



ELSEVIER

Available online at www.sciencedirect.com

SCIENCE @ DIRECT®

Environmental and Experimental Botany 54 (2005) 202–212

Environmental
and Experimental
Botany

www.elsevier.com/locate/envexpbot

Differential stress responses to NaCl salt application in early- and late-maturing diploid potato (*Solanum* sp.) clones

Javad Shaterian^{a,1}, Doug Waterer^a, Hielke De Jong^b, Karen K. Tanino^{a,*}

^a Department of Plant Sciences, University of Saskatchewan, 51 Campus Dr., Saskatoon, Sask., Canada S7N 5A8

^b Potato Research Centre, Agriculture and Agri-Food Canada, 850 Lincoln Rd., P.O. Box 20280, Fredericton, NB, Canada E3B 4Z7

Received 11 September 2003; received in revised form 9 July 2004; accepted 12 July 2004

Abstract

Cultivated tetraploid potatoes (*Solanum tuberosum* L.) are moderately salt sensitive but greater stress tolerance exists in diploid wild types. However, little work has been published on salt-tolerance in diploid potato. This study utilized sensitive and tolerant diploid potatoes as well as a commercially cultivated potato to investigate mechanisms of stress tolerance. Stem cuttings from salt-tolerant (T) and sensitive (S) clones of early-maturing (EM) and late-maturing (LM) diploid potato clones were stressed for 5 days at the tuber initiation stage with 150 mmol NaCl in a hydroponic sand culture under greenhouse conditions. The stress responses of the early- and late-maturing potato clones were distinctly different. Under stress, early-maturing clones accumulated Na⁺ in the leaf tissues while late-maturing clones generally excluded Na⁺ from the leaf tissues. Salt tolerant clones of both maturity types were able to tolerate high levels of Na⁺ in the leaf tissues. The lower leaves accumulated more Na⁺ than the upper leaves in both maturity types. The potassium to sodium ratio was significantly greater in the leaves of the late-maturing types, reflecting differences in Na⁺ accumulation rather than alterations in K⁺ levels. Proline levels increased upon salt exposure but were not clearly associated with salinity tolerance. Tolerance was manifested in maintenance of vegetative growth, tuber yield, and reduced leaf necrosis. These responses require efficient uptake of water and source–sink translocation. Maintenance of stomatal conductance under stress was not associated with these responses but tuber yield was related to lower-leaf osmotic potential (OP) in both early- and late-maturity types. Salt tolerant clones of both maturity types also had less negative tuber OP under salt stress than sensitive types. High yielding EMT and LMT clones either minimized tuber yield loss or even increased yield after exposure to salt stress. Mechanistic studies and screening experiments for salt tolerant clones should consider maturity type, leaf position and source–sink relationships enhancing tuber yield.

© 2004 Elsevier B.V. All rights reserved.

Keywords: Diploid potato; Early maturity; Late maturity; Salt stress; *Solanum tuberosum*

* Corresponding author. Tel.: +1 306 966 8617; fax: +1 306 966 5015.

E-mail address: tanino@sask.usask.ca (K.K. Tanino).

¹ Present address: Seed and Plant Improvement Institute (SPII), P.O. Box 4119, Mardabad Road, Karaj 31585, Iran.

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). The majority of potatoes (*Solanum tuberosum* L.) are grown under irrigation and are a significant source of food world-wide (Poehlman and Sleper, 1995). Cultivated tetraploid potatoes are moderately sensitive to soil salinity, with damage thresholds ranging from 15 to 30 mmol of NaCl (Mass and Hoffman, 1977). Considerable salt stress resistance occurs in wild diploid *Solanum* potato relatives. In spite of the importance of potato, whole plant salt resistance mechanisms in diploid potato have not been extensively studied (Sabbah and Tal, 1995).

Resistance to salinity-induced osmotic stress is defined as: (a) avoidance of water deficits, either through a very early short life cycle or osmo-adaptation; (b) tolerance of water deficits via production of antioxidants, protective solutes or turgor maintenance (Paleg and Aspinall, 1981); and (c) water-use-efficiency mechanisms (Kramer and Boyer, 1995). These mechanisms can be exerted at whole plant to cellular levels. At the cellular level, sodium and chloride ion sequestration into vacuoles was a factor in salt adaptation in tobacco cells (Binzel et al., 1987). Overexpression of Na⁺/H⁺ antiport channel controlling K⁺/Na⁺ ratio conferred salt-tolerance in a transgenic tomato line (Zhang and Blumwald, 2001). While salt-tolerance mechanisms ultimately function at the cellular level, whole plant adaptive responses are also critical (Adams et al., 1992).

Potatoes are typically grouped into early- and late-maturing types. Early-maturing potato cultivars have a higher early growth rate than late-maturing types (Kleinkopf et al., 1981) with earlier tuber set and tend to have a determinate growth habit. Late-maturing cultivars are more productive in a longer growing season than early-maturing clones (Love et al., 1995). Late-maturing types typically produce more vegetative biomass, exhibit late tuber initiation and are less responsive to environmental stress cues which reduce shoot vegetative growth compared to early-maturing types. Tuber set and enlargement of late-maturing potato clones are more adversely affected by high N levels early in the growing season than early-maturing cultivars (Kleinkopf et al., 1981).

In spite of these differences between early- and late-maturing potato types, there are no previous studies comparing salinity responses based on maturity. Our previous work identified early and late salt tolerant and sensitive diploid potato clones. This current work tests the hypothesis that early- and late-maturing diploid potato express different salt stress response mechanisms.

2. Materials and methods

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). Stem cuttings were propagated from stock plants of seven diploid potato clones as well as the early-maturing tetraploid cultivar 'Norland'. The diploid clones were selected for variation in salt-tolerance and maturity (Table 1) based on preliminary screening trials (Shaterian, 2002). Cuttings were rooted in a mist chamber in Sun-Shine soil-less mix #4 (Sungro Hort. Inc., Bellevue, WA) in the College of Agriculture, University of Saskatchewan greenhouses.

