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Methodologies and Traits for Evaluating the Salt Tolerance in Diploid Potato Clones

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Abstract Efforts to incorporate the salt stress tolerance expressed by certain wild diploid potato species into improvement programs for cultivated tetraploid potatoes are hampered by a lack of quick, efficient and representative salt stress tolerance screening methods capable of evaluating diverse potato genotypes. This study developed screening methods and evaluated phenotypic and physiological responses as indicators of salt tolerance in diploid potato clones (*Solanum tuberosum* L. x wild relatives) utilizing a hydroponic sand-based system. When diploid clones known to vary in their salt tolerance were treated at tuber initiation with salinities ranging from 0 to 300 mM NaCl for 30 days, treatment with 100–150 mM NaCl allowed for rapid differentiation between salt tolerant and sensitive clones. Differences in relative salt tolerance were more clearly illustrated by changes in shoot growth and water content than by changes in shoot or root dry weights. Salt tolerances of 22 diploid potato clones were then evaluated by exposure to 100 to 150 mM NaCl stress for 28 days beginning at tuber initiation. Cluster analysis was used to segregate the clones into discrete groups based on the relative similarity of their responses to salt stress. Eight clones were subsequently selected to reflect a range in salinity tolerance and maturity. The eight clones were exposed to salinity stress for the reduced stress period of 7 days and then grown through to maturity. The 7 days stress period still allowed differentiation of tolerant versus sensitive clones. Growth index in both the 30 and 7 days salt stress trials was a good predictor of absolute tuber yield but not tuber yield tolerance to salt stress (tuber yield under stress/tuber yield under non-stress conditions). Tuber yield tolerance to salt stress was positively correlated with time to maturity. When the genotypes were ranked based on cluster analysis of a range of readily measured phenotypic and physiological responses to salt stress, the ranking was effective at predicting the relative impact of a salt stress event on tuber yield at maturity. While growth index may be a useful initial rapid selection tool, maturity and cluster analysis based on readily measured phenotypic characteristics were more effective in predicting relative yield tolerance to salt stress than individual phenotypic or physiological responses.

Resumen Los esfuerzos para incorporar tolerancia a las sales expresadas por ciertas especies diplodes de papa silvestre en los programas de mejoramiento para papa cultivada tetraploide se ven obstaculizados por falta de métodos rápidos, eficientes y representativos de tamizado para tolerancia a las sales, capaces de evaluar diversos genotipos de papa. El presente estudio desarrolló métodos de tamizado y evaluó las respuestas fisiológicas como indicadoras de la tolerancia a las sales en clones diploides de papa (*Solanum tuberosum* L. x parientes silvestres), utilizando un sistema hidropónico basado en arena. Cuando los clones diploides conocidos por su tolerancia a las sales fueron tratados al inicio de la tuberización con salinidades de 0 a 300 mM Na CI por 30d; el tratamiento con 100–

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150 mM Na CI permitió una rápida diferenciación entre clones tolerantes y sensibles. Las diferencias en la tolerancia relativa a las sales fue más claramente ilustrada por cambios en el crecimiento de los brotes y el contenido de agua, más que en cambios en el peso seco de brotes o raíces. Se evaluó la tolerancia en 22 clones diploides de papa por exposición de 100 a 150 mM de Na CI por 28d, comenzando con la iniciación del tubérculo. Se usó análisis de conglomerados para separar los clones en grupos basados en la relativa similaridad de sus respuestas al estrés por sales. Se seleccionaron ocho clones que reflejan un rango de tolerancia a la salinidad y madurez. Los ocho clones se sometieron al estrés de salinidad por un periodo reducido de 7d y luego dejados crecer hasta la madurez. Los 7d de estrés permitieron la diferenciación de clones tolerantes versus sensibles. El índice de crecimiento en las pruebas de estrés tanto de 30d como de 7d fue un buen pronóstico de rendimiento absoluto de tubérculos pero no del rendimiento de tubérculos tolerantes a la sales (rendimiento de tubérculos bajo estrés/ rendimiento de tubérculos en condiciones sin estrés). El rendimiento de tubérculos bajo condiciones de estrés por sales fue positivamente correlacionado con el tiempo hasta la madurez. Cuando el genotipo fue clasificado en base al análisis de agrupamiento en un rango de genotipo de fácil identificación y respuesta fisiológica al estrés por sales, la clasificación fue efectiva a la predicción del impacto relativo al evento de estrés de sales sobre el rendimiento de tubérculos a la madurez. Mientras el índice de crecimiento pueda ser una herramienta útil de rápida selección inicial, la madurez y el análisis de agrupamiento basado en la facilidad de medir las características fenotípicas, fueron más efectivos para predecir la relativa tolerancia de la producción al estrés de sales que al fenotipo individual o las respuestas fisiológicas.

