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Comparison of tissue culture and whole plant responses to salinity in potato

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Abstract

Salt sensitivities of six potato cultivars using six levels of sodium chloride (0.0 to 0.25M) were studied in a greenhouse. Responses of these cultivars were also determined in tissue culture by studying rooting of stem segments, increase in length of cultured roots and inhibition of growth of cell suspension cultures using similar salt concentrations. Responses of cultured stem segments and cell suspensions differed from those expressed by whole plants. A close similarity was observed between the salt stress response of whole plants and of cultured roots. The latter technique may provide a preliminary screening method for assessing salt tolerance in potato genotypes.

Abbreviations: BA – 6-benzyladenine, GA_3 – gibberellic acid, I_{50} – concentration which inhibits growth 50%, MS – Murashige and Skoog (1962) medium, NAA – naphthaleneacetic acid

Introduction

Potatoes (*Solanum tuberosum* L.) possess a low to moderate tolerance to salinity, a soil problem caused by excessive concentrations of mainly Na^+, Ca^{++}, Mg^{++}, C^{l-}, SO_4^- and HCO_3^- ions (Bresler et al. 1982). Growth in most potato cultivars is adversely affected by salinity levels when the electrical conductivity of the saturated soil extract is about 2–3 dS/m (Mass & Hoffman 1977). Yields of potato are reduced to 50% when the conductivities of sulfatic-chlorodic and sulfatic saline soils are 2.16 and 3.38 mmhos cm^{-1}, respectively (Bilski et al. 1987). Potato cultivars and wild potato species have been shown to vary in their response to elevated levels of NaCl and Na_2SO_4, the most common salts associated with saline soils (Bilski et al. 1988a,b). However, there are no reports on systematic breeding attempts for salt tolerance in potato and the conventional breeding systems have met with limited success in improving the response of many other crops to salt stress (Epstein et al. 1980). Therefore the use of unconventional crop improvement methods such as tissue culture selection or the screening of existing germplasm for obtaining salt tolerant potato plant types should be explored.

Besides its use as a tool for obtaining salt tolerant plants through selection of salt tolerant cell lines (Beloualy & Bouharmont 1992; Kirti et al. 1991; McHughen 1987; Sumaryati et al. 1992; Vajrabhaya et al. 1989 among others), plant tissue culture techniques may offer a potential for quick evaluation of germplasm against salt stress. If tissue culture methods are to be used it is necessary for the salt tolerance expressed by whole plants to also be manifested at the level of cells or isolated organs such as roots or stem segments. In the present paper we describe a

comparative study of the growth of whole plants under greenhouse conditions with that of stem segments, isolated roots and cell suspensions in tissue culture under similar salt (NaCl) concentrations.

Materials and methods

The six potato cultivars, Kennebec, Norchip, Red Pontiac, Russet Burbank, Russet Norkotah and Superior were tested with six levels of NaCl (0.0, 0.5, 0.10, 0.15, 0.20 and 0.25 M) in all experiments with the data expressed as % of the control without NaCl.

Whole plants

The whole plant work was conducted in a greenhouse, with 16 h days produced by General Electric Lucalox LV-400 high pressure sodium lights suspended 1.5 m above the bench surface, 30°C average maximum and 18°C average minimum temperatures. Tubers for this experiment were kindly supplied by the Wisconsin Seed Potato Certification Program from Lelah Starks Elite Foundation Seed Potato Farm, Rhinelander, Wiscosin (USA). Five scooped eyes, each with a growing sprout were planted in a 30 cm dia. pot. Each pot contained 6 kg of a mixture of soil: pear moss: perlite (1:1:1 v:v:v). Each treatment consisted of two pots for each cultivar. The salt treatments were initiated after 70% of the sprouts had emerged (9 DAP) and the levels of NaCl were raised by increments = <0.05 M each day until the final concentrations were reached. The plants were irrigated daily to keep the soil moist with 300 ml (<field capacity) of deionized water or salt solutions. Once a week all treatments were watered with 500 ml fertilizer mixture containing 2.5 g l^{-1} Peters 20:20:20 and microelements of Hoagland-Arnon (1938) solution. Plants were grown for 25 days following the first application of salt solution and the fresh weight of plant tops (haulms) were measured. At the end of the experiment the average electrical conductivities of rhizosphere soil samples (1:1, soil:deionized water) were 1.16, 4.55, 6.33, 8.41, 9.50 and 13.43 mmhos cm^{-1} in ascending order of NaCl levels. The electrical conductivity of the growth medium before planting was 0.58 mmhos cm^{-1}.