Two-week-old rooted cuttings 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 not prone to salt accumulation. The tubs with pots were arranged in a randomized complete block design with four replicates and two treatments (control and salt stressed). The tubs were automatically flooded and then drained once or twice a day, depending on the evapotranspiration demand, with 20–20–20 (Plant Products Co. Ltd., Orinda Road, Brampton, Ont.) nutrient solution at a concentration of 1.28 g l⁻¹ (osmotic potential of -0.14 MPa, EC of 1.27 dS m⁻¹ and pH of 6.5–6.6). Greenhouse conditions were: 490–550 (mol m⁻² s⁻¹) (PAR) natural

Table 1
 Characteristics of pre-selected diploid potato clones and the tetraploid cultivar 'Norland'

Clones	Salt-stress-tolerance	Maturity type	Tuber yield under non-stress conditions
'Norland'	Intermediate tolerance (IT)	EM	High yielding
10908-05	Sensitive (S)	EM	High yielding
10909-18	Tolerant (T)	EM	High yielding
F20-1D	Sensitive	LM	Low yielding
9506	Tolerant	LM	Low yielding ^a
11374-01	Intermediate tolerance	LM	High yielding
10602-02	Intermediate tolerance	LM	Medium yielding
9788-03	Tolerant	LM	Low yielding

Diploid potato clones (all clones except 'Norland') were developed at the Agriculture and Agri-Food Canada Potato Research Centre in Fredericton, NB, courtesy of Henry deJong and characterized for maturity and salinity tolerance (Shaterian, 2002). EM: an early-maturing clone; LM: a late-maturing clone; 'Norland' = a commercial tetraploid cultivar.

^a Zhang and Donnelly, 1997.

light intensity supplemented with sodium vapour pressure lamps, a 15 h photoperiod and 25/15 °C day/night temperatures. The diploid clones and 'Norland' cultivar tested all have *S. tuberosum* in their pedigree and tuberized under this photoperiod. Once the plants were established, the rate at which fertilizer was applied was halved to stimulate tuber production.

The intensity and impact of stress injury depends upon the phase of plant growth at the time of salt exposure. It is critical to impose stress at a consistent stage of development and for a sufficient period to stimulate long-term effects (Munns, 1993). Our previous tests (Shaterian, 2002) indicated 5 days exposure to 150 mmol NaCl produced measurable differences in height and visual injury to the lower leaves of salt sensitive clones. Stolon growth and tuber initiation are the most drought sensitive growth stages in potatoes (Hukkeri et al., 1970; Nelson and Hwang, 1975; Haverkort et al., 1990). Therefore, all clones regardless of maturity type were stressed at the same tuber initiation stage of development. Previous information on maturities of the clones (Shaterian, 2002) was used to predict the time of stolon growth and tuber initiation. For 'Norland' and line 10908-05, this occurred at 39 days after planting (DAP); line 10909-18 at 43 DAP; 11374-01 at 54 DAP; 10602-02 at 62 DAP; line F20-1D at 66 DAP; lines 9788-03 and 9506 at 73 DAP. The tuber initiation stage was also verified by gently examining media around the plant collar after transplanting the seedlings.

Beginning at tuber initiation, half of the plants were challenged for 5 days with 150 mmol NaCl salt added

to the nutrient solution ($\psi_{\text{salt medium}} = -0.73$ MPa, EC of 16.27 dS m⁻¹, pH 6.7). The control treatments were watered with the standard nutrient solution during this time.

The first harvest was performed after plant establishment and before initiation of the salt stress, i.e. prior to tuber initiation. The second harvest occurred at the commencement of the salt stress treatment. A final set of plants were harvested at senescence of the early-maturing clones, or after 120 days for the late-maturing clones. Plant height, tuber weight, shoot fresh and dry weight, % harvest index [(tuber weight/fresh plant biomass) × 100], and tuber bulking rate (tuber yield/number of days from tuber initiation to final harvest) were recorded at tuber initiation and the final harvest. Changes in height and shoot dry weight from tuber initiation to the final harvest were also calculated. All plants were tied upright to bamboo-support sticks and plant height was measured based on the distance from the plant collar to the uppermost leaf node on the tallest stem. Dry weights of the shoots were determined after air-drying at 65 °C for 48 h. Plant biomass was estimated from fresh shoot and tuber weights.

Stomatal conductance (g_s) was measured by placing the detector clamp of a steady state Porometer (Li-1600, Li-cor Inc., Lincoln, NE, USA) on the abaxial side of the fourth leaf from the apical meristem and on the oldest non-wilted leaves between 10:00 a.m. and 12:00 noon on day 5 of the stress period. Stomatal conductance (g_s) refers to the rate of water vapour exchange between the leaf and its boundary layer. Higher

values of g_s indicate more rapid transpiration. Osmotic potentials (OP) (vapour pressure osmometer (VPO), Wescor 5500, Wescor Inc., Logan, UT, USA) were measured between 11 a.m. and 12 p.m. at the end of the 5-day salt stress period on the lowest fully expanded non-wilted leaves and the youngest expanding leaf. OPs were adjusted for full turgor. The observed OP's were adjusted using the RWC, $[(FW - DW)/(TW - DW) \times 100]$, of each line, to allow for genotypic comparisons. Osmo-adaptation was calculated based on differences between OP under control conditions and salt stress. RWC, FW, TW and DW represent leaf relative water content, fresh weight, turgor weight and dry weight, respectively.