Keywords Salt tolerance · Diploid potato · Tetraploid potato

Introduction

Cultivated tetraploid potatoes are sensitive to soil salinity, with damage thresholds ranging from electrical conductivity (EC) of 1.5 to 3.0 dS m^{-1} (Maas and Hoffman 1977). Some wild species of diploid potato appear more salt tolerant (Hawkes 1992; Zhang and Donnelly 1997), however, efforts to incorporate this salt stress tolerance into potato improvement programs are hampered by a lack of quick, efficient and representative methods for evaluating the relative salt tolerance of genetically and phenotypically diverse germplasm. Bilski et al. (1988) concluded that both survival and growth were good indictors of salt tolerance, based on the performance of six wild potato species exposed to NaCl or Na_2SO_4-containing hydroponic solutions under greenhouse conditions. Relative growth following exposure to 40 to 100 mM salt solutions was used to evaluate the salt tolerance of diploid clones (Zhang and Donnelly 1997) and tetraploid potato varieties (Khrais et al. 1998) in vitro. When potato plants were exposed to salinity ranging from 1.5 to 6.0 dS m^{-1}, fresh weight, plant height and leaf area were the most sensitive indicators of salt stress (Heuer and Nadler 1998). Plant height, growth and water content have also been used as indices of salt stress in other studies of potato (Levy 1986; Levy et al. 1988; Ashraf 1994; Gong et al. 1996). Salt tolerance of six potato cultivars was closely related to relative growth rates of the roots following exposure to the salt stress (Naik and Widholm 1993). A positive relationship between root fresh weight and salt stress tolerance was also observed for ten potato clones exposed to salt stress in vitro and in the field (Morpurgo 1991).

Evaluating relative salt tolerance based on an individual phenotypic or physiological parameters represents a convenient but simplistic approach to a complex problem. Simultaneous analysis of multiple phenotypic and physiological responses to a stress event likely represents a more robust approach. Multiple selection criteria have been useful in screening grain and fruit crops for salt tolerance (Blum 1988). Khrais et al. (1998) used multivariant cluster analysis of a number of physical and physiological responses to salt stress to rank the relative salt tolerance of potato genotypes over a range of salt stress levels in vitro. It is important to demonstrate that this approach provides a valid predictor of yield responses to salt stress in whole plants under field conditions.

Effective screening for salt tolerance hinges on the application of an appropriate level of salt stress at a common and representative stage of the crop's development. Tuber development appears to be the most salt sensitive stage in the development of potato (Levy et al. 1988). Imposition of relatively short-term salt stress at tuber initiation may therefore represent an efficient means for evaluating overall stress responses.

In this study, diverse diploid *Solanum* genotypes were stressed with NaCl at tuber initiation. The objectives of this study were to: (a) develop a simple, yet accurate method for segregating salt tolerant and sensitive genotypes; (b) identify phenotypic and physiological parameters indicative of yield tolerance to salt stress.

Materials and Methods

Genetic Background of Genotypes Tested

The diploid potato genotypes evaluated represented advanced selections from hybrids created by Agriculture and

Agri-Food Canada Potato Research Centre in Fredericton, New Brunswick, Canada (courtesy H. de Jong) by crossing diploid *Solanum tuberosum* clones with a range of other diploid types. These clones were selected based on variation in their growth habit and relative salt tolerance as determined in preliminary screening trials (Shaterian 2002). Brief pedigrees of some of the key clones follow;

