Relative Salinity Tolerance of Potato Cultivars Assessed By *In Vitro* Screening

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ABSTRACT

One hundred and thirty European and North American potato cultivars were assayed in vitro for salinity (NaCl) tolerance. A modified single-node cutting bioassay was used in which cultivars were exposed to a range of NaCl levels (0, 40, 80, and 120 mM), in a Murashige and Skoog-based medium, for 1 month. Evaluations were performed twice for each cultivar at each salt level, using five single-node cuttings. Six vegetative growth parameters (shoot and root lengths, fresh and dry weights) were measured at the time of harvest and corrected for differences in cultivar vigor. These relative values were subjected to multivariate cluster analysis. The sum of the relative rankings at 40, 80, and 120 mM NaCl partitioned the cultivars into 8 units. The cultivars Amisk, BelRus, Bintje, Onaway, Sierra, and Tobique were in the most salinity tolerant unit and in the top cluster group for vigor with the exception of Tobique. These cultivars can be recommended for further study.

RESUMEN

Se hicieron experimentos in vitro con ciento treinta cultivares de papa europeos y norteamericanos para analizar su tolerancia a la salinidad (NaCl). Se utilizó un bioensayo modificado de esquejes de un sólo nudo en el cual los cultivares fueron expuestos a un rango de niveles de NaCl (0, 40, 80 y 120 mM), en un medio Murashige y Skoog durante un mes. Las evaluaciones se realizaron dos veces por cada cultivar en cada nivel de sal, empleando cinco esquejes con un solo nudo. Se midieron seis parámetros vegetales de crecimiento (longitud de las raíces y de los brotes, peso fresco y seco) en la cosecha y se corrigieron debido a sus diferencias en el vigor de la planta. Estos valores relativos fueron sujetos a un análisis de multivariación de grupos. La suma de los rangos relativos a 40, 80 y 120 mM NaCl separaron los cultivares en 8 unidades. Los cultivares Amisk, Belrus, Bintje, Onaway, Sierra y Tobique estuvieron en la unidad con mayor tolerancia a la salinidad y en el grupo con mayor vigor, con excepción de Tobique. Se recomienda el empleo de estos cultivares para un estudio futuro.

INTRODUCTION

A limited number of potato cultivars (cvs.) have been evaluated for salinity tolerance in vitro (Arslan et al., 1987; Elhag, 1991; Morpurgo, 1991; Morpurgo and Rodriguez, 1987; Naik and Widholm, 1993; Zhang et al., 1993). The common technique has been to subject stem cuttings with one or more axillary buds, excised from in vitro plantlets, to salt stress in vitro. The validity of in vitro screening procedures was based on correlations between vegetative growth in vitro and haulm growth (Naik and Widholm, 1993) or yield parameters (Elhag, 1991; Morpurgo, 1991) in the field. NaCl was mainly used in assessing salt tolerance in vitro. There is some inconsistency in the literature concerning the precise level suitable for effective in vitro screening. The use of one relatively high level of NaCl (103 or 154 mM) compared with a 0-level control failed to quantify differences among cultivars in vitro (Morpurgo, 1991; Morpurgo and Rodriguez, 1987). Progressive reduction in two parameters, shoot length and shoot fresh weight, occurred as NaCl levels increased from 0 to 40, 80, and 120 mM (Elhag, 1991). Evaluation at a range of salinity levels appears useful and may involve differential gene expression at different stress levels (Ashraf, 1994).

Different morphological parameters have been recommended for ranking cultivars *in vitro* for salt tolerance

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weight were significantly correlated with each other, in both *in vitro* and *in vivo* experiments, and with tuber yield *in vivo* (Elhag, 1991), while root fresh weight *in vitro* was positively correlated with tuber yield *in vivo* (Morpurgo, 1991).

The objective of this research was to evaluate the salinity (NaCl) tolerance *in vitro* of 130 European and North American potato cvs. and to rank them based on *in vitro* growth parameters.

MATERIALS AND METHODS

Multiplication of In Vitro Material

In vitro plantlets of 130 cvs. (Table 1) were received from the New Brunswick Plant Propagation Centre, New Brunswick Dept. of Agric. & Rural Devel., Canada. The plantlets were micropropagated in 25 x 150 mm Pyrex glass culture tubes on 15 ml of agar-solidified (0.7%; Anachemia, Lachine, QC) modified MS (Murashige and Skoog, 1962) basal salt medium supplemented with organic addenda as previously described (Zhang *et al.*, 1993). The pH was adjusted to 5.7 prior to autoclaving at 121 C for 15 min. Cultures were incubated under 16/8 h D/N, 40 µmolm⁻²s⁻¹ photon flux density (cool-white fluorescent light), with temperatures of 25 ± 2 C.