Plant injury was visually ranked at the termination of the 5-day stress treatment. The ranking ranged from a low of 1 for leaves showing only 5–10% chlorotic spots to a ranking of 5 for leaves with 80–90% necrosis. At the end of the stress period, 10–15 (g) from the young developing leaves and lower leaves was collected to measure water content, proline and glucose measurements, Na^+ , K^+ composition (ASOC method, Thomas et al., 1967). Each Na^+ and K^+ sample was run through a calibrated atomic absorption spectrometer/flame photometer (Perkin-Elmer-3100). Proline levels in the leaf samples were measured according to Bates (1973). Filtrate of homogenized plant samples were reacted with dissolved ninhydrin at 100 °C. Mixture was extracted with toluene and chromophore containing toluene was aspirated and read by spectroscopy at 520 nm. Glucose content of the tissue samples was measured according to Dubios et al. (1956). Phenol was added to the extracts of homogenized plant materials in a test-tube. Concentrated sulfuric acid was then added and the tubes were allowed to stand for few minutes, then shaken and placed in a water bath at 30 °C for 10–20 min before reading. Intensity of chromophore was measured at 490 nm.

Analyses of variance for yield, changes in height and shoot dry weight (from tuber initiation to final harvest) and time to maturity were determined by GLM, using the SAS statistical method (SAS, 1997). Fisher's protected LSD ($P \leq 0.05$) was used for mean separation. Pre-planned, single degree of freedom contrasts were also used to evaluate specific treatment effects. Correlation coefficients were calculated between leaf osmotic potential and solute components, using the Pearson correlation procedure in SAS (Table 3).

3. Results

3.1. Sodium accumulation

Early-maturing clones and the early-maturing cultivar 'Norland' accumulated Na^+ in the leaf tissues (Fig. 1a) in response to salt/osmotic stress while late-maturing clones did not accumulate Na^+ in this tissue. The potassium to sodium ratio in the leaves was significantly greater in the late-maturing potato types than in the early-maturing types under both control and salt stressed treatments (Fig. 1c). This difference was associated with differences in Na^+ accumulation and not alterations in K^+ levels (Fig. 1b).

Under stress, both early- and late-maturing potato clones accumulated more Na^+ in the lower leaves compared to the upper leaves (Fig. 1a). Lower leaf damage ratings followed this response, with lower leaves showing more salt damage than upper leaves (Table 2). This Na^+ accumulation and associated leaf damage response was most distinct in the tetraploid cultivar 'Norland'. In addition, lower leaves in the salt tolerant types showed less damage than sensitive types even with comparable levels of tissue Na^+ (Fig. 1a). Both the EMT and LMT clones tolerated more Na^+ in the lower leaf tissue than their sensitive counterparts, with the sodium accumulating EMT type also tolerating more Na^+ in the upper leaves than the sensitive types.

3.2. Water relations

Stress altered the relative water content in the lower leaves but did not change the RWC in the upper leaves (Table 2). Stem diameter (data not shown) and lower leaf RWC actually increased in the EMT lines under stress while stomatal conductance declined significantly at $P = 0.05$ (Table 2). Osmotic potential of the upper and lower leaves was consistently positively correlated with both Na^+ and proline levels in all maturity types (Table 3). Glucose was positively correlated with OP in the upper leaves only.

3.3. Growth and yield

Stress significantly reduced shoot growth in both salt sensitive and intermediately tolerant early- and late-maturing types (Fig. 2). The sensitive F20-1D clone also showed a 50% reduction in growth under stress

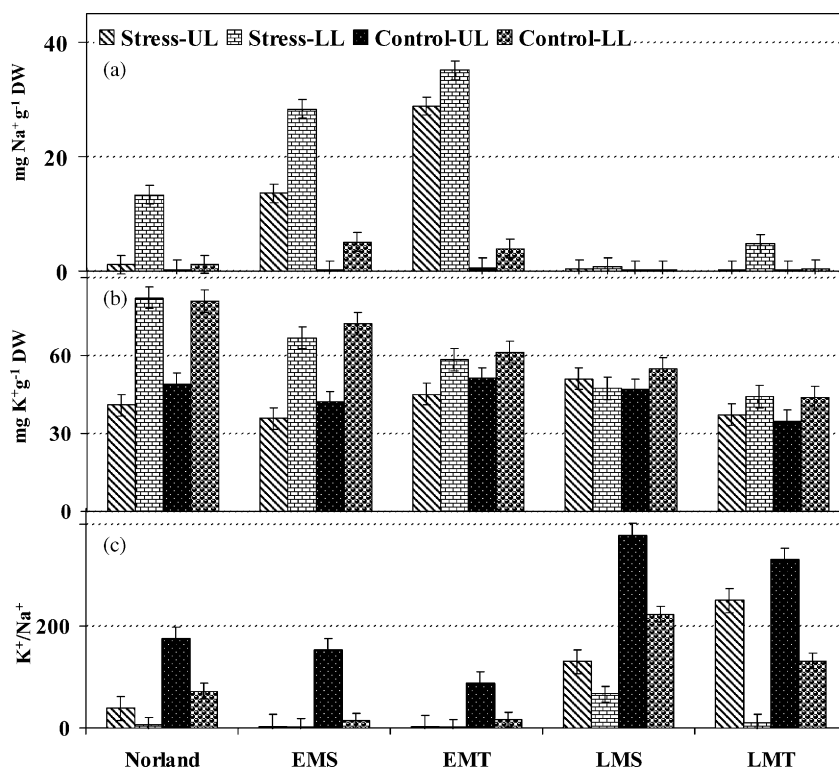


Fig. 1. Leaf Na⁺, K⁺ and Na⁺/K⁺ ratio measured at the end of the test period in four diploid potato clones and the tetraploid cultivar 'Norland' exposed to control conditions or treated with 150 mmol NaCl salt stress for 5 days. EMS = early-maturing sensitive clone (10908-05); EMT = early-maturing tolerant clone (10909-18); LMS = late-maturing sensitive clone (F20-1D); LMT = late-maturing tolerant clone (9506). UL = upper leaf; LL = lower leaf. LSD ($P \leq 0.05$). Values represent the mean of four replications.

but due to a high standard error, could not be detected (data not shown). However, shoot growth was not significantly depressed in the more tolerant types of both early- and late-maturing potato. While continued uptake of water through transpiration is required for long-term growth under salt stress, the observed differences in growth were not associated with differences in stomatal conductance (Table 2).

