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Screening Potato Cultivars and Wild Species to Abiotic Stresses Using an Electrolyte Leakage Bioassay

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ABSTRACT

Ten *Solanum tuberosum* cultivars and accessions from 11 wild *Solanum* species were evaluated for their tolerance to salt (200 mM NaCl or 100 mM Na_2SO_4), drought (35% PEG), cold (-4ºC) and heat (37ºC). Evaluation was based on electrolyte leakage from detached leaves of *in vitro* plantlets. Testing involved a rinsing treatment with three changes of distilled water to remove electrolytes from leaf and petiole surfaces, an exposure period of 24 hours for salt and PEG stress and four hours for temperature stress and a rehydration period of 24 hours in distilled water followed by measurement of electrical conductivity into distilled water to determine the effects of stress. Significant differences were observed between *Solanum* genotypes for all types of stresses. Among the *S. tuberosum* cultivars, stress tolerance was great to NaCl and PEG in Alpha and Bintje; to Na_2SO_4 in Agria and to heat and cold in Norland. Among the wild species, tolerance to all stresses was great in *S. demissum*, to all stresses except heat in *S. acaule* and to heat and cold in *S. commersonii*. The correlations among types of stress tolerance were significant for all stresses except for heat and drought. The level of tolerance in some wild species was significantly greater compared with the cultivars tested. There appears to be a wide genetic base available to improve the stress tolerance of cultivated potato.

Keywords*:* Abiotic stresses, Electrolyte leakage, Potato.

INTRODUCTION

Environmental stresses, including extreme temperatures, salinity and drought, affect plant growth and productivity in many crop species including potato. The loss to agricultural and horticultural industries as a result of exposure of plants to adverse environmental conditions is estimated in the billions of dollars annually (Senaratna, 2003).

Cold stress sensitivity is a major factor limiting potato production; freezing damage to foliage results in reduced tuber yield and limits cultivation. In the US, one sixth of the total cultivated area is subjected to cold-induced limitation (Boyer, 1982). It is not possible to adjust planting times to avoid damage since frosts occur sporadically or throughout the growing season (Richardson and Estrada, 1971). The development of frost tolerant potato cultivars could greatly expand potato production; areas that are currently marginal due to low temperatures could be brought into production and the season extended in areas where production is limited by cold.

High temperatures are also a major limitation on potato production in many developing countries, which prevails during most of the year (Dodds, 1990). Reductions in leaf area, tuber number and tuber weight have been reported as symptoms of elevated temperatures during the growing season of potato plants (Menzel, 1985). However, potato

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cultivars or clones which are able to maintain relatively high yields at high temperatures have been identified in field trials (Levy, 1984; Malik *et al*., 1992).

Salinity is also a serious problem for commercial agriculture, particularly in arid and semi-arid regions. Six of 14 billion ha of arable land available in the world are located in these areas, and out of this, about one billion ha are affected by excess salt (Christiansen, 1982). With increasing world population, the use of irrigation has increased by 300% during the last 3-4 decades (Iyengar and Reddy, 1994). The most common salts responsible for toxicity and associated with saline soils are NaCl and Na_2SO_4 (Cramer and Spuir, 1986).

Moisture stress also severely affect potatoes' growth and productivity as documented in reviews by Singh (1969). Water stress during tuber initiation has been reported to reduce tuber set (Lynch and Tai, 1989; Struik and Van Voorst, 1986). The development of drought tolerant cultivars is one of the major breeding objectives in hot tropical environments where moisture is insufficient during the growing season.

Examining the field performance of genotypes under a particular stress is the usual method for evaluation, but, the results are often inconclusive. In the case of salt, a field trial is normally associated with the spatial distribution of salt, non-uniform moisture availability and temperature fluctuations during the growing season. In the case of low or high temperatures, depending on the severity of a particular season for cold or heat evaluation, either the complete survival or death of all genotypes might result. For drought tolerance, the line source sprinkler irrigation technique, which creates a continuous and linear moisture gradient, has been used extensively to screen drought stress evaluations (Levy, 1986). This method not only depends on weather changes from season to season but also involves considerable space, time, labor, equipment and planting material resources.