Stem cuttings

In vitro plantlets of potato cultivars obtained from Valley Tissue Culture, Halstad, Minnesota (USA) were micropropagated and maintained through nodal cuttings on propagation medium [basal Murashige & Skoog (1962) medium (MS) supplemented with 2 mg l^{-1} calcium pantothenate, 0.1 mg l^{-1} gibberellic acid (GA_3), 0.01 mg l^{-1} naphthaleneacetic acid (NAA) and solidified with 0.8% agar]. To study the effect of salt stress on % rooting and number of roots per cutting, ten 1 cm long cuttings [5 single-node cuttings and 5 apical cuttings] taken from 20 d old *in vitro* plantlets, were grown at 25°C and 16 h illumination (30 μmol m^{-2} s^{-1}) for 15 days in a magenta box containing 75 ml of propagation medium. Each treatment and cultivar consisted of four replicates of one magenta box each.

Roots

Healthy sprouts with well developed root primordia were detached from mother tubers, sterilized with 20% (v/v) Clorox (1.05% sodium hypochlorite) and rooted for six days on filter paper wicks in a culture tube containing 10 ml of liquid propagation medium. One cm long apical root segments with growing meristem tips were cut under aseptic conditions and inoculated on 25 ml of solid propagation medium in a petri dish (dia. 9 cm). Five root tips were cultured per petri dish and each treatment and cultivar consisted of 8 petri dishes (4 replications of 2 petri dishes each). These were grown at 28°C in the dark and the increase in root length was measured after 8 days.

Cell suspensions

Callus cultures were established from leaf rachis explants of greenhouse-grown potato plants on basal MS medium supplemented with 5 mg l^{-1} NAA at 28°C in the dark. Approximately 1–2 g callus was dispersed in 125 ml Erlenmeyer flasks containing 50 ml liquid PS medium [MS salts, Nitsch & Nitsch (1969) vitamins, 2 mg l^{-1} NAA,

0.5 mg l^{-1} 6-benzyladenine (BA), 40 mg l^{-1} adenine sulfate and 30 g l^{-1} sucrose] under continuous shaking on rotary shaker at 130 rpm. Cell suspensions were maintained by subculturing (0.5–1.0 g fresh cell weight) in fresh medium at 10–15 d intervals, depending on rate of growth. Cell growth studies on various salt concentrations were carried out by pipetting 0.5 g fresh weight of cells into 50 ml liquid medium in a 125 ml Erlenmeyer flask and allowing the cells to grow for 12 days. Cells were harvested by vacuum filtration on Miracloth filters and fresh weights were determined. Four flasks (each as a replication) were used for every treatment and cultivar.

Statistical analysis

ANOVA, Duncan Multiple Range Test and correlations were performed using arc sine transformed data for analysis.

Results

Growth in whole plants

A comparison of fresh weights of haulms for the six potato cultivars after growth for 25 days with various NaCl treatments showed a drastic reduction in fresh weights under saline conditions with all cultivars (Table 1). The extent of the reduction was 30–72% at 0.05 M NaCl and 61–79% at 0.25 M NaCl. When averaged over the treatments, the cultivars differed in their individual responses to saline irrigation with cv. Russet Norkotah being clearly the most sensitive, and Red Pontiac and Norchip the least sensitive.

These results are consistent with those of Bilski et al. (1988a) who found that Red Pontiac and Norchip plants tolerated watering with NaCl solutions better than Russet Burbank. Superior, Kennebec and Russet Norkotah were not used in their studies.

Rooting of stem segments

When the rooting behavior of stem segments was measured, the percent rooting and number of roots per cutting showed a decrease in all cultivars with increasing salt concentrations (Table 2). When averaged over all salt treatments the cv. Russet Norkotah showed the greatest decrease in these parameters which correlates with the whole plant results. The other five cvs., however, were not strikingly different in their responses although there were some statistically significant differences. Then reductions in % rooting and no. of roots per cutting were more conspicuous in single node cuttings than in apical

Table 1. Effect of watering with salt solutions for 25 days on the fresh weight on potato plants (haulms) of six cultivars grown in the greenhouse.