Clone 9506-04 resulted from a composite of crosses of diploid *Solanum chacoense* Bitt., *S. tuberosum* L., *Solanum phureja* L. and *Solanum microdontum* and was shown to be salt tolerant in vitro (Zhang and Donnelly 1997). *Clone 9507* was derived from a cross between individual selections of clone 9506-04 and clone 82.771. *Clone 82.771* is the result of a cross between *S. chacoense* and *S. tuberosum* and was selected for the high dry matter content of its tubers. *Clone F20-1D* is a diploid early maturing *S. tuberosum*, with no integration from other species. *Clones 10909-18 and 10908-05* are progenies of crosses between clone 9507-3 (a selection from clone 9507) and H412-1 (a diploid *S. tuberosum* selection). *Clone 11374-01* was derived from a cross between 10238-02 (a diploid hybrid with a complex pedigree involving cultivated diploids such as *S. phureja*, *S. stenotomum* and *S. tuberosum*) and 9787-07. The ancestry of *clone 9787-07* includes diploid *S. chacoense* and *S. tuberosum* species. It was selected for its resistance to potato virus X, potato virus Y and for high dry matter production.

Clone 9120-05 is an abscisic acid (ABA)-deficient mutant. This trait is determined by a single recessive gene, *dr* (droopy). The *dr* alleles in clone 9120-05 can be traced back to a complex hybrid of cultivated diploids (including *S. phureja* and *S. tuberosum*; De Jong et al. 2001). *Clone 9120-18* is an ABA-normal sibling (Drdr) of clone 9120-05. '*Norland*,' an early maturing commercially cultivated tetraploid potato cultivar was used to evaluate the response of more advanced selections to the test conditions and stress treatments.

Salinity Treatments

Three diverse clones were used to determine the best level of salt stress that most effectively differentiated between salt sensitive and tolerant genotypes. Clone 9506-04 is known to be salt tolerant (Zhang and Donnelly 1997). The ABA-deficient clone 9120-05 was expected to be salt sensitive (De Jong et al. 2001), while its ABA-normal sibling (9120-18) was included as a putative intermediate type.

This experiment was conducted under greenhouse conditions at the Swift Current research station of Agriculture and Agri-Food Canada. Plants were vegetatively propagated using rooted stem cuttings. Single, 10 cm tall cuttings were transplanted into 1.5 L pots containing Ottawa white sand (75.5% very coarse with 1 to 2 mm particle diameter, 24.4% coarse with 0.5 to 1 mm diameter and 0.1% less than 0.5 mm diameter). The pots were placed in large containers connected to nutrient tanks. The containers were flood irrigated five times a day, using half strength Hoagland's nutrient solution (Hoagland and Arnon 1950). The EC of the Hoagland's solution was 1.55 dS m^{-1}. Day/night temperatures were 21/18°C and the photoperiod was extended to 16 h at a mean irradiance of 200 μM m^2 s^{-1} photosynthetically active radiation. Once the plants were established and stolons initiated (visual observation after gently removing the media from around each plant), the plants were stressed by adding sufficient NaCl to the nutrient solution to achieve concentrations of: 0, 100, 150, 175, 200 or 300 mM. The conductivities of the resulting nutrient solutions were 1.5, 10.2, 14.5, 17.6, 20.4 and 28.6 dS m^{-1}, respectively. The plants were harvested after 30 days exposure to the stress treatments. Shoot growth over the stress period was evaluated by comparing the height of the tallest stem at the beginning of the stress period relative to the height after the 30 days stress event. At harvest, shoot fresh weight, shoot and root dry weights (after 48 h drying at 65°C) and percentage shoot water content ((shoot fresh−dry weight)/fresh weight)×100 were evaluated. Tuber weights in this particular experiment were not determined as the 16 h photoperiod inhibited tuberization.

The experiment was a six (salinity levels) × three (genotypes) factorial with three replicates, with a single plant from each clone in each treatment replicate. Treatment means were separated by Fisher's protected least significant difference (LSD) test, $P \leq 0.05$ and the general linear model (GLM) procedure in Statistical Analysis System (SAS) statistical method was used for analysis of variance (SAS 1997).