Therefore, a number of evaluation methods have replaced costly and labor intensive field trials. To predict freezing tolerance, controlled freezing is usually followed by observations on plant recovery; often a lengthy, tedious and destructive process requiring a large number of plants. Plant tissue water has also been used to screen for freezing tolerance in wheat and rye (Brule-Babel and Fowler, 1989). The water content percentage of fully acclimated plants (crowns and leaves) has been correlated with field evaluation, but the technique was not able to detect small differences between cultivars without excessive replication. Alternatively, the dielectric properties of plant tissues can be used as an indicator of cold survival. This involves measurement of the proportion of bound water on structural proteins in the plant's terminal meristem. This method is neither rapid nor simple and does not measure a particular physiological parameter associated with freezing stress. *In vitro* methods for the evaluation of potato genotypes to salt and drought were proposed as alternatives to the costly, labor-intensive field based evaluations. These have used single node cuttings (Zhang and Donnelly, 1997; Arvin, 1992), five node cuttings (Morpurgo, 1991), root tip segment or suspension culture (Naik and Widholm, 1993) or callus growth (Arvin, 1992) and measured one or more growth parameters at one or more salinity or osmoticum level *in vitro*. More recently, Khrais *et al*. (1998) determined the relative tolerance of cultivars based on a multivariate analysis of the relative means of six growth parameters of *in vitro* plantlets over a range of salinity levels. While promising, these *in vitro* assays are also labor intensive. Genetic improvement of crop plants requires the identification of appropriate stress tolerance mechanisms and particularly the development of suitable methodologies for their measurement in large breeding populations.

Electrolyte leakage tests have been widely used to assess the level of plant tolerance to various stresses. These tests determine the degree of cell membrane injury caused by stress based on electrolyte leakage from the cells. The technique is relatively simple, re-

peatable and rapid and requires inexpensive equipment, can be used on plant material from a variety of cultural systems and it is suitable for the analysis of large numbers of samples. For example, it has been used to quantify damage to cell membranes in various abiotic stress conditions such as high temperatures (Ismail and Hall, 1999; Maheshwary *et al*., 1999; Saelim and Zwiazekk, 2000; Rahman *et al*., 2004; Blum, 1981), low temperatures (alfalfa; Sulc *et al*., 1991), air pollution (Garty *et al*., 2000), sodium chloride salt (Chen *et al*., 1999), soil acidity (Spencer and Ksander, 1999), heavy metals (De and Mukherjee, 1996) and even in response to biotic stresses such as wheat leaf rust (Adam *et al*., 2000) or rice sheath blight (Sriram *et al*., 2000).

The objective of this study was to examine the relative tolerance of 10 *S. tuberosum* cultivars and accession from 11 wild *Solanum* species to abiotic stresses based on electrolyte leakage and also to examine the possible relationship between the various stress tolerances.

MATERIALS AND METHODS

Plant Materials

These experiments were conducted in the tissue culture lab of the Plant Science Department of McGill University in Canada. True potato seeds from 11 wild species (accession number in brackets) were received from the United States Department of Agriculture Research Service, Inter-Regional Potato Introduction Station, at Sturgeon Bay, WI., USA and held at fridge temperature (4º C) until required. *Solanum* species included: *S. acaule* (4n, PI 266386), *S. berthaultii* (2n, PI 473331), *S. chacoense* (2n, PI 209411), *S. commersonii* (2n, PI 472833), *S. demissum* (6n, PI 186562), *S. hjertingii* (4n, PI 251065), *S. lycopersicoides* (2n, PI 265378), *S. microdomtum* (2n, PI 473171), *S. phureja* (2n, PI 243461), S. *stenotonum* (2n, PI 195214), and *S. stoloniferum* (4n, PI 230477). Seeds were disinfested for 20 minutes in 10% household bleach (5.25% sodium hyperchlorite), rinsed several times with sterile distilled water, and aseptically transferred to a micropropagation medium for *in vitro* germination.

Ten potato cultivars were received as *in vitro* plantlets: Caribe, Carlton, Eramosa and Jemseg were received from Potato Gene Resources, New Brunswick, Agriculture and Agri-Food, Canada; Agria from Seed Potato Specialist, AAFRD, Crop Diversification Centre North, Edmonton and Kennebec; and Alpha, Norland, Bintje and Russet Burbank from the Plant Propagation Centre, Fredericton, NB, Canada. All were acquired in November, 2003. The *Solanum* spp. seedlings and the *S. tuberosum* plantlets were micropropagated using single node cuttings.