NaCl concentration (M)	Haulm fresh weight (% of control)					
	Kennebec	Norchip	Red Pontiac	Russet Burbank	Russet Norkotah	Superior
0.00	100.00 (40.7 ± 1.8)	100.00 (27.1 ± 2.3)	100.00 (32.8 ± 2.6)	100.00 (39.4 ± 1.7)	100.00 (20.8 ± 1.7)	100.00 (31.6 ± 0.4)
0.05	46.2 (18.8 ± 0.8)	70.1 (19.0 ± 1.0)	69.2 (22.7 ± 2.6)	55.3 (21.8 ± 2.3)	28.4 (5.9 ± 0.3)	61.1 (19.3 ± 2.9)
0.10	42.3 (17.2 ± 2.6)	54.6 (14.8 ± 1.3)	62.2 (20.4 ± 1.0)	55.8 (22.0 ± 1.4)	26.9 (5.6 ± 0.2)	55.1 (17.4 ± 1.9)
0.15	40.8 (16.6 ± 1.6)	50.9 (13.8 ± 2.1)	51.2 (16.8 ± 1.9)	46.2 (18.2 ± 0.6)	25.0 (5.2 ± 0.6)	45.9 (14.5 ± 0.6)
0.20	33.2 (13.5 ± 2.1)	50.5 (13.7 ± 0.8)	48.2 (15.8 ± 0.5)	47.5 (18.7 ± 0.6)	23.4 (4.9 ± 0.5)	44.9 (14.2 ± 1.1)
0.25	27.0 (11.0 ± 1.0)	39.1 (10.6 ± 1.6)	37.2 (12.2 ± 2.3)	22.6 (8.9 ± 0.8)	21.2 (4.4 ± 0.3)	35.8 (11.3 ± 1.0)
Average (excluding control)	37.9 (15.4 ± 0.9)	53.0 (14.4 ± 1.0)	53.6 (17.6 ± 0.9)	45.5 (17.9 ± 1.0)	25.0 (5.2 ± 0.3)	48.6 (15.3 ± 0.8)

() Mean fresh weight (g) per plant ± Standard error.

Table 2. Rooting behavior (% rooting and number of roots per cutting) of potato stem segments of six cultivars on salt media after 15 days.

NaCl conc. (M)	Kennebec	Norchip	Red Pontiac	Russet Burbank	Russet Norkotah	Superior	Average
(I) *Percent rooting*							
0.00	100.0	100.0	100.0	100.0	100.0	100.0	100.0
0.05	100.0	100.0	100.0	100.0	75.0	100.0	95.8a
0.10	60.0	65.0	75.0	65.0	40.0	55.0	60.0b
0.15	25.0	15.0	20.0	35.0	0.0	10.0	17.5c
0.20	0.0	0.0	10.0	0.0	0.0	0.0	1.7d
0.25	0.0	0.0	0.0	0.0	0.0	0.0	0.0d
Average (excluding control)	37.0bc	36.0c	41.0a	40.0ab	23.0e	33.0d	
C.V. (%) 10.94							
(II) *No. of roots per cutting (% of control)*							
0.00	100.0 (6.9)*	100.0 (6.4)	100.0 (6.7)	100.0 (6.7)	100.0 (6.3)	100.0 (4.8)	100.0 (6.3)
0.05	78.1	88.4	81.3	76.2	67.1	95.3	81.1a
0.10	49.6	46.1	48.5	65.2	26.4	36.1	45.3b
0.15	9.8	15.3	9.7	11.4	0.0	3.1	8.2c
0.20	0.0	0.0	1.9	0.0	0.0	0.0	0.3d
0.25	0.0	0.0	0.0	0.0	0.0	0.0	0.0d
Average (excluding control)	27.5b	30.0a	28.3a	30.6a	18.7c	26.9b	
C.V. (%) 10.79							

()* Average number of roots per plantlet in control.
Ten cuttings with four replicates made up each treatment.
(Means with the same letter are not significantly different at $p = 0.05$).

Table 3. Effect of NaCl on the increase (% of control) in cultured root length of six potato cultivars measured after 8 days with 1 cm initial length.