In the most tolerant types of the late-maturing clones, tuber yield actually increased in response to stress. By contrast, the sensitive LMS clone F20-1D had a 42% tuber yield reduction under stress. Although tuber yields of the EMT clone 10909-18 were significantly reduced under stress, this clone had a high and stable harvest index, the highest bulking rate and produced the highest final tuber yield under both control and stress conditions (Table 4).

High yielding EMT and LMT clones maintained a less negative OP in their tubers compared to sensitive types and showed less leaf necroses and slower leaf senescence than the EMS and LMS clones after exposure to stress (Table 5). OP of the lower but not upper leaves was positively correlated to EM tuber yield and negatively correlated in LM clones (Fig. 3). Proline and Na⁺ but not glucose were the major contributors to lower leaf OP (Table 3). The observed negative relationship between lower leaf OP and tuber yield in the late-maturing clones may be due to the lower proline content after stress in the salt tolerant type compared to the sensitive clone (Fig. 4). Glucose levels increased with stress in the lower leaves of the late-maturing types and could have provided the carbohydrate source for tuber yield (Fig. 5).

Table 2

Stomatal conductance of the abaxial surface of upper and lower leaves, water content and rating of salt-stress-tolerance measured at the end of the test period in four diploid potato clones and the tetraploid cultivar ‘Norland’ exposed to control conditions or treated with 150 mmol NaCl salt stress for 5 days

Tissues sampled	Clones	Stomatal conductance (mmol H ₂ O m ⁻² s ⁻¹)		RWC (%)		RWC changes	Leaf damage rating	
		Control	Salt stress	Control	Salt stress		Control	Salt stress
Lower leaves	Norland	0.003	0.00	82.5	67.3	–**	2.5	5.0
	EMS	0.043	0.029	80.0	62.2	–**	2.0	5.0
	EMT	0.045	0.028	72.2	79.8	+*	2.0	3.5
	LMS	0.048	0.00	83.7	81.6	ns	3.0	5.0
	LMT	0.029	0.022	85.5	63.0	–**	2.0	3.0
Upper leaves	Norland	0.088	0.086	73.1	65.2	ns	1.5	2.0
	EMS	0.073	0.040	65.4	68.8	ns	1.0	3.0
	EMT	0.082	0.050	67.5	66.2	ns	1.0	2.0
	LMS	0.085	0.032	60.0	60.3	ns	2.0	4.0
	LMT	0.049	0.027	70.2	61.2	–*	1.0	2.0
LSD*								
Lower leaves		0.007	0.007	7.2	7.2			
Upper leaves		0.015	0.015	9.0	9.0			

RWC = relative water content. Youngest expanding leaves were measured for upper leaf-water content; youngest fully expanded leaves were measured for stomatal conductance; lowest non-wilted leaves were measured for both water content and stomatal conductance. Leaves showing 5–10% chlorotic or necrotic spots were ranked as 1 and leaves with 80–90% chlorotic or necrotic were ranked 5. EMS = early-maturing sensitive clone (10908-05); EMT= early-maturing tolerant clone (10909-18); LMS = late-maturing sensitive clone (F20-1D); LMT = late-maturing tolerant clone (9506).

* LSD ($P \leq 0.05$) for comparing within similar tissue types.

** $P \leq 0.01$

Table 3

Correlations between osmotic potential, Na⁺, proline and glucose concentrations measured at the end of the test period in the upper and lower leaves of early- and late-maturing clones after exposure to control conditions and treatment with 150 mmol NaCl salt stress for 5 days

Solutes	Upper leaves			Lower leaves		
	Na ⁺	OP	Proline	Na ⁺	OP	Proline
EMS						
OP	–0.95**	–	–	–0.95**	–	–
Proline	0.96**	–0.99**	–	0.97**	–0.96**	–
Glucose	0.76*	–0.86**	0.86**	0.06	0.04	0.07
EMT						
OP	–0.94**	–	–	–0.96**	–	–
Proline	0.95**	–0.89**	–	0.99**	–0.96**	–
Glucose	0.94**	–0.81**	0.94**	0.68	–0.67	0.68
LMS						
OP	–0.96**	–	–	–0.87**	–	–
Proline	0.89**	–0.89**	–	0.93**	–0.98**	–
Glucose	0.95**	–0.93**	–0.93**	0.87**	–0.94**	0.96**
LMT						
OP	–0.61	–	–	–0.89*	–	–
Proline	0.81**	–0.81*	–	0.80*	–0.95**	–
Glucose	–0.81*	0.79*	–0.94**	0.09	0.05	0.13

Unit of measurement: osmotic potential (OP) = (MPa); Na⁺ and glucose = mg g⁻¹ DW; proline = μM g⁻¹ DW. Asterisks indicate significant difference at: * $P \leq 0.05$; ** $P \leq 0.01$. No sign before numbers indicates a positive relation. EMS: early-maturing sensitive clone (10908-05); EMT: early-maturing tolerant clone (10909-18); LMS: late-maturing sensitive clone (F20-1D); LMT: late-maturing tolerant clone (9506).