In Vitro Screening

Single-node cuttings (Arslan et al., 1987; Elhag, 1991; Zhang et al., 1993) had no apparent disadvantage over fivenode cuttings (Morpurgo, 1991; Morpurgo and Rodriguez, 1987), were more economical of plant material, and therefore, were employed in this study. Apical cuttings were less influenced by salt than single-node cuttings from lower on the stem (Naik and Widholm, 1993) so were discarded in this study. Single-node cuttings were tested on micropropagation medium as described above, containing a range of NaCl levels including 0, 40, 80, and 120 mM (Elhag, 1991; Zhang et al., 1993). After 4 wk, the plantlets were destructively harvested and six vegetative growth parameters were measured. The shoot and root lengths were measured, from the point of emergence of the lateral bud on the original single-node cutting or the point of emergence of the roots, to the tip of the organ, respectively. The shoot and root fresh and dry weights were measured at the time of harvest and after 60 h in a 60 ± 2 C convection oven, respectively.

Experimental Design and Data Analysis

A completely randomized design factorial experiment was conducted involving 520 treatment combinations of cultivars and salt levels. To prevent confounding of treatment effects with micro environmental effects for this large number of culture tubes, racks of test tubes were re-randomized on a weekly basis. Evaluations were performed twice, for each cultivar, at each salinity level, with each replicate consisting of five single-node cuttings.

Ward's minimum-variance cluster analysis (SAS Institute, 1989) was selected for its distinct advantage of presenting the ranking, based on several growth parameters, of a large number of cultivars in a single dendrogram. Growth data at each NaCl level were corrected for differences in cultivar vigor *in vitro*, using values from the 0-level control medium. The data matrix was then standardized prior to the analysis to remove the arbitrary effects due to the different scales of measurement of the variables. Relative salinity tolerance of the 130 cultivars was determined based on multivariate analysis of the relative means of the six growth parameters for each level of NaCl (40, 80, or 120 mM).

RESULTS

Multivariate analysis on the means of the six growth parameters in 0-NaCl control medium divided the cultivars into three cluster groups. These represented the relative vigor of each cultivar with 1 being the greatest and 3 the least (Table 1, 0 mM NaCl). Multivariate analysis on the relative means of six growth parameters indicated three cluster groups at 40 and 120 mM NaCl and four cluster groups at 80 mM NaCl (Table 1) with 1 being the most tolerant and 3 or 4 the least. As salinity levels increased, the number of cultivars in the topranked cluster group decreased. Ranking based on the relative means of six growth parameters, averaged over the three salt levels, can not be recommended. This overestimated the salinity tolerance of most cultivars, especially their ability to tolerate the higher NaCl levels. A more credible ranking was achieved by summing the cluster group rankings for each of the three NaCl levels (adding columns 2 to 4), as done by Elhag (1991) for two growth parameters (Table 1, Sum). Based on these sums, the cultivars were divided into eight units, with a sum of 3 (unit 1) the most tolerant, and a sum of 10 (unit 7) the least tolerant (Table 1, Unit). The cvs. Amisk, BelRus, Bintje, Onaway, Sierra, and Tobique were the most salt tolerant of the 130 cvs., at all levels of NaCl (Table 1, unit 1) These six cultivars can be recommended for further study.

TABLE 1.—Relative salinity tolerance for 130 Europeanand North American cultivars tested in vitro,with Units 1 through 8 containing cultivarswith the highest to lowest salinity tolerancelevels, respectively. The units were derived fromthe sum (Sum) of the cluster group rankingsfrom multivariate cluster analysis at eachsalinity level tested (40, 80, 120 mM NaCl).