Screening the Salt Tolerance of Diverse Diploid Clones

Salt tolerance of 22 diploid clones and the tetraploid cultivar 'Norland' were evaluated using the best salinity stress level identified in the preliminary study. This experiment was conducted in the University of Saskatchewan greenhouses under the conditions previously described except photoperiod was reduced to 15 h and temperature regime was 25/17°C day/night under which all clones tuberized. At 14 days after planting, four uniform-sized plants from each clone were transferred into 1.5 L pots containing the previously described white Ottawa sand. Beginning at the tuber initiation time coinciding for each genotype, each genotype was then challenged with 100 mM salt (10 dS cm^{-1}) for 2 weeks, using the previously described hydroponics system. After 2 weeks, the concentration of the salt solution was increased to 150 mM for an additional 2 weeks since

plants did not express marked salt stress symptoms. Non-stressed control plants were watered with a nutrient solution as previously outlined.

The height of the tallest stem on each plant was measured at the onset and conclusion of the salt stress treatments. Shoot fresh and dry weights, shoot water content, WC_s/WC_c (ratio of percent shoot water content under salt stress/percent water content under control conditions), tuber yields and growth index (GI) were also measured after the salt stress treatment. Growth index was calculated as: (shoot height after salt stress−height at the beginning of the stress treatment)/(final shoot height under non-stressed conditions−height at the beginning of treatment). Days to maturity were determined in another trial (Shaterian 2002), with maturity defined as the point where 70% of the leaves had senesced. Cluster analysis based on the hierarchical nearest neighboring single-linkage technique (Sharma 1996) was used to compare and categorize the different clones in terms of how much each of the measured growth and yield parameters (stem height, fresh weight, shoot dry matter, shoot water content, tuber yield and growth index) changed in response to the salt stress event.

Screening for Salt Tolerance Based on Yield at Maturity

While our previous trials utilized vegetative growth parameters or tuber yield prior to maturity as indicators of relative tolerance of salt stress, this trial looked at the impact of short-term salt stress on tuber yields at maturity. Seven diploid clones shown in the previous trials to differ in both their rate of development and salt stress tolerance were selected for testing. The cultivar 'Norland' was again included as a standard reference type.

The plants were grown from stem cuttings under greenhouse conditions in the previously described Ottawa sand-based hydroponic system. The plants were watered and fertilized once or twice a day, depending on evapotranspiration demand. At the onset of stolon development (visual observation), fertilizer was excluded from the irrigation solution on alternating days for 2 weeks to stimulate tuberization. Beginning at the tuber initiation time for each genotype, half of the plants of each clone were challenged by adding 150 mM NaCl (16.3 dS m^{-1}) salt to the nutrient solution for 7 days, after which standard watering and fertilization were resumed. The control treatments were watered with the standard nutrient solution throughout the trial.

Two plants from each treatment replicate were harvested for determination of shoot weights just prior to initiation of the salt stress, while the remaining plants were harvested at senescence or after 120 days, whichever came earlier. Shoot and tuber fresh weight were recorded at the final harvest. The harvest index was calculated as (tuber weight/total plant biomass)×100. The relative shoot growth index, relative shoot yields and relative tuber yields were calculated as previously described. The experiment was conducted as a factorial of eight (clones) × two (harvests) × two levels of salt stress with four replicates and four plants per replicate. Analyses of variance were conducted using the GLM, procedure of SAS (SAS 1997). Fisher's protected LSD ($P \leq 0.05$) was used for means separation.

Evaluation of Salt Stress Indicators

Ideally any phenotypical or physiological parameter used to evaluate relative salt tolerance must be both easily evaluated and an accurate predictor of the impact of stress on yields. The degree of association between the phenotypical and physiological parameters measured in the various studies and salt tolerance as indicated by relative growth and tuber yields (stressed versus control) were determined using Pearson correlation product-moment analysis. The relationship between these indicators of salt stress tolerance and the relative ranking of the clones based on cluster analysis under stress conditions was also assessed.