NaCl conc. (M)	Kennebec	Norchip	Red Pontiac	Russet Burbank	Russet Norkotah	Superior	Average
0.00	100.0 (5.19)*	100.0 (5.15)	100.0 (3.20)	100.0 (4.29)	100.0 (4.75)	100.0 (6.20)	100.0 (4.80)
0.5	84.2	90.2	95.6	71.5	73.6	84.3	83.2a
0.10	77.4	71.3	88.4	57.2	54.0	74.9	70.5b
0.15	45.1	51.6	59.6	45.1	31.3	52.1	47.5c
0.20	11.3	32.6	30.4	0.0	14.2	25.5	19.0d
0.25	0.0	13.5	0.0	0.0	0.0	0.0	2.3e
Average (excluding control)	43.6c	51.8a	54.8a	34.8d	34.6d	47.4b	
C.V. (%) 5.74							

()* Average increase in root length (cm) in control.
Each treatment consisted of four replicates of two Petri dishes with five roots each.
(Mean with the same letter are not significantly different at $p = 0.05$)

cuttings (data not shown). Thus the rooting response does not correlate very well with the responses observed with whole plants.

Growth of cultured roots

When cultured roots of the six potato cultivars were grown with different salt concentrations, the increase in root length averaged over treatments and cultivars (Table 3) appeared to be less sensitive to salt stress than the rooting parameters scored in stem segments (Table 2). The sensitivity trends of cultured roots of the cultivars were similar to those expressed by whole plants in the greenhouse except Kennebec and Russet Burbank were reversed in order with Russet Burbank roots being more sensitive. However, the salt sensitivities of these cultivars measured with both methods were not much different. Thus the growth of cultured roots does correlate well with the whole plant responses.

Cell growth

Cell suspension cultures from all the cultivars were initiated in PS medium and cell fresh weight was used as a convenient indicator for studying the effect of salt stress (Table 4). Though cell suspensions varied in their growth rates in the control medium without NaCl, there was a decrease in cell growth with increasing salt concentrations in all cases. The most sensitive to salt stress were Russet Norkotah, Kennebec and Red Pontiac with the three other cvs. clearly less sensitive (Table 4). The differential sensitivities of cells of potato cultivars can be seen more clearly through inhibition curves (Fig. 1) with I_{50} values ranging from 0.063 M NaCl for Kennebec to 0.113 M NaCl for Norchip, Russet Burbank and Superior.

Clearly the relative sensitivities to NaCl

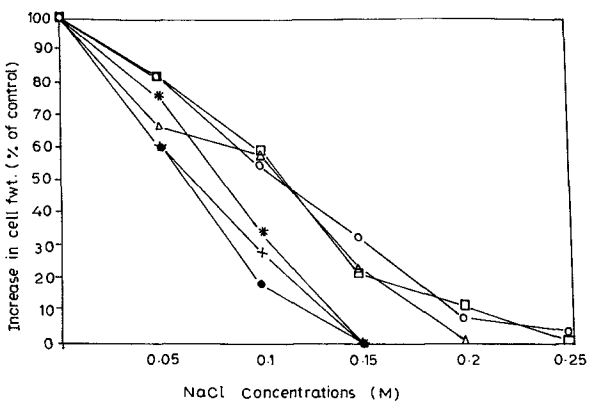

Fig. 1. Growth of cell suspension cultures of Kennebec (●–●), Norchip (○–○), Red Pontiac (*–*), Russet Burbak (□–□), Russet Norkotah (+ – +), and Superior (△–△), on PS medium containing different NaCl concentrations after 12 days of growth. The inoculum was 0.5 g fresh weight of cells and each treatment consisted of four flasks.

Table 4. Increase in fresh weight (% of control) of suspension cultures of six potato cultivars at different salt concentrations after 12 days.

NaCl conc. (M)	Kennebec	Norchip	Red Pontiac	Russet Burbank	Russet Norkotah	Superior	Average
0.00	100.0 (5.81)*	100.0 (9.10)	100.0 (5.42)	100.0 (4.89)	100.0 (2.81)	100.0 (4.45)	100.0 (5.41)
0.05	60.9	82.1	76.0	82.6	60.6	67.2	71.6a
0.10	18.7	55.5	34.1	59.4	28.4	58.2	42.4b
0.15	0.0	32.1	0.0	21.7	0.0	23.0	12.8c
0.20	0.0	8.3	0.0	10.8	0.0	0.8	3.3d
0.25	0.0	3.6	0.0	0.3	0.0	0.0	0.7e
Average (excluding control)	15.9f	36.3a	22.0d	35.0b	17.8e	29.8c	
C.V. (%) 7.56							

()* Average increase in cell fresh weight (g) per flask in control.
The initial cell weight was 0.5 g fresh weight per flask with 4 replications.
(Mean with the same letter are not significantly different at $p = 0.05$)

growth inhibition of the suspension cultures does not correlate with that of whole plants.