	¹ Cluster group rankings							
Cultivar	0	40	80	120	Sum	Unit		
Amisk	1	1	1	1	3	1		
BelRus	1	1	1	1	3	1		
Bintie	1	1	1	1	3	1		
Onaway	1	1	î	1	3	1		
Sierra	1	1	1	Ĩ	3	1		
Tobique	2	1	î	1	3	1		
Conestoga	1	1	1	2	4	2		
Donna	2	1	ĩ	2	4	2		
Superior	1	1	î	2	4	2		
Tolaas	1	1	î	2	4	2		
Lilster Scentre	1	1	1	2	4	2		
Belleisle	î	1	1	3	5	3		
Blue Mac	2	1	1	3	5	3		
Denali	1	1	1	3	5	2 2		
Disco	1 9	1	1	ม จ	5	ว ว		
Fetima	2	1	1	3 2	5	3 9		
ESUIIIA E89065	2 1	1	1	ა ი	5	ა ი		
roouoo Croon Mountain	1	1	1	0 9	5	อ จ		
Green Mountain	1	1	1	3 9	5 5	ა ი		
La Unipper	1	1	1	0	9 5	3 9		
ND800-2	1	1	1	3	5	ა ი		
NemaRus	1	1	1	3	5	3		
NorQueen	1	1	1	3	5	3		
Ofelia	1	1	I	3	5	3		
Rhinered	2	2	2	1	5	3		
Trent	1	1	1	3	5	3		
Frontier Russet	1	1	2	3	6	4		
Islander	2	2	2	2	6	4		
Michigold	2	2	2	2	6	4		
MN9632	2	2	2	2	6	4		
Premiere	2	1	2	3	6	4		
Radosa	2	1	3	2	6	4		
Ropta I-1234	2	2	2	2	6	4		
Rose Gold	2	2	2	2	6	4		
Russett Norkotah	2	2	2	2	6	4		
Saginaw Gold	2	2	2	2	6	4		
Ute Russet	1	1	3	2	6	4		
AC Brador	2	2	3	2	7	5		
Acadia Russet	1	2	3	2	7	5		
Adora	2	2	2	3	7	5		
Amanda	2	2	2	3	7	5		
Aminca	2	2	2	3	7	5		
Anosta	1	1	3	3	7	5		
Atlantic	2	2	3	2	7	5		
Ausonia	2	2	2	3	7	5		
Chaleur	2	2	2	3	7	5		
Cupids	$\frac{1}{2}$	2	$\overline{2}$	3	7	5		
Draga	$\frac{-}{2}$	2	$\overline{2}$	3	7	5		
			-	-	-	-		

Dundrod	3	2	2	3	7	5
Dundrum	2	2	2	3	7	5
Eide Russet	2	2	2	3	7	5
Erontestolz	2	$\overline{2}$	$\overline{2}$	3	7	5
Gloria	1	2	3	2	, 7	5
Goldrush	2	2	2	2	7	5
Hampton	2	2	2	5	7	5
Hampion	4	0	2	2	(7	5
Hillite Russet	4	4	3	Z	1	D F
Katandin	3	z	2	3	7	5
Keswick	2	2	2	3	7	5
Lenape	1	2	2	3	7	5.
Lesita	2	2	3	2	7	5
Marfona	2	2	2	3	7	5
Mirton Pearl	2	2	2	3	7	5
Mouraska	2	1	3	3	7	5
Prior	2	1	3	3	7	5
Rubinia	2	2	2	3	7	5
Russette	2	$\frac{1}{2}$	2	3	7	5
Russet Burbank	2	3	3	1	7	5
Russet Nugget	2	2	9	2 2	7	5
Santo	2	2	2	2	7	5
Same	4	4	2	3	2	0 ~
Sebago	2	4	2	3	(9 -
Somerset	z	2	2	3	<u>7</u>	5
Spartan Pearl	2	2	2	3	7	5
Spunta	2	2	2	3	7	5
Sunrise	2	2	2	3	7	5
Tejon	3	2	2	3	7	5
Vital	2	2	2	3	7	5
Yankee Chipper	3	2	2	3	7	5
Annika	1	2	3	3	8	6
Avanti	2	2	3	3	8	6
AC Belmont	2	2	3	3	8	6
Allegany	$\frac{-}{2}$	2	3	3	8	6
Campbell-13	2	2	3	3	8	6
Caribe	2	2	ğ	3	8	6
Castilo	2.	2	3	2	0	6
Casule	ວ ດ	2	3	2	0	0 C
Cherokee	4	2	3	3	8	0
Coastal Chip	2	2	3	3	8	6
Concorde	1	2	3	3	8	6
Foreston Russet						
Burbank	2	2	3	3	8	6
Hertha	2	3	2	3	8	6
Hudson	2	2	3	3	8	6
Irish Cobbler	2	2	3	3	8	6
Kanona	2	2	3	3	8	6
New Red Norland	2	1	4	3	8	6
Red LaSoda	2	2	3	3	8	6
Red Pontiac	2	2	3	3	8	6
Redsen	$\overline{2}$	$\frac{1}{2}$	3	ŝ	8	6
Rideau	2	2	3	3	8	6
Ponta F 815	2	2	4	0	0	6
Compla 1-015	2	2	4	2	0	e
General Deals Deal Mealand	ე ი	3	4	2	0	0
Dark Red Norland	2	2	4	3	9	
Hulda	Z	2	4	3	9	7
Junior	2	2	4	3	9	7
Kennebec	2	3	4	2	9	7
La Rouge	3	2	4	3	9	7
Lily	1	2	4	3	9	7
Ropta J-418	3	3	3	3	9	7
Snowden	2	3	3	3	9	7
Suncrisp	3	3	3	3	9	7
Viking	3	3	3	3	9	7

TABLE 1-Cont.