Results and Discussion

Effective Salt Concentration for Screening for Salt Tolerance

Efforts to screen populations for relative salt tolerance hinge on identification of stress treatments and stress indicators which effectively and rapidly differentiates between genotypes with differing levels of tolerance. When three genotypes putatively representing diverse sensitivity to salt stress were exposed at tuber initiation to salinity levels ranging from 0–300 mM NaCl for 30 days, the 150 mM NaCl treatment allowed effective differentiation between the three levels of salt sensitivity (Fig. 1). Higher salt concentrations (175, 200 or 300 mM), were fatal to the more sensitive clone, while responses to lower concentrations of NaCl were too subtle to be of use in rapid screening. Differences in the relative salt tolerance of the intermediate and salt sensitive clones were more clearly illustrated by changes in their shoot growth and shoot water content in response to salt stress than by changes in shoot or root dry weights (Fig. 1). Impairment of water uptake, leading to lower tissue water content and reduced growth is a reliable indicator of many different types of plant stress (Levitt 1980).

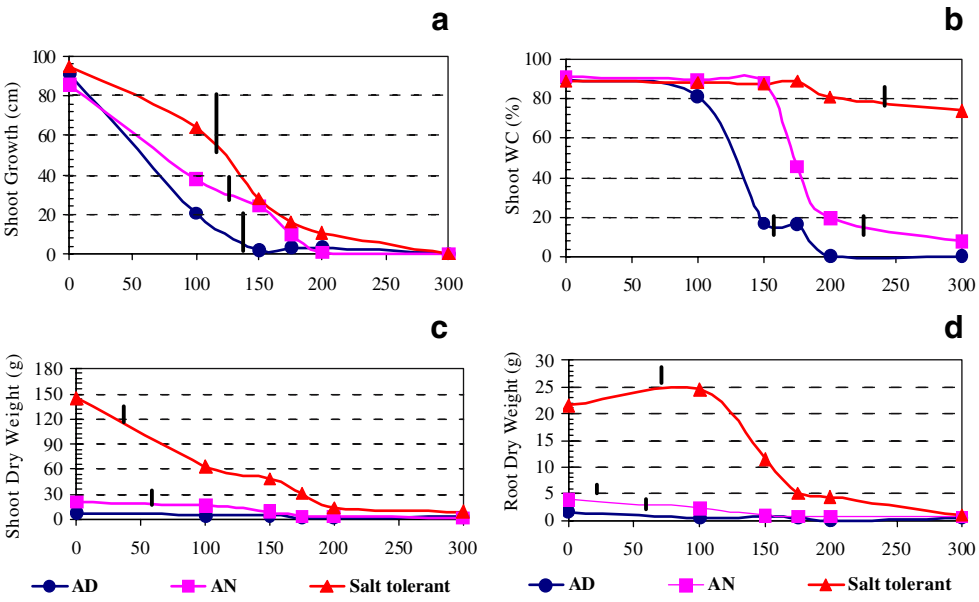

Fig. 1 Shoot growth (**a**), shoot water content (**b**), shoot (**c**) and root (**d**) dry weights of three diploid potato clones after 30 days exposure to different salt concentrations (millimolar NaCl). *Vertical bars* represent LSD ($P \leq 0.05$). LSD values are only applicable to the points along the same line. *AD* = ABA-deficient clone 9120-05; *AN* = ABA-normal clone 9120-18; *Salt tolerant* = clone 9506-04. *WC* = water content

Preliminary Screening of Diverse Diploid Potato Clones

Exposure to 100–150 mM salinity stress for 30 days, beginning at tuber initiation, resulted in a range of phenotypical and physiological responses in the 22 diploid clones tested (Table 1). GI showed a wide response range to the salt stress (GI stressed versus non-stressed=10.9% to 109.3%). Tuber yield showed an even wider range of responses to the salt stress, however, tuber yields were highly variable and many clones failed to set tubers under either stressed or non-stressed conditions. By contrast, shoot water content under stressed conditions did not vary significantly from control conditions. Time to maturity varied from 98 days to over 123 days and was distributed into four statistically discrete categories (Table 1).

The single-linkage method of cluster analysis (Sharma 1996) was used to segregate the potato genotypes tested into groups based on the relative similarity of their measured responses to a specific level of salt stress (Fig. 2). The closer two genotypes are in the cluster analysis, the more nearly they resemble each other in their response to salt stress. Early and late maturing genotypes were scattered throughout the clusters, suggesting a lack of association between maturity and cluster analysis of six responses. However, maturity was associated with relative yield tolerance to salt stress (Table 3).