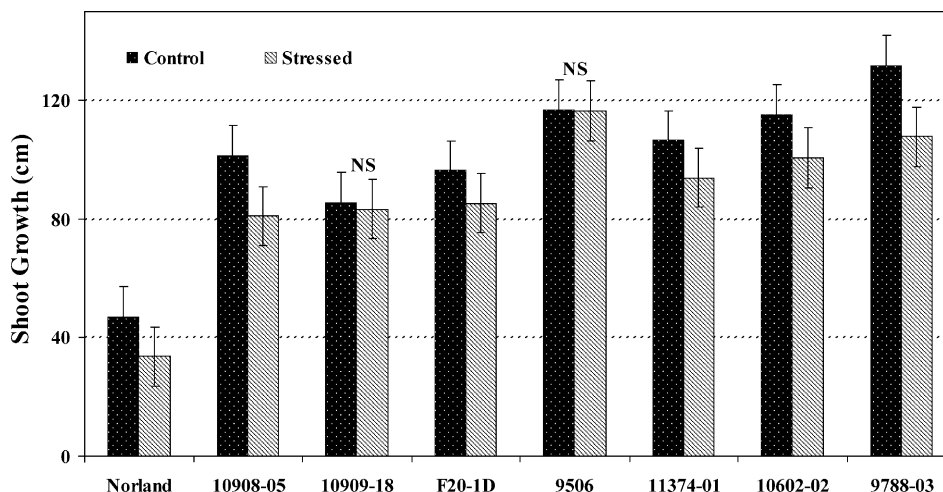


Fig. 2. Effect of salt stress on shoot growth (final height) between tuber initiation and senescence of seven diploid potato clones and the tetraploid cultivar 'Norland' after exposure to 150 mmol NaCl salt stress for 5 days. LSD ($P \leq 0.05$). Note: NS indicates differences in heights of control and stressed lines are not significant at $P < 0.05$.

Table 4

Effect of salt stress on final tuber yield, bulking rate, tuber yield reduction and final harvest index of seven diploid potato clones and the tetraploid cultivar 'Norland' after exposure to 150 mmol NaCl for 5 days at the tuber initiation stage

Clones	Maturity and tolerance	Final tuber yield (g)		Bulking rate (g day ⁻¹)		TYR (%)	Final harvest index (%)	
		Control	Stress	Control	Stress		Control	Stress
'Norland'	EMIT	266.2	98.7*	4.6	2.2*	-63	82.1	54.2*
10908-05	EMS	241.4	71.5*	5.1	1.5*	-70	56.5	36.5*
10909-18	EMT	265.8	167.5*	5.5	4.1*	-37	64.3	58.7
F20-1D	LMS	74.7	45.9*	1.2	0.8	-39	21.8	13.0
9506	LMT	100.0*	137.2	0.9	3.0*	+37	25.9	35.7*
11374-01	LMIT	225.0	59.2*	4.1	1.0*	-74	42.0	15.2*
10602-02	LMIT	70.5	51.3	1.2	0.7*	-27	25.8	19.8
9788-03	LMT	12.5	29.7	0.3	0.7	+137	3.2	10.6

EM = an early-maturing clone; LM = a late-maturing clone (Shaterian, 2002); S = a salt-sensitive clone; T = a salt-tolerant clone; IT = intermediate salt-tolerant clone. Bulking rate under salt stress = tuber yield/days from tuber initiation to harvest; TYR = tuber yield reduction. Asterisks (*) indicate significant difference at $P \leq 0.05$ for means within a row.

4. Discussion

This study demonstrated a distinct difference in salt stress response between early- and late-maturing potato clones. Early-maturing clones and the early-maturing cultivar 'Norland' accumulated Na⁺ in the leaf tissues in response to salt (ionic/osmotic) stress. However, late-maturing clones did not accumulate Na⁺ in these tissues. Subsequent studies showed that late-maturing clones accumulated Na⁺ in their roots (Etehadnia and Tanino, unpublished). The large fibrous root

mass of the LMT clone (data not shown) may have also contributed to its sodium exclusion properties. In *Citrus reticulata* L., development of a fibrous root mass was associated with continued water uptake and Na⁺-exclusion from the leaves (Blum, 1988; Storey, 1995). Late-maturing potato types typically have larger rooting systems which directly increases nutrient uptake, while early-maturing cultivars develop a small root system which may lead to a susceptibility to nutrient deficiency (Widders and Lorenz, 1979). The wild potato species used in the study by Sabbah and Tal (1995) also

Table 5

Osmotic potential of leaves, tubers and roots measured at the end of the test period in four diploid potato clones and the tetraploid cultivar ‘Norland’ exposed to control conditions or treated with 150 mmol NaCl salt stress for 5 days

Tissues sampled	Clones	Control (MPa)	Salt stress (MPa)
Lower leaves	Norland	-0.77	-1.05
	EMS	-0.60	-1.05
	EMT	-0.74	-1.47
	LMS	-0.96	-1.70
	LMT	-0.86	-1.40
Upper leaves	Norland	-0.48	-0.58
	EMS	-0.48	-0.82
	EMT	-0.55	-0.96
	LMS	-0.71	-1.17
	LMT	-0.89	-1.24
Tubers	Norland	-0.70	-0.94
	EMS	-0.81	-1.58
	EMT	-0.78	-1.03
	LMS	-0.70	-1.35
	LMT	-0.99	-1.17
Roots	EMS	-0.62	-1.33
	EMT	-0.51	-1.00
	LMS	-0.70	-1.47
	LMT	-0.85	-1.68
LSD			
Lower leaves		0.15	0.15
Upper leaves		0.14	0.14
Tuber/root		0.16	0.16

LSD ($P \leq 0.05$) for comparing effects of clones and salt stress within each tissue type. EMS = early-maturing sensitive clone (10908-05); EMT = early-maturing tolerant clone (10909-18); LMS = late-maturing sensitive clone (F20-1D); LMT = late-maturing tolerant clone (9506).

accumulated more Na^+ than cultivated types but it is not known if these wild types were early or late maturing.

The different potassium to sodium ratio in the leaves under stress was associated with differences in Na^+ accumulation and not alterations in K^+ levels. The stable K^+ levels in the lower leaves under stress is consistent with recent evidence of specific K^+ uptake maintained under high salinity thereby alleviating K^+ starvation in the halophytic ice plant (Su et al., 2002). In our study, the upper leaves showed some variation in K^+ accumulation, with higher K^+ levels maintained in the salt tolerant early but not in the tolerant late-maturing types. Early- and late-maturing potato clones appear to have different nutritional requirements and/or uptake/utilization of K^+ under salt stress.

