American-Eurasian J. Agric. & Environ. Sci., 3 (2): 159-164, 2008
ISSN 1818-6769

In vitro Screening of Potato (*Solanum tuberosum* L.) Cultivars for Salt Tolerance Using Physiological Parameters and RAPD Analysis

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Abstract: *In vitro* screening of ten potato cultivars (White Desiree, Russet Burbank, Cosima, Agria, Concord, Diamant, Maradona, Kennebec, Marfona and Main) for salt tolerance at different concentration of NaCl (0, 30, 60, 90 and 120 mM) was investigated. To select the salt tolerant and salt sensitive cultivar, Physiological parameters such as, Shoot Length (SL), Root Length (RL), Shoot Dry Weight (SDW), Root Dry Weight (RDW), Leaf Area (LA), number of Rooted Shoots (RS) and RDW/SDW were measured. Comparing of the mean values for physiological parameters using ANOVA showed that Cultivar Kennebec and Concord are salt tolerant and salt sensitive respectively. A dendrogram based on dry weight led to clustering of potato cultivars in 3 groups, the salt tolerant (Kennebec, Main, Cosima), the moderately salt tolerant (White Desiree, Russet Burbank, Maradona, Diamant) and the salt sensitive cultivars (Agria, Marfona, Concord). RAPD analysis was also conducted using 10mer random primers. Dendrograms based on DNA banding pattern showed polymorphism by FPK2-19 primer. Banding pattern of RAPD confirmed a distinct polymorphism between salt sensitive and salt tolerant cultivars. The clustering pattern of the potato cultivars in this study suggests that: the salt tolerance and salt sensitivity of some potato cultivars are due to the genotype variation and possibly not epigenetic adaptation under salt stress condition.

Key words: Potato · *Solanum tuberosum* · salt stress · *in vitro* screening · RAPD analysis

INTRODUCTION

Soil salinity is a critical environmental constraint to crop productivity. Six out of the 14 billion ha of arable land available in the world are located in arid and semi-arid areas and of this; about 1 billion ha are affected by excess salt [1]. Salt stress induces several alterations on cell growth and cell division and enzymatic activities. Salt tolerance of plants depending primarily on the genotype that determines alterations on processes such as exclusion of the salt, uptake and transport of salts by roots, together with metabolic and physiological events occurring at cellular level [2].

Screening large number of genotypes for salinity tolerance in the field is difficult, due to spatial heterogeneity of soil chemical and physical properties and the seasonal fluctuations. Improving the salt tolerance of crop and pasture species requires access to new genetic diversity (either natural or transgenic) and efficient techniques for identifying salt-tolerance genes or physiological mechanisms [3].

The finding that *Solanum* species possess genetic differences in stress tolerance is interesting not only for breeding of potato to abiotic stress but also provides better material for studying the stress tolerance mechanism [2]. Potato as a glycophyte plant has been classified as moderately salt tolerant to moderately salt sensitive compared with other crops. It is an autotetraploid ($2n = 4x = 48$) with tetrasomic inheritance and a species where commercial cultivars are often sterile. Potato improvement by traditional breeding methods is slow and unpredictable. In potato, although salt tolerance is known to be a polygenic trait, the mechanism responsible for salt tolerance has not been studied in detail [4].

The salt tolerance of only small number of potato genotypes has been evaluated under outdoor, green house conditions for example [1, 2, 5-9]. *In vitro* evaluations of salt stress of potato cultivars were recently proposed as alternatives to the costly, labor-intensive and sometimes problematic field-based evaluations [5]. *In vitro* axillary bud cultures for studying the phylogeny

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of salt tolerance in some potato cultivars and other crops has been used by a number of researchers for example, [6, 11]. The most important advantage of *in vitro* cultures is their rapid regeneration under controlled environment after their exposure to stress [8].

Investigation of relatedness among traditional cultivars based on physiological and genetic characters has been studied in Maize [7, 11, 12] and Tomato [13]. Chollares *et al.* [5] used DNA pattern using RAPD-PCR and some characters "such as isozymes pattern and soluble proteins in order to study the possible relationship between genotypes and physiological parameters. So far no report has been published on combination analysis of growth parameters and RAPD for potato cultivars.