Screening for Salt Tolerance Based on Tuber Yield at Maturity

When genotypes selected for differences in their growth habit and relative tolerance of salinity were exposed to 150 mM NaCl for just 7 days at tuber initiation, the salt stress significantly reduced final shoot weights of some clones but had no effect on others (Table 2). Tuber yields of most clones were also reduced by transient salt stress at tuber initiation (Table 2), however, short-term exposure to salt stress actually increased tuber yield of clones 9506-04 and 9788-03. Exposure to salt stress reduced the harvest index (HI) of most clones (Table 2), which supports previous studies indicating that tuber growth is more sensitive to salt stress than top growth (Hukkeri et al. 1970; Nelson and Hwang 1975). Salt stress actually increased the HI of clone 9506-04 relative to control conditions. This may reflect superior remobilization of carbohydrates from the leaves of this clone (Griffith 1986).

Clone 10909-18, whose pedigree includes 9506-04, was as high yielding as the standard cultivar Norland under control conditions, but its tuber yields appeared to be more tolerant of salt stress (Table 2). Similarly, the early maturing clone 7506-1 was relatively salt tolerant according to the cluster analysis. Identification of clones with excellent yield potential under both non-stress and salt stress conditions is the ultimate objective of this type of screening program.

Evaluation of Salt Stress Indicators

Time to maturity was not correlated with absolute tuber yields under salt stress but was positively correlated with relative tuber yield tolerance to salt stress (Table 3). A long subsequent period of growth and development may have allowed the late maturing genotypes to recover from the transient 7 days salt stress applied at tuber initiation. Genotypes that produced large tops at maturity under control conditions also showed high tuber yield tolerance

Table 1 Phenotypic traits measured at the end of the test period for 22 diploid potato clones and the tetraploid cultivar 'Norland' exposed to control conditions or 100–150 mM salt stress for 30 days starting at tuber initiation

Clones	Shoot growth[a] (cm)		GI[b] (%)	Tuber yield (g)		WC_s/WC_c[c]	Maturity[d] (days)
	Control	Stress		Control	Stress		
9120-05	76.0	8.3	10.9	0.5	1.0	0.99	113.1 b
9120-18	84.0	26.2	22.0	5.0	1.0	0.90	115.0 b
10911-02	47.0	17.0	36.2	46.5	5.5	0.99	113.8 b
9787-07	47.0	17.0	36.2	1.5	0.0	1.01	123.1 a
10908-06	87.5	32.0	36.6	10.5	1.0	0.99	103.8 c
CH072.03	38.5	15.0	39.0	19.5	0.0	0.99	114.1 b
10602-02	35.0	13.0	37.1	41.5	16.5	1.00	112.1 b
7506-1	53.0	24.0	45.3	4.0	0.0	1.02	98.7 d
9788-03	52.5	25.0	47.6	8.5	1.0	0.98	123.2 a
9787-01	54.5	26.0	47.7	0.0	0.0	1.02	114.3 b
F20-1D	31.0	15.5	50.0	21.5	1.0	0.88	113.8 b
8675-21	43.0	23.0	53.5	6.0	0.0	1.02	114.2 b
9507-04	47.0	25.5	54.3	0.5	0.0	1.03	123.5 a
11374-01	39.0	21.5	55.1	15.0	8.5	0.97	114.3 b
H412-01	24.5	14.5	59.2	25.0	0.0	0.98	113.8 b
Norland	39.5	23.5	59.5	15.5	1.0	0.98	99.0 d
10910-08	46.5	30.0	64.5	22.0	0.5	1.01	113.7 b
10908-05	45.5	30.0	65.9	49.0	1.0	0.99	103.8 c
9506-04	40.0	27.5	68.8	0.0	0.0	1.03	123.5 a
9507-03	63.0	44.0	69.8	0.0	0.0	1.01	123.5 a
11379-03	40.5	31.0	76.5	23.0	0.0	1.01	114.3 b
10909-18	41.5	39.0	94.0	27.0	3.0	1.02	98.8 d
10612-03	27.0	29.5	109.3	33.0	0.0	1.01	114.1 b
Mean	47.0	23.0	53.6	16.1	1.4	1.00	–
$LSD_{5\%}$	14.0	7.5	15.6	1.1	1.0	NS	–