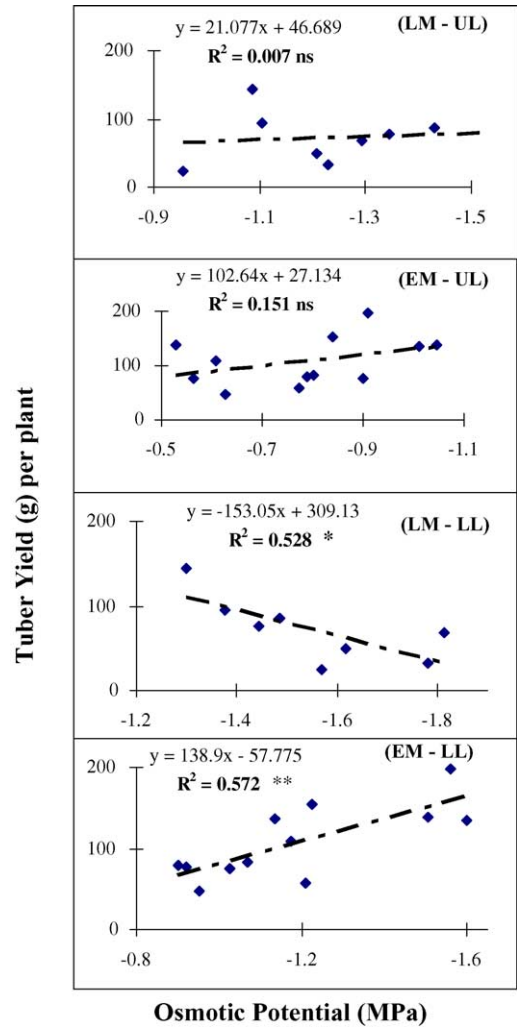


Fig. 3. Relationship between osmotic potentials of the upper or lower leaves and tuber yield of the early-maturing (EM: 10908-05, 10909-18) and late-maturing (LM: F20-1D, 9506) potato clones exposed to control conditions or treated with 150 mmol NaCl salt stress. UL = upper leaves; LL = lower leaves.

Similarly, young, upper expanding leaves exhibit a different stress response compared to older, lower expanded leaves (Greenway and Munns, 1980). Old leaves generally dehiscence earlier, acting as ion sinks, accumulating Na^+ away from younger, expanding leaves (Munns, 1993). Both the EMT and LMT clones tolerated more Na^+ in the lower leaf tissue than their sensitive counterparts, with the sodium accumulating EMT type also tolerating more Na^+ in the upper leaves

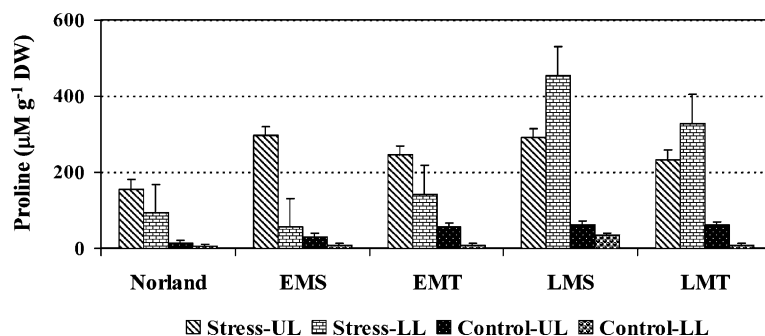


Fig. 4. Leaf proline concentrations measured at the end of the test period in four diploid potato clones and the tetraploid 'Norland' cultivar exposed to control conditions or treated with 150 mmol NaCl salt stress for 5 days. EMS = early-maturing sensitive clone (10908-05); EMT = early-maturing tolerant clone (10909-18); LMS = late-maturing sensitive clone (F20-1D); LMT = late-maturing tolerant clone (9506). UL = upper leaves; LL = lower leaves. LSD ($P \leq 0.05$). Values represent the mean of four replications.

than the sensitive types. According to Hasegawa et al. (2000), the ability to partition Na^+ on a whole plant level enhances water uptake and transport, and decreases metabolic costs of osmolyte biosynthesis but at the expense of the metabolic costs of pumping Na^+ into the vacuole.

Chauhan et al. (2000) reported a coordinated induction of Na^+/H^+ antiporters and $\text{Na}^+/\text{myo-inositol}$ symporters which transferred Na^+ from vacuolated root cells into the leaf mesophyll in the halophytic ice plant (*Mesembryanthemum crystallinum*) and lowered osmotic potential. In our study, osmotic potential of the upper and lower leaves was consistently positively correlated with both Na^+ and proline levels in all maturity types (Table 3). Internal solute content may either actively or passively increase in response to drying soil or salt stress (Kramer and Boyer,

1995). Although many protective osmolytic characteristics were attributed to proline accumulation under salt stress (Stewart and Hanson, 1980; Yancey et al., 1982; Smirnov and Cumbes, 1989), proline, along with leaf glucose, did not differentiate between relative salt-tolerance in either potato maturity types tested in this trial. Adams et al. (1992) also reported proline increases were insufficient to account for protection against severe salt stress. However, using an in vitro system, Martinez et al. (1996) suggested proline to be a biochemical marker of salt stress tolerance in potato.

