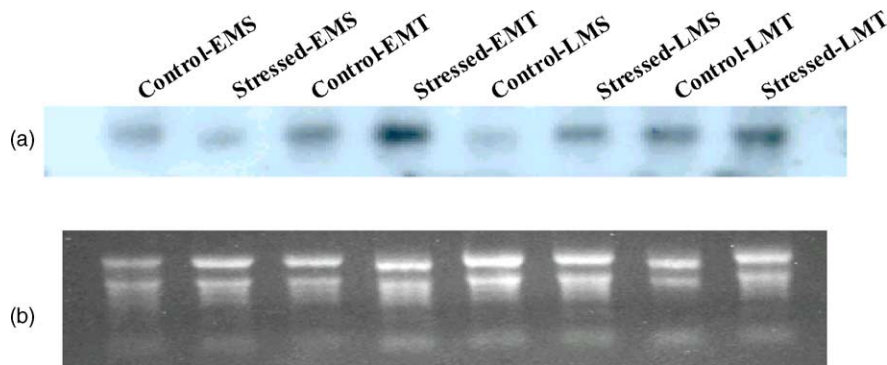


Fig. 1. Northern analysis of RNA extracted from upper leaves of four potato clones after 5 days of exposure to 150 mM NaCl salt-stress. EMS and EMT, early-maturing sensitive and tolerant clones, respectively; LMS and LMT, late-maturing sensitive and tolerant clones, respectively. Probe used was a full-length *CR* cDNA from *B. napus* L. (a) onto autoradiograph and (b) Northern gel (formaldehyde agarose gel). The results were based on mean response of 24 plants per treatment from four replicates.

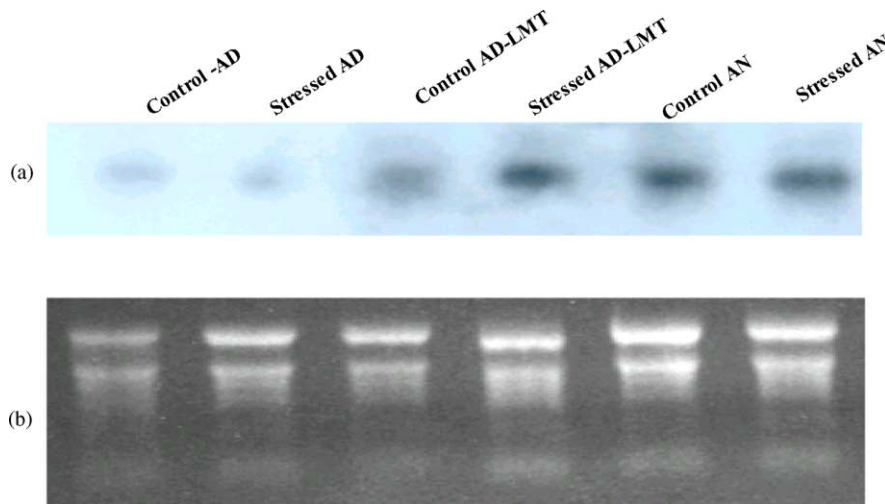


Fig. 2. Northern analysis of RNA extracted from upper leaves of the ABA-deficient clone, ABA-deficient clone grafted onto the LMT clone and ABA-normal clone after 5 days exposure to 150 mM NaCl salt-stress. Probe used was a full-length *CR* cDNA from *B. napus* L. (a) radiograph and (b) Northern gel (formaldehyde agarose gel). The results were based on mean response of 24 plants per treatment from four replicates.

Table 5

Leaf  $\text{Ca}^{2+}$  concentrations after exposure to with 150 mM NaCl salt-stress for 5 days in the upper and lower leaves of diploid potato clones

Tissues sampled (leaves)	Clones	Control ( $\text{mg g}^{-1}$ ) DW	Salt-stress ( $\text{mg g}^{-1}$ ) DW	Difference (stressed – control)
Lower	EMS	18.6	17.9	–0.7
	EMT	16.5	15.3	–1.2
	LMS	5.1	3.4	–1.7
	LMT	10.7	11.6	+0.9
Upper	EMS	6.8	5.6	–1.2
	EMT	10.2	10.6	+0.4
	LMS	5.3	4.1	–1.2
	LMT	3.4	3.9	+0.5

DW, dry weight; lower leaves, most mature, fully expanded leaf; upper leaves, immature, expanding leaves. LSD ( $P \leq 0.05$ ) for: lower leaves = 2.0; upper leaves = 1.5. The results were based on mean response of 24 plants per treatment from four replicates.