Clones are ranked in ascending order according to GI%

[a] Shoot growth=change in plant height from onset of stress treatment until the final harvest

[b] Growth index=(shoot height after 4 weeks of salt stress−height at the beginning of the stress treatment)/(shoot height after 4 weeks under non-stressed control conditions−height at the beginning of the control treatment)×100

[c] WC_s/WC_c=ratio of percent shoot water content of stressed plants/percent shoot water content of control plants

[d] Maturity values from Shaterian (2002) ranked by Duncan method ($P<0.05$). Group (a) did not mature before the first frost at 124 days after planting.

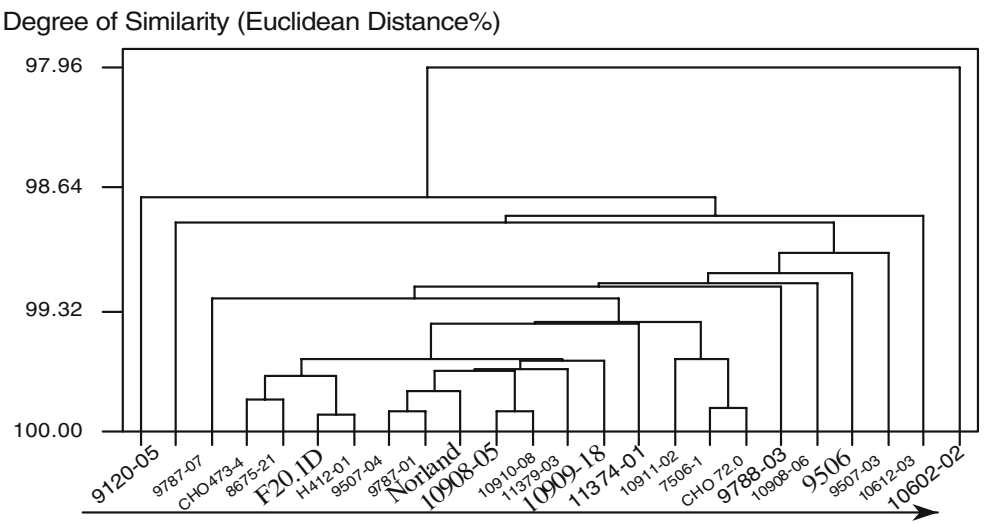

Fig. 2 Grouping of 22 diploid potato genotypes and the tetraploid cultivar 'Norland' after 14 days exposure to 150 mM NaCl salt stress based on cluster analysis of stem height, fresh weight, shoot dry matter, shoot water content, tuber yield and growth index. The *arrow* indicates increasing salt tolerance. *Bolded clones* were selected for further testing

Table 2 Plant vegetative growth, tuber yield and harvest indices for seven diploid potato clones and the tetraploid cultivar 'Norland' exposed to control conditions or 150 mM salt stress for 7 days at tuber initiation

Clones	GI[a] (%)	Final plant shoot dry weight (g)		RSY[b] (%)	Tuber yield (g)		RTY[c] (%)	Harvest index[d] (%)	
		Control	Stress		Control	Stress		Control	Stress
Norland	52	10.8	7.4	68	266.2	98.7[e]	37	82.1	54.2[e]
10908-05	85	19.2	13.2[e]	68	241.4	71.5[e]	29	56.5	36.5[e]
10909-18	96	16.0	16.8	105	265.8	167.5[e]	63	64.3	58.7
F20-1D	60	42.2	42.3	100	74.7	45.9[e]	60	21.8	13.0
9506-04	107	52.6	41.3[e]	78	100.0	137.2[e]	137	25.9	35.7[e]
11374-01	54	45.5	45.6	100	225.0	59.2[e]	26	42.0	15.2[e]
10602-02	61	31.5	27.7	87	70.5	51.3	73	25.8	19.1
9788-03	70	50.7	41.0[e]	81	12.5	29.7	237	3.2	10.6
Mean	*73*	*33.6*	*29.4*	*88*	*157.0*	*82.6*	*53*	*40.2*	*30.3*

GI Growth index, *RSY* relative shoot dry weight yield, *RTY* relative tuber yield

[a] GI=(shoot height after salt stress−height at the beginning of the stress treatment)/(shoot height after treatment period under control conditions−height at the beginning of the treatment period)×100

[b] RSY=(stressed/non-stressed)×100.