Plants counteract the imbalances in water relations caused by salt stress through osmotic adjustment, via biosynthesis of compatible solutes and/or accumulation of ions from the environment (Niu et al., 1995). Halophytes compartmentalize salt in their leaf vacuoles, thereby protecting critical enzyme systems from

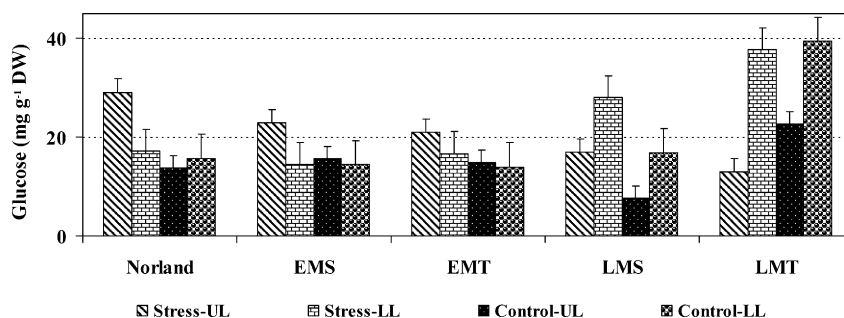


Fig. 5. Leaf glucose concentrations measured at the end of the test period in four diploid potato clones and the tetraploid 'Norland' cultivar exposed to control conditions or treated with 150 mmol NaCl salt stress for 5 days. EMS = early-maturing sensitive clone (10908-05); EMT = early-maturing tolerant clone (10909-18); LMS = late-maturing sensitive clone (F20-1D); LMT = late-maturing tolerant clone (9506). UL = upper leaves; LL = lower leaves. LSD ($P \leq 0.05$). Values represent the mean of four replications.

salt damage (Volkmar et al., 1998). According to Yeo (1998), the most important aspect of salt-tolerance in plants is the ability to maintain net influx of water with the transpiration stream. In this study, stress altered the relative water content in the lower leaves but did not change the RWC in the upper leaves. Since root mass of the EMS and EMT clones were comparable (data not shown), a higher root conductivity in the EMT clone may have been responsible for the higher RWC in this tolerant clone compared to the EMS clone. The OP of the roots in the EMT clone was also less negative compared to that of the EMS clone (Table 5). Recent research on water channel proteins indicate water flow may not solely be related to ion fluxes alone but may also be influenced by aquaporin quantity and localization under salt stress (Kirch et al., 2000).

To ensure continuous growth of the sinks that represent yield, a continued uptake of water and an osmotic pressure gradient also has to be maintained between the sink and source sites (Wolswinkel, 1985; Patrick et al., 1986; Ernst Steudle, 2001, personal communication). In potato, natural senescence contributes to the export of nutrients and assimilates from leaves to the sinks (Jones, 1992; Penarrubia and Moreno, 1994). Translocation normally occurs from the closest source to the sink. While maintenance of stomatal conductance under salt stress was not associated with these responses, the OP of the lower leaves and not the upper leaves, was related to tuber yield in both early- and late-maturing clones. High yielding EMT and LMT clones either minimized tuber yield loss or even increased yield under salt stress conditions compared to the EMS and LMS clones after exposure to salt stress. Minimizing yield losses in the EMT clone may relate to maintenance of a less negative OP in its tubers and showed less leaf necroses and slower leaf senescence (Table 2) than the EMS clone after exposure to salt stress.

This study supports the hypothesis of distinct differences in salt stress responses between early- and late-maturing potato clones. Under salt stress, early-maturing clones accumulated Na^+ into the leaf tissues while late-maturing clones generally excluded Na^+ from the leaves. Salt tolerant clones of both maturity types were able to tolerate greater leaf Na^+ accumulation compared to sensitive types with the lower leaves accumulating more Na^+ than the upper leaves. Relatively higher yield stability (early maturing) or even yield increase (late maturing) in tolerant clones after

exposure to salt stress indicates positive influencing traits regulated by source–sink relationships in potato plants. Efficient source–sink relationships should also be considered as a marker in screening tests. The results of this study show that future examination of the mechanisms of stress resistance in potato, particularly yield under salt stress, should distinguish maturity type, source–sink relationships, and leaf position when sampling the whole plant.

Acknowledgments

Thanks to the financial support from the Iranian Higher Education and Research and to the College of Agriculture, University of Saskatchewan; to Karl Volkmar (Agriculture and Agri-Food Canada, Brandon), to Tom Ward, John Peters, Mary Lee and Carolyn Ouellet of the Department of Plant Sciences, College of Agriculture, University of Saskatchewan.

References

- Adams, P., Thomas, J.C., Vernon, D.M., Hohnert, H.J., Jensen, R.G., 1992. Distinct cellular and organismic responses to salt stress. *Plant Cell Physiol.* 33 (8), 1215–1223.
- Bates, I.S., 1973. Rapid determination of free proline for water-stress studies. *PLSOA* 39, 205–207 (short communication).
- Binzel, M.L., Hasegawa, P.M., Rhodes, D., Handa, S., Handa, A.V., Bressan, R.A., 1987. Solute accumulation in tobacco cells adapted to NaCl. *Plant Physiol.* 84, 1408–1415.
- Blum, A., 1988. *Plant Breeding for Stress Environments*. CRC Press Inc., Florida.
- Chauhan, S., Forsthoefel, N., Ran, Y., Quigley, F., Nelson, D.W., Bohnert, H.J., 2000. Na^+ /myo-inositol symporters and Na^+ / H^+ antiporters in *Mesembryanthemum crystallinum*. *Plant J.* 24 (4), 511–522.
- Dubios, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28, 350–356.
- Greenway, H., Munns, R., 1980. Mechanisms of salt tolerance in non-haophytes. *Annu. Rev. Plant Physiol.* 31, 149–190.
- Hasegawa, P.L., Bressan, R.A., Zhu, J.K., Bohnert, H.J., 2000. Plant cellular and molecular responses to high salinity. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 51, 463–499.
- Haverkort, A.J., Van de Waart, M., Bodlaender, K.B.A., 1990. The effect of early drought stress on numbers of tubers and stolons of potato in controlled and field conditions. *Potato Res.* 33, 89–96.
- Hawkes, J.G., Hjerting, P.J., 1969. The Potatoes of Argentina, Brazil Paraguay and Uruguay: A Biosystematic Study. *Ann. Bot. Mem.* No. 3. Oxford University Press, Oxford, UK.