Culture Conditions and Medium

Cultures were incubated at 25±2ºC with 16/8 h D/N at 40 µmol m^{-2} s^{-1} photon flux density (cool white fluorescent light). For micropropagation, MS (Murashige and Skoog, 1962) basal salt solution was used, supplemented with 3% sucrose and 0.7% agar. The medium was adjusted to pH 5.7 prior to autoclaving at 121ºC for 20 minutes.

Sampling

All stress tolerance tests were performed using leaves with attached petioles of *in vitro* plantlets grown under non-stress conditions. A single sample consisted of 10 randomly selected leaves from two plantlets (four weeks old and approximately 10 cm long), avoiding the top and bottom leaves. The leaves were gently pinched by hand and introduced into test tubes.

Salt and Drought Tolerance Test

The method of Sullivan and Ross (1979) was used with minor modification according to the type of stress tested. Samples were

washed with three changes of distilled water to remove solutes from leaf surfaces and damaged areas. Ten leaves were placed in a Pyrex test tube (15 × 25mm) into 15 ml solution of a given concentration of PEG for drought and NaCl or Na_2SO_4 for salinity tests. The control tubes contained distilled water. Samples were incubated at 10ºC for 24 hours, after which media were drained and samples washed with five changes of distilled water. Samples were incubated with 15 ml of distilled water and kept at 10ºC for 24 hours in the dark**.** Tubes were then brought to 25ºC and the electrical conductivity (EC) of the incubation medium was read using a conductivity meter (conduct meter; Radiometer, Copenhagen) after a vigorous mixing of the tube's contents by hand. Following the initial reading, samples were autoclaved for 15 minutes to kill leaf tissues, brought to 25ºC and a final reading was obtained. Calculation of the percentage injury of was performed as follows:

% Injury = 1- [1- (T1/T2)/ 1- (C1/C2)] ×100

Where T and C refer to the EC values of stress treated and control tubes and 1 and 2 refer to the initial and final EC, respectively.

Preliminary experiments with six genotypes (three cultivars and three wild species) and four replications were conducted to select an appropriate treatment under which maximum genotypic differences could be observed for each stress (data are not presented). For PEG, concentrations of 0, 30, 35 and 40% of PEG 8000, for NaCl 0, 100, 150, 200 and 250 mM and for Na_2SO_4 0, 50, 100, and 150 mM were tested. Maximum genotypic differences were observed with 35 % PEG, 200 mM NaCl and 100 mM Na_2SO_4. Therefore, those concentrations were used for the reported experiments.

Heat Tolerance Test

The method of Blum and Ebercon (1981) was used with minor modifications. The treatment temperature and duration test was conducted as follows; The tubes containing leaves were placed in a glycol bath (NESLAB Instruments, INC. USA) at 33, 35, 37 and 39ºC for a period of 2, 3, 4 and 5 hours after which tubes were removed and kept at 10ºC for 24 hours before initial conductivity was measured at 25ºC. For final reading, the tubes were autoclaved for 15 minutes at 121ºC and brought to 25ºC. The combination of temperature and duration treatments exposing maximum genotypic differences was 37ºC for 4 hours, which was selected for the reported experiments.

Cold Tolerance Test

The method used for this test was that of Sekozawa *et al*. (2003) with modifications. Tubes containing leaves were submerged in a glycol bath at 5ºC and then 0ºC each for 30 minutes, cooled down at the rate of 1ºC h^{-1} and removed at -2, -3, -4 or -5ºC from the bath, thawed on ice overnight in a fridge. Ice was added to each tube for initiating ice nucleation after 30 minutes at -1ºC. Control tubes were kept in a fridge at 5ºC without subjecting them to freeze-thaw treatment. Initial and final EC readings were performed as mentioned before. Leaves were suspended in 15 ml of distilled water and kept at 10ºC for 12 hours before initial EC reading was made at 25ºC. The final reading was made after autoclaving. Maximum genotypic differences were observed at -4ºC, which was selected for the reported experiments.