The objectives of this study were to assess the potential for *in vitro* screening of ten widely growing potato cultivars for salt tolerance in IRAN using physiological parameters associated with RAPD analysis to understand the possible relationship among these cultivars based on genetic or physiological adaptation of salt tolerance or salt sensitivity.

MATERIALS AND METHODS

Plant material and culture condition: Sprouted healthy tubers of ten widely growing of potato cultivars in Iran (White Desiree, Russet Burbank, Cosima, Agria, Concord, Diamant, Maradona, Kennebec, Marfona and Main) were planted in 500ml pots containing steam stilled soil, then nodal sections of pot grown plants were used as primary explants. Stem-cuttings consisting of a single node and a leaf were surface sterilized in a solution of 10% v/v sodium hypochlorite for 15 minutes. They were then rinsed with sterile distilled water 3 times and were transferred on 35 ml MS medium [6]. Cultures were then maintained and sub-cultured under 16/8 hour light/dark photoperiod with 150 μmol m^{-1} s^{-1} at 25±1°C. *In vitro* grown plants were propagated by sub-culture with 4 weeks interval.

Single nodes then were transferred to MS medium containing 0, 30, 60, 90 and 120 mM NaCl with five replicates in each treatment in a Completely Random Design (CRD). After five weeks, the physiological parameters including Shoot Length (SL), Root Length (RL), Shoot Dry Weight (SDW), Root Dry Weight (RDW), Leaf Area (LA), number of Rooted Shoots (RS) and the ratio of RDW/SDW were measured. Results were statistically analyzed using ANOVA and Turkey's tests.

DNA extraction: Small segments of leaf tissue was homogenized after freezing in liquid nitrogen by a mixed of glass beads and 250 μl DNA extraction buffer (1.4 M NaCl, 20 mM EDTA, 100 mM Tris PH: 8, 3% W/V CTAB). Then mixture was vortexed and incubated at room temperature for 15-20 minutes. 250 μl of chloroform/IAA (24:1) was added and mixed well then spined at 13000 rpm for 10 minutes and the aqueous layer was transferred to fresh tube. Then 140 μl Iso-Propanol was added, mixed well and spined at 13000 rpm for 7 minutes and supernatant was discarded and the pellet was washed by 1ml of 70% ethanol. Finally, spined for one minute and pellet dried and dissolved in 100 μl of sterile distilled water.

RAPD analysis: Six 10-mer primers including OPB-07, OPAA-14, FPK2-19, OPAA-20, FDK1-05 and OPAA-03 (Technologies Inc) were used for PCR amplifications and identification of polymorphic marker. DNA amplification was also subjected to four primers including ISSR1, ISSR2, ISSR3 and ISSR4. DNA amplification procedure was a modification of that reported by Williams *et al.* [12].

Total DNA of every plant was amplified twice. Amplification was carried in a final volume of 20 μl containing: 2 μl PCR buffer (10X), 0.8 μl MgCl2 (50 mM), 2 μl dNTPs (2 Mm), 1.5 μl of primer (5 ng μl^{-1}), 3 μl of DNA templet (5 ng μl^{-1}) and 1 unit of Taq polymerase. Amplification was performed as 94°C of 2 min. (one cycle) then, 94 °C for 15 sec., 35°C for 15 sec., 72°C for 30 sec.

Table 1: Total mean values of physiological characters for 10 potato cultivars, 5 weeks after treatment. SL: shoot length, RL: Root length, LA: Leaf area, RDW: Root dry weight, SDW: Shoot dry weight, RDW/SDW: Root dry weight/shoot dry weight, R.S: the number of rooted shoots.Mean values of characters