- Hukkeri, S.B., Dastane, N.G., Chauhan, D.S., 1970. Effect of soil-moisture stress at different stages of growth on the yield of potato (*Solanum tuberosum* L.). *Indian J. Agric. Sci.* 40, 318–325.
- Jones, H.G., 1992. Plants and microclimate. In: *A Quantitative Approach to Environmental Plant Physiol.* Cambridge University Press, Cambridge, UK.
- Kirch, H.H., Vera-Estrella, R., Gollmack, D., Quigley, F., Michalowski, C.B., Barclay, B.J., Bohnert, H.J., 2000. Expression of water channel proteins in *Mesembryanthemum crystallinum*. *Plant Physiol.* 123 (1), 111–124.
- Kleinkopf, G.E., Westermann, D.T., Dwelle, R.B., 1981. Dry matter production and nitrogen utilization by six potato cultivars. *Agron. J.* 73, 799–802.
- Kramer, P.J., Boyer, J.S., 1995. *Water Relations of Plants and Soils.* Academic Press Inc., New York, USA.
- Love, S.L., Eberlein, C.V., Stark, J.C., Bohl, W.H., 1995. Cultivar and seedpiece spacing effects on potato competitiveness with weeds. *Am. Potato J.* 72, 197–213.
- Martinez, C.A., Maestri, M., Lani, E.G., 1996. In vitro salt tolerance and proline accumulation in Andean potato (*Solanum* spp.) differing in frost resistance. *Plant Sci. (Limerick)* 116 (2), 177–184.
- Mass, E.V., Hoffman, G.K.J., 1977. Crop salt tolerance-current assessment. *J. Irrig. Drain. Div.* 103, 115–134.
- Munns, R., 1993. Physiological processes limiting plant growth in saline soil: some dogmas and hypotheses. *Plant Cell Environ.* 16, 15–24.
- Nelson, S.H., Hwang, K.E., 1975. Water usage by potato plants at different stages of growth. *Am. Potato J.* 52, 331–339.
- Niu, X., Bressan, A., Hasegawa, P.M., Pardo, J.M., 1995. Ion homeostasis in NaCl stress environments. *Plant Physiol.* 109 (3), 735–742.
- Paleg, L.G., Aspinall, D., 1981. Proline accumulation: physiological aspects. In: Paleg, L.G., Aspinall, D. (Eds.), *The Physiology and Biochemistry of Drought Resistance in Plant.* Academic Press, New York, USA.
- Patrick, J.W., Jacobs, E., Offler, C.E., Cram, W.J., 1986. Photosynthesis unloading from seed coats of *Phaseolus vulgaris* L.—Nature and cellular location of turgor-sensitive unloading. *J. Exp. Bot.* 37, 1006–1019.
- Penarrubia, L., Moreno, J., 1994. Senescence in plants and crops. In: *Handbook of Plant and Crop Physiology.* Marcel Dekker Inc., New York, NY.
- Poehlman, J.M., Sleper, D.A., 1995. *Breeding Field Crops.* Iowa State University Press, Ames, USA.
- Sabbah, S., Tal, M., 1995. Salt tolerance in *Solanum kurzianum* and *S. tuberosum* cvs Alpha and Russet Burbank. *Potato Res.* 38 (3), 319–330.
- Serrano, R., Gaxiola, R., 1994. Microbial models and salt stress tolerance in plants. *Crit. Rev. Plant Sci.* 13, 121–138.
- Shaterian, J., 2002. Salt tolerance in diploid potato clones. Ph.D. dissertation. University of Saskatchewan, Saskatoon, Sask., Canada.
- Smirnoff, N., Cumbes, Q.J., 1998. Hydroxyl radical scavenging activity of compatible solutes. *Phytochemistry* 28, 1057–1060.
- Stewart, C.R., Hanson, A.D., 1980. Proline accumulation as a metabolic response to water stress. In: Turner, N.C., Kramer, P.J. (Eds.), *Adaptation of Plants to Water and High Temperature Stress.* John Wiley and Sons, New York, USA.
- Storey, R., 1995. Salt tolerance, ion relations and the effect of root medium on the response of citrus to salinity. *Aust. J. Plant Physiol.* 22, 101–114.
- Su, H., Gollmack, D., Zhao, C., Bohnert, H.J., 2002. The expression of HAK-type K⁺ transporters is regulated in response to salinity stress in common ice plant. *Plant Physiol.* 129 (4), 1482–1493.
- Thomas, R.L., Sheard, R.W., Mayer, J.R., 1967. Comparison of conventional and automated procedures for nitrogen, phosphorous and potassium analysis of plant material using a single digestion. *Agron. J.* 59, 240–243.
- Volkmar, K.M., Hu, Y., Steppuhn, H., 1998. Physiological responses of plants to salinity: a review. *Can. J. Plant Sci.* 78, 19–27.
- Widders, I.E., Lorenz, O.A., 1979. Tomato root development as related to potassium nutrition. *J. Am. Soc. Hort. Sci.* 104, 216–220.
- Wolswinkel, P., 1985. Phloem unloading and turgor-sensitive transport: factors involved in sink control of assimilate partitioning. *Physiol. Plant.* 65, 331–339.
- Yancey, P.H., Clark, M.E., Hand, S.C., Bowlus, R.D., Somero, G.N., 1982. Living with water stress: evolution of osmolyte systems. *Science* 217, 1222–1241.
- Yeo, A., 1998. Molecular biology of salt tolerance in the context of whole-plant physiology. *J. Exp. Bot.* 49, 915–929.
- Zhang, H.X., Blumwald, E., 2001. Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit. *Nat. Biotechnol.* 19, 765–768.