Statistical Analysis

All experiments were conducted twice with four replicates each, using a completely randomized design. Data were combined and subjected to ANOVA using SAS (SAS, 1988) (Table 1) and the means were separated by Duncan's Multiple Range test at 5% level.

Table 1. ANOVA for percentage of ion leakage (percentage of injury) of potato cultivars and wild species in response to types of stress (VMS and EMS represent mean squares of stress treatment and error, respectively).

Source	VMS	EMS	F Value	CV
NaCl	676.09	52.94	12.77**	13.3%
Na_2SO_4	541.56	28.04	19.31**	7.7%
PEG	803.82	51.69	15.55**	12.1%
Heat	386.35	51.36	7.52**	14.0%
Cold	612.10	80.89	7.57**	16.6%

** Significant at 1%.

RESULTS AND DISCUSSION

NaCl

Significant differences existed between genotypes (Table 2). Little difference was found between overall performance of cultivars and wild species (56.0 vs 53.6). Agria, Alpha, Bintje, Caribe and Norland showed above average tolerance compared with the other cultivars while the rest of the cultivars, especially Carlton and Russet Burbank, were less tolerant than the average. These results are in accordance with a number of published reports; Alpha was relatively tolerant to NaCl, both *in vitro* and in a greenhouse pot trial (Arvin, 1992). Bintje was in the most tolerant cultivar group based on *in vitro* growth measurements (Khrais *et al*., 1998). Russet Burbank was not tolerant to NaCl in a greenhouse pot trial (Bilski *et al*., 1988a).

S. acaule and *S. demissum* had significantly greater tolerance to NaCl, compared with the other wild *Solanum* spp. and cultivars tested. Both were highly salt tolerant *in vitro* and in a greenhouse pot experiment (Arvin, 1992). *S. lycopersicoides, S. phureja* and *S. stoloniferum* were comparatively salt sensitive while the rest of accessions were moderately salt tolerant.

Na_2SO_4

Agria was more tolerant to Na_2SO_4, compared with the other cultivars. More variation was found among the wild species than among the cultivars as judged by the calculated range (26 vs 55.3) (Table 2). *S. acaule* was the most tolerant of the species tested followed by *S. commersonii*, *S. demissum, S. stenotonum* and *S. stoloniferum*.

When genotype responses to NaCl and Na_2SO_4 were compared, it is clear that the effect of Na_2SO_4 is more severe (68.3 vs 54.7) (Table 2). The severity of this effect is likely to be due to the sulfate ion as the two salts are iso-osmotic with equal amounts of Na. The correlation between genotype tolerance to NaCl and Na_2SO_4 was formally significant (r= 0.44**) (Table 4). This indicated that some genotypes responded differently to the two salts (Table 3). Cv. Carlton showed greater tolerance to Na_2SO_4, Bintje, Alpha, Eramosa and Norland showed relative susceptibility to Na_2SO_4 and the rest of the cultivars responded similarly to the two salts. Furthermore, *S. commersonii*, *S. lycopersicoides, S. stoloniferum*, *S. chacoense* and *S. phureja*, were relatively more tolerant to the sulfate salt, while *S. berthaultii* and *S. hjertingii* were relatively more tolerant to NaCl; *S. demissum*, *S. acaule* and *S. stenotonum,* on the other hand, responded similarly to both salts. Similar variation in response to different salts was reported for four potato cultivars by Bilski *et al*. (1988a, b). They found that cv. Russet Burbank and Norchip were more severely affected by Na_2SO_4 compared with NaCl whereas Red Pontiac and Norgold Russet responded similarly to NaCl and Na_2SO_4.

Drought (PEG)

Significant differences were observed between genotypes (Table 2). Alpha, Bintje, *S. acaule*, *S. demissum* and *S. stenotonum* were significantly more drought tolerant compared with the other genotypes. High levels of drought tolerance were also reported for *S. acaule* and *S. demissum in vitro* and in greenhouse pot trials (Arvin, 1992).

There were highly significant correlations between PEG and NaCl tolerance (r= 0.74**) (Table 4). It is likely therefore that genotype response to NaCl is via an osmotic effect. *S. commersonii* was the only exception; it was relatively salt tolerant but very drought sensitive (Table 3).