Discussion

Plants can adapt to salinity stress by several mechanisms at the cell, tissue and organ level in coordination to cope with salt stress. Some of the mechanisms require anatomical organization which exists in whole plants to avoid excess ion concentrations so that resistance at the cellular level may not be expected (Greenway & Munns 1980). There are several equivocal reports on the correlations between whole plant and callus/cell culture responses for salt tolerance with several species (Barlass & Skene 1981; Orton 1980; Smith & McComb 1981; Strogonov et al. 1968; Tal et al. 1978; Trivedi et al. 1991). These studies indicate that correlation of salt tolerance of a plant and cultured cells is observed only if the tolerance of the plant is predominately due to cellular mechanisms. Therefore, we studied this phenomenon at the whole plant, organized explant and cell levels in order to identify a suitable tissue culture system for screening for salt stress tolerance in potato.

The NaCl sensitivities of whole potato plants measured in this study are consistent with those of Bilski et al. (1988a) with the cvs. tested in common, Red Pontiac, Norchip and Russet Burbank. However, the correlation of the whole plant results with those found with the different tissue cultures, were significant for all cvs. only for the increase in cultured root length (Table 5). The respective NaCl concentrations also had less effect on the cultured roots than on stem rooting and suspension culture growth. This suggests that roots may be actively involved in the salt stress adaptation, and increases in cultured root length can be taken as a suitable parameter for salt stress screening of potato genotypes. Less salt sensitivity in cultured roots may also imply that roots have a mechanism for exclusion of excess ions. Energy dependent Na^+ exclusion by roots has been reported in *Zea mays* (Drew & Dikumwin 1985). Recently, differential regulation of root proteins expression has been shown to exist in salt-tolerant and salt-sensitive cultivars of barley (Ramagopal 1987) and La Rosa et al. (1989) have found that the NaCl tolerance of NaCl-adapted tobacco suspension cultures is associated with increased levels of a NaCl-induced protein, osmotion.

A response similar to salt stress in whole plants was expected in stem segments because they could be considered to be mini-replicas of a plant having the anatomical organization and ability to root and grow into a complete plant. However, the poor correlations observed in the present study (Tables 2 and 5) do not support this view. At higher salt concentrations yellowing and thickening of the stems and leaves was observed in the stem segments. Increase in leaf or stem volume associated with succulence im-

Table 5. Correlations between salt sensitivities of six potato cultivars grown in tissue culture (Y-variable) and sensitivities determined with greenhouse grown plants (X-variable).

Cultivar	Correlation coefficients for			
	% rooting	No. of roots per cutting	Increase in cultured root length	Increase in cell fresh weight
Russet Burbank	0.703NS	0.734NS	0.911*	0.859NS
Russet Norkotah	0.872NS	0.865NS	0.988**	0.871NS
Kennebec	0.898*	0.891*	0.977**	0.753NS
Superior	0.928*	0.913*	0.954**	0.930*
Norchip	0.911*	0.909*	0.948*	0.919*
Red Pontiac	0.979**	9.971**	0.974**	0.906*
Total	0.665**	0.644**	0.691**	0.674**

NS Not significant. * Significant at $p = 0.05$. ** Significant at $p = 0.01$.

proves water use efficiency (water transpired/carbon dioxide fixed) and is an additional adaptive feature under salt stress conditions (Jennings 1976). During routine *in vitro* maintenance of potato cultivars using stem segments, apical cuttings always rooted earlier and grew faster than single node cuttings (data not shown). This might be attributable to the differences in age (apical cuttings are younger than single node cuttings) or different endogenous levels of growth hormones, among other possibilities. In the present study single node cuttings were observed to be more affected by salt than apical cuttings.

Our results also show that salt tolerance in intact plants is not reflected in cultured cells, as the cells of cvs. Red Pontiac and Kennebec grew as poorly as the cells of the most sensitive cv. Russet Norkotah (Fig. 1). This is in agreement with lack of correlation between the tolerance of the entire plant and callus derived from it (Smith & McComb 1981; Strogonov et al. 1968).

Conclusions

These studies show that the relative NaCl sensitivities noted at the whole plant level with six potato cvs. correlates well with the growth response of cultured roots but not with the rooting of stem segments or growth of suspension cultures. The similarities in response to salt stress between cultured roots and whole plants established in these studies suggest that root cultures may be useful for rapid evaluation and screening of potato genotypes. The screening method involving root culture is also more rapid and simpler than the other culture methods, taking 2–3 weeks if started with sprouted tubers and it does not require extensive transfers and manipulations.

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