Cultivars	Groups	SL (cm)	RL (cm)	LA (mm^2)	RDW (mg)	SDW (mg)	RDW/ SDW	RS
Kennebec 1	1	2.83^{a}	11.08^{a}	141.30^{b}	0.021^{a}	0.045^{a}	0.41^{b}	2.85^{a}
Main	1	2.89^{b}	13.11^{a}	150.30^{b}	0.023^{a}	0.037^{a}	0.60^{a}	2.75^{a}
Cosima	1	2.19^{c}	11.25^{a}	143.70^{b}	0.019^{b}	0.030^{c}	0.57^{a}	3.10^{a}
W. Desiree	2	3.15^{b}	9.12^{b}	194.70^{a}	0.0132^{b}	0.043^{a}	0.28^{c}	2.7^{a}
R. Burbank	2	3.08^{b}	7.67^{b}	123.60^{b}	0.011^{c}	0.033^{c}	0.31^{c}	2.55^{a}
Maradona	2	3.10^{b}	11.03^{a}	135.70^{b}	0.016^{b}	0.041^{a}	0.31^{c}	2.85^{a}
Diamant	2	1.73^{d}	7.25^{b}	117.03^{b}	0.012^{b}	0.025^{c}	0.39^{b}	1.55^{b}
Agria	3	3.06^{b}	9.41^{b}	73.00^{c}	0.010^{c}	0.024^{d}	0.44^{b}	2.75^{a}
Marfona	3	2.52^{c}	6.79^{c}	79.10^{c}	0.011^{c}	0.024^{d}	0.29^{a}	2.06^{b}
Concord 3	3	1.63^{d}	4.49^{d}	76.05^{c}	0.009^{c}	0.018^{d}	0.23^{c}	1.90^{b}

1: The most salt tolerant,: The most salt sensitive

1: Salt tolerant, 2: salt moderate, 3: salt sensitive

Different letters in each column show significant difference ($p<0.05$)

(39 cycle) followed by 72°C for 5 min. DNA banding were revealed by electrophoresis at 90V on 1% agarose gels using ethidium bromide staining (7 µl of a solution of 10 mg ml^{-1} for 70 ml of gel) and photographed under UV light (302 nm) using a Polaroid camera and a photo print image visualizer.

Clustering of potato cultivars based on their response to salinity: Grouping of the cultivars was conducted based on dry weight as a representative of all physiological characters (Table 1). The potato cultivars were also classified according to RAPD banding using NTYSIS-PC soft ware.

RESULTS

Screening of potato cultivars based on physiological parameters: Five weeks after salt treatment of plants, physiological characters including: Shoot Length (SL), Root Length (RL), Shoot Dry Weight (SDW), Root Dry Weight (RDW), Leaf Area (LA), number of rooted shoots (RS) and RDW/SDW were measured. Then the value for each parameter for plants ranked and finally the overall value for each character considered as a final data for ranking of the cultivars for salt response.

According to the response of plants under salt stress (Table 1), potato cultivars calcified in three distinct groups, (1) Tolerant, including Kennebec> Main> Cosima. (2) Moderately tolerant including: White Desiree> Diamant> Maradona> Russet Burbank, (3) Sensitive: including: Agria> Marfona >Concord respectively.

There was a significant difference ($p<0.05$) in response to salinity between Kennebec and the other cultivars, consequently Kennebec was selected as a salt tolerant. In contrast, Concord with the lowest value ($p<0.05$) of physiological parameters considered as sensitive cultivar. As Fig. 1 showing, cultivar Concord showed dramatic decrease in dry weight as well as other physiological characters when the concentration of salt in the medium increased. At 60 to 120 mM NaCl Concord showed sever damages on leaves and did not grow, while cultivar Kennebec at the same condition rooted very well and did not show leaf necrosis (Fig. 2).

Clustering of the potato cultivars based on their response to salt stress according to dry weight showed, four clusters (Fig. 3), Cluster 1 consist of White Desiree, Russet Burbank and Diamant. Cluster 2 including Cosima and Main. Cluster 3 consist of Kenebec and Maradona. Cluster 4 including Marfona, Agria and Concord. In this grouping, three cultivars (Marfona, Agria and Concord) are calcified as sensitive cultivars with 100% similarity. Cultivar White Desiree, Russet Burbank and Diamant are moderately salt tolerant group. In this cluster Russet Burbank