Heat

Significant differences were found in genotype response to heat. Compared with the other cultivars, Norland and Bintje showed more tolerance to heat. Among the wild species, *S. chacoense*, *S. commersonii*, *S. demissum*, and *S. hjertingii* showed more tolerance. No correlation was found between heat and drought stress tolerance. This indicated that different mechanism(s) are involved for heat and drought stresses. This supports the work of Bilski *et al*. (1988b) who reported that no relationship existed between the drought and salt tolerance of various accessions of wild potato species. A similar finding was also reported for wheat cultivars but not for sorghum cultivars (Blum and Ebercon, 1981).

Cold

There were few differences among cultivar responses to cold (Tables 2 and 3). Cultivar Norland was the most tolerant and

Table 2. Means of percentage of ion leakage (percentage of injury) of potato cultivars and wild species as affected by types of stress.

Genotype	NaCl	Na_2SO_4	PEG	Heat	Cold
Agria	44.5gh	58.1ih	55.5cd	50.0cdef	62.2abcd
Alpha	39.8h	78.1bcd	38.8e	53.4cde	55.4bcd
Bintje	39.4h	67.3fgh	36.4e	44.5cdefg	54.0bcd
Caribe	53.2efgh	73.9cdef	66.2abcd	59.0bc	75.5a
Carlton	72.1abc	70.5defg	72.7ab	60.5bc	60.5abcd
Eramosa	59.9bcde	84.7ab	56.3cd	55.4cd	65.4abc
Jesmeg	65.7bcde	78.6bcd	69.0abc	50.6cdef	55.4bcd
Kennebec	64.0bcde	80.0bcd	67.7abcd	56.2cd	75.5a
Norland	52.2efgh	77.5bcde	58.1cd	40.0efgh	50.4cd
Russet. B	69.2abc	81.7bc	57.5cd	72.9a	63.6abc
Range	(39.4-72.1)	(58.1-84.7)	(36.4-72.7)	(40.0-72.9)	(50.4-75.5)
Mean(cultivar)	**56.0**	**75.0**	**57.8**	**54.2**	**61.9**
Acaule	24.6i	37.5j	27.4e	57.0bcd	20.1e
berthaultii	64.2bcde	92.8a	75.9ab	59.6bc	55.0bcd
chacoense	58.3cdefg	61.5ghi	62.2bcd	36.0gh	63.4abc
commersonii	47.6fgh	74.0cdef	55.0d	37.5fgh	45.2d
demissum	25.4i	52.0i	29.5e	33.4h	28.4e
hjertingii	46.1fgh	74.0cdef	55.0d	37.5fgh	45.2d
lycopersiocoide	79.9a	68.0efg	74.4ab	69.8ab	65.4abc
microdontum	55.0defg	71.8cdef	74.8ab	43.3defgh	63.7abc
phureja	72.6ab	74.4a	72.6ab	67.1fgh	59.2bc
stenotonum	47.2fgh	54.7i	38.8e	58.7bc	50.2cd
stoloniferum	68.5abcd	53.0i	76.1ab	48.4cdefg	55.1bcd
Range	(24.6-79.9)	(37.5-92.8)	(29.5-78.8)	(32.1-69.8)	(20.1-65.4)
Mean (W.species)	**53.6**	**62.2**	**60.7**	**51.3**	**47.9**

Different letters in each column indicate significant differences by Duncan's multiple range test at 5%.

Kennebec and Caribe the least tolerant compared with the other cultivars. However, the wild species showed highly significant differences with respect to cold tolerance. *S. acaule, S. commersonii*, and *S. demissum* showed very high levels of cold tolerance relative to the other *Solanum* species and cultivars tested. These rankings, based on EC, support the field results of Vega and Bamberg (1995), who found high levels of cold tolerance in these three species. Cold tolerance showed significant correlations with other stresses, though the coefficient was not very high (Table 4).

CONCLUSION

This study showed that 1) The electrolyte leakage bioassay is able to detect differences among genotypes to many environmental stresses as the results are in agreement with many published results. 2) Variation existed between cultivars and, to a larger extent, between wild species for many stresses. 3) Generally, correlations existed among types of stress, especially between NaCl and PEG. This indicates that it might be possible to breed cultivars tolerant of more than one stress using multi stress tolerant wild species such as *S. acaule* and *S. demissum*. 4) Many

Table 3. Ranking of genotypes as affected by types of stress.