WF31-4	3	3	3	3	9	7
Yukon Gold	3	3	3	3	9	7
AC Domino	3	3	4	3	10	8
Agria	3	3	4	3	10	8
Cardinal	3	3	4	3	10	8
Chipeta	3	3	3	3	10	8
Coastal Russet	3	3	4	3	10	8
NY85	3	3	4	3	10	8
Delcora	3	3	4	3	10	8
Delta Gold	3	3	4	3	10	8
Diamant	3	3	4	3	10	8
Fontenot	3	3	4	3	10	8
Fundy	3	3	4	3	10	8
Idole	3	3	4	3	10	8
Jemseg	3	3	4	3	10	8
Mainechip	3	3	4	3	10	8
Matilda	3	3	4	3	10	8
Monona	3	3	4	3	10	8
Norchip	3	3	4	3	10	8
NY73	3	3	4	3	10	8
Ocenia	3	3	4	3	10	8
Red Gold	3	3	4	3	10	8

¹Cultivars within the same cluster groups within each column are not significantly different in growth performance by Ward's Minimum Cluster Analysis. Within each column, cluster group 1 contains the cultivars with the greatest growth performance while cluster groups with higher numbers indicate cultivars with reduced growth.

DISCUSSION

Six cvs. Diamant, Draga, Erntestolz, Kennebec, Marfona, and Spunta were evaluated for salt tolerance at the same NaCl levels and ranked based on shoot length and fresh weight by Elhag (1991). We found no differences between the top-ranked cv. Erntestolz, and the lower-ranked Draga, Marfona, and Spunta (Table 1, unit 5). The six cvs. Kennebec, Norchip, Red Pontiac, Russet Burbank, Russet Norkotah, and Superior were tested in vitro by Naik and Widholm (1993). We did not find the higher-ranked Russet Norkotah (Table 1, unit 4), Norchip (Table 1, unit 8), or Red Pontiac (Table 1, unit 6) to be very salt tolerant, compared with the six top-ranked cvs. reported here. The disparity in ranking for salt tolerance between these two studies and ours is likely due to the relatively small number of cvs. screened and the few growth parameters measured in previous studies, and the method of statistical analysis.

In conclusion, multivariate analysis, using the relative means of six growth parameters evaluated at a range of salinity levels, enabled ranking of a large population of potato cvs. for salinity tolerance. The sum of the relative rankings at 40, 80, and 120 mM NaCl partitioned the cvs. into 8 units. The cvs. Amisk, BelRus, Bintje, Onaway, Sierra, and Tobique were in the top cluster groups at all levels of NaCl and comprised the top-ranked unit in the sum of rankings (Table 1, unit 1). Field testing of these promising cultivars must precede recommendations for growers in salt-affected areas of the world.

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LITERATURE CITED

- Arslan N., G. Mix, and N.E. Bassam. 1987. Salt tolerance determination in vitro of wild species and varieties of potatoes. Landbauforsch Volkenrode 37:128-131.
- Ashraf, M. 1994. Breeding for salinity tolerance in plants. CRC Crit Rev Plant Sci 13:17-42.
- Elhag, A.Z. 1991. Eignung von *in vitro* verfahreh zur charakterisierung der salztoleranz bei Solanum-arten. Vom Fachbereich Gartenbau der Universitat Hannover. 148 pp.
- Morpurgo, R. 1991. Correlation between potato clones grown *in vivo* and *in vitro* under sodium chloride stress conditions. Plant Breed 107:80-82.
- Morpurgo, R. and D.S. Rodriguez. 1987. *In vitro* differential response of the potato (*Solanum tuberosum* L.) under sodium chloride stress conditions. Riv Agric Subtrop Trop 81:73-77
- Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473-497.
- Naik, P.S. and J.M. Widholm. 1993. Comparison of tissue culture and whole plant responses to salinity in potato. Plant Cell Tiss Org Cult 33:273-280.
- SAS Institute Inc. 1989. SAS/STAT User's Guide, Version 6. 4th ed. Vol. 1, SAS Inst. Inc. Cary, NC. pp. 943.
- Zhang, Y., M. Brault, V. Chalavi, and D.J. Donnelly. 1993. In vitro screening for salinity tolerant potato. In: Biometeorology. Proc. of the 13th Int. Cong. of Biometeorology. Sept. 12-18, 1993. Calgary, AB Canada. Part 2, 2:491-498.