#### 4. Discussion

Calcium plays an important role in salt-stress tolerance and thus, the regulation of calcium storage through proteins such as *CR* is predicted to be significant. In our study, *CR* expression was generally higher in the young leaves of salt tolerant clones compared to sensitive types. Furthermore, higher *CR* expression was evident in the salt-stressed LM clones which accumulate  $\text{Na}^+$  within the roots (Etehadnia and Tanino, unpublished) and exclude  $\text{Na}^+$  from the shoots (Shaterian, 2002). These results support the

hypothesis that *CR* expression is related to salt-stress tolerance and suggests that the  $\text{Na}^+$  accumulation within the roots may be a factor in triggering this expression. It also supports the model outlined by Zhu (2002) of high  $\text{Na}^+$ -induced SOS3 (calcium binding protein) which subsequently regulates salt-stress tolerance in *Arabidopsis*. Baluska et al. (1999) also found increased *CR* protein in the root tips of maize plants challenged with mannitol.

Unlike the normal clones, the ABA-deficient clones were characterized by low *CR* levels, that did not increase after stress. However, AD grafted onto the

normal LMT rootstock increased *CR* expression in the AD portion of the graft combination. Plant growth regulators may be transmitted across the graft union (Murfet, 1985). Salt-stress tolerance is positively associated with ABA and salt-stress-induced *CR* expression. Long-distance signaling from root to leaf tissues in terms of ABA flux within the plant is regulated by pH changes and environmental stress (Sauter et al., 2001) and may be important in induction of *CR*. The apparent ABA-dependent *CR* mRNA expression found in this study is in contrast to Georges et al. (1999) who found exogenous ABA application reduced *CR* mRNA in young seedlings of *B. napus*. Nevertheless, they also found NaCl salt-stress markedly induced *CR* mRNA and suggested multiple pathways for *CR* mRNA induction. Induction may be developmentally dependent and species-specific.

Cytosolic-calcium levels typically increase under salt-stress, as a function of the release from the bound calcium pool (Allen et al., 1999). Our experiment found no changes in total  $\text{Ca}^{2+}$  concentration on exposure to salt treatment, but this does not exclude alterations of intracellular  $\text{Ca}^{2+}$  distribution in bound versus free pools. Furthermore,  $\text{Ca}^{2+}$  concentration was greater in the tolerant early and late-maturing clones than their corresponding sensitive clones and calcium levels did not increase in any of the salt sensitive clones after salt treatment. More extensive localization, time-course studies need to be performed to appropriately address this area.

In this study, the osmotic potential of the EMS scions became less negative when grafted onto the LMT clone compared to the EMS/EMS grafts. This may reflect the extensive rooting system and  $\text{Na}^+$ -inclusion of the LMT root system which was associated with a reduction of  $\text{Na}^+$  from the scions (Shaterian, 2002). Grafting EMS scions onto EMT rootstocks and their reciprocal grafts did not alter OP compared to grafting onto the sodium including LMT rootstocks. By contrast, the ion-excluding root mechanism of the EM clones under salt-stress conditions may have allowed export out of the roots to the shoot tissues where  $\text{Na}^+$  ions were observed to accumulate (Shaterian, 2002).

Sodium exclusion from the roots is more often associated with complete restriction of  $\text{Na}^+$  uptake. Entry of  $\text{Na}^+$  or  $\text{Cl}^-$  ions into the leaves of lemon (*C. limon* L.) can be stopped at the rhizosphere of

salt-excluding lines and consequently shoot cytoplasm can be protected from the toxic ions under NaCl or  $\text{Na}_2\text{SO}_4$  stresses (Walker et al., 1993). Grafts of a salt sensitive commercial citrus cultivar onto a  $\text{Cl}^-$ -excluding rootstock reduced  $\text{Cl}^-$  uptake by three fold relative to the same scions on poor  $\text{Cl}^-$ -excluding rootstocks. There are few published papers on graft transmissibility of salt tolerance, especially on herbaceous species. Intra-specific reciprocal graftings of bean (*P. vulgaris* L.) was studied for dehydration tolerance (Sanders and Markhart, 1990). While osmotic potential ( $\Psi_s$ ) of stressed plants (scions) was regulated by the roots of bean lines, the  $\Psi_s$  of non-stressed plants (scions) was governed by the shoot line. Graft-compatibility of interspecific potato lines provides an opportunity to determine the role of the root in salt tolerance of the shoots and offers a practical method of quickly enhancing salt-stress tolerance through the use of automated grafting equipment.

The hypothesis that calreticulin expression is involved in ABA-induced salt tolerance and that both salt tolerance and the trigger for *CR* expression is regulated by the roots appears to be supported by this study. Salt tolerant traits at the macromolecule level were translocatable from root to shoot. Sodium accumulation within the roots may be necessary for full *CR* expression in the leaves under salt-stress. Zhang and Blumwald (2001) have already successfully increased salt tolerance in tomato by overexpressing a vacuolar  $\text{Na}^+/\text{H}^+$  antiport which accumulated  $\text{Na}^+$  in leaf tissue. Another promising approach to enhance salt tolerance in potato clones may involve grafting sensitive scions onto rootstocks which accumulate  $\text{Na}^+$ .

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