Genotype	NaCl	Na2SO4	PEG	Heat	Cold
Agria	5	6	7	9	13
Alpha	4	16	3	12	11
Bintje	3	11	2	6	7
Caribe	10	13	12	16	20
Carlton	10	13	14	17	13
Eramosa	13	20	7	13	17
Jesmeg	16	17	13	11	12
Kennebec	14	18	19	14	21
Norland	9	15	10	7	5
Russet. B	17	19	9	21	17
acaule	2	1	1	15	1
berthaultii	14	21	18	17	7
chacoense	12	7	11	3	16
commersonii	8	2	20	1	2
demissum	1	3	5	2	3
hjertingii	6	13	6	4	4
lycopersiocoide	21	9	15	20	17
microdontum	11	12	17	5	13
phureja	20	8	15	17	7
stenotonum	7	5	3	9	5
stoloniferum	17	4	21	8	7

Table 4. Pearson's coefficient correlations for genotypes to types of stress.

	NaCl	Na_2SO_4	PEG	Heat	Cold
NaCl	-	0.44**	0.74**	0.45**	0.55**
Na_2SO_4	-	-	0.33**	0.25**	0.53**
PEG	-	-	-	0.08 ^{Ns}	0.35**
Heat	-	-	-	-	0.39**

** Significant at 1%.
Ns: not significant

cultivars could be relatively easily evaluated by this method for immediate use in areas with environmental limitations. Similarly, more wild species and accessions could be screened for the identification of suitable parental lines with improved stress tolerance.

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استفاده از روش نشت یونی برای غربال ارقام و گونه های وحشی سیب زمینی به تنشهای محیطی

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چکیده

دریک سری آزمایش‌ها، دو رقم سیب زمینی و نژادهای مختلف از یازده گونه وحشی سیب‌زمینی برای مقاومت به تنشهای شوری (۲۰۰ میلی مول کلرورسدیم یا ۱۰۰ میلی مول سولفات سدیم)، خشکی (۳۵ درصد پلی اتیلن گلیکول)، سرما (۴- درجه سانتی‌گراد) و گرما (۳۷ درجه سانتی‌گراد) ارزیابی گردید. ارزیابی مقاومت بر اساس میزان نشت یون از برگهای جدا شده از گیاهان درون شیشه‌ای بود. روش انجام آزمایش شامل سه بار شستشوی برگها با آب مقطر برای از بین برن یونها از سطوح برگها و دمبرگها، قرار دادن برگها در معرض تنش‌های شوری و خشکی برای مدت ۲۴ ساعت و مدت ۴ ساعت برای تنش های گرما و سرما، سپس نگهداری بافت‌ها در آب مقطر به مدت ۲۴ ساعت و نهایتاً اندازه‌گیری هدایت الکتریکی محلول به منظور تعیین مقدار تنش بود. نتایج آزمایش ها نشان داد اختلافات معنی‌داری بین ژنوتیپهای آزمایش شده برای انواع تنشها وجود دارد. از بین ارقام آزمایش شده، مقاومت به کلرور سدیم و خشکی در رقمهای آلفا و بینژه، مقاومت به سولفات سدیم در رقم آگریا و مقاومت به گرما و سرما در رقم نورلند نسبت به اکثر ارقام بیشتر بود. در گونه های وحشی مقاومت به کلیه تنشها در *S. demissum*، به کلیه تنشها به غیر از گرما در *S. acaule* و مقاومت به گرما و سرما در *S. commersonii* بیشتر از اکثر گونه‌های وحشی بود. ضرایب همبستگی برای کلیه تنشها به جز برای گرما و خشکی معنی دار بود. میزان مقاومت به تنش‌ها در بعضی از گونه های وحشی بسیار بیشتر از ارقام بود و به نظر می‌رسد که منابع بسیار مهم مقاومت به تنش‌ها در ارقام وحشی وجود دارد که برای اصلاح ارقام می‌تواند مورد استفاده قرار گیرد.