[c] RTY=(stress/non-stressed)×100.

[d] Harvest index=(tuber yield/total plant biomass)×100.

[e] Significant difference at $P \leq 0.05$ for pairwise comparison of means within a row.

to salt stress. This may again reflect the ability of slower maturing types with an indeterminant growth habit to recover from transient stress events. It is noteworthy that relative shoot dry weight was not a good predictor of either absolute or relative tuber yields. However, in nature, early maturity generally enables plants to avoid late seasonal drought-associated stresses (Larcher 2003). Thus, the findings in this present study that longer maturity time is positively correlated with relative tuber yield needs to be confirmed in field trials.

Salt stress reduced the harvest index but the degree of reduction varied amongst the clones tested. Growth index measured over the period that the plants were exposed to the salt stress was positively correlated with absolute but not relative tuber yields under stress in both the 30 and 7 days salt stress trials (Table 3). Selection for a high growth index during stress may be a useful tool for initial rapid screening for yield potential even under stress. When the genotypes were ranked based on cluster analysis of a range of readily measured phenotypic and physiological responses to salt stress, the ranking did predict the relative impact of a salt stress event on tuber yields at maturity. This corresponds with previous findings (Blum 1988; Khrais et al. 1998) that multiple selection criteria represent a more robust means of evaluating relative salt tolerance than reliance on individual indicators.

Table 3 Correlation between growth and physiological parameters measured in various trials and tuber yield tolerance to 150 mM salt stress imposed at tuber initiation for seven diploid potato clones and the tetraploid cultivar 'Norland'

	Days to maturity	Growth index[a] (%)		Relative shoot dry weight at final harvest[b]	Relative shoot water content[c]	Cluster ranking[d]
	1	2	3	3	2	2
Absolute tuber yield under stress	−0.24	0.89[e]	0.69[f]	0.10	0.59	0.01
Relative tuber yield under salt stress[g]	0.67[f]	−0.18	0.26	−0.12	0.18	−0.68[f]
Relative total biomass	0.39	−0.12	−0.33	0.43	−0.21	−0.41

1 Data from Shaterian 2002, *2* data from preliminary experiment (30 days stress), *3* data from advanced screening experiment (7 days stress)

[a] Growth index=(shoot height after salt stress−height at the beginning of the stress treatment)/(shoot height after treatment period under control conditions−height at the beginning of the treatment period)×100.

[b] Relative shoot dry weight=(shoot dry weight at final harvest following salt stress−dry weight at the beginning of the stress treatment)/(shoot dry weight at the final harvest in the control treatments−dry weight of the controls at the beginning of the stress treatment)×100.

[c] Relative shoot water content=ratio of percent shoot water content of plants in salt stress treatments/percent shoot water content of control plants.

[d] Cluster ranking from most to least salt tolerant based on multivariate analysis.

[e] Significant at $P<0.01$

[f] Significant at $P<0.05$

[g] Relative tuber yield=(tuber yield in salt stress treatments/yield under non-stress conditions)×100.

Conclusions

In trials utilizing a hydroponic system under greenhouse conditions, exposure to 150 mM NaCl stress for as little as 7 days at tuber initiation appeared to produce sufficient salt stress to allow for effective differentiation of diploid potato clones for their tuber yield tolerance to salt stress under controlled conditions. Relative tuber yield tolerance to salt stress could be predicted using readily measured phenotypic characteristics—in some cases without having to expose the plants to salt stress. For example, while not an absolute guideline, days to maturity was positively correlated with tuber yield tolerance to salt stress. Growth index may be a useful initial rapid screening tool while cluster analysis based on an array of readily measured phenotypic characteristics was more effective in predicting relative yield tolerance to salt stress than individual phenotypic or physiological responses. These findings may facilitate rapid screening of germplasm if it can be demonstrated that the results obtained from this short-term exposure to salt stress under greenhouse conditions accurately reflect the responses that occur under field situations in which salt stress is induced over a longer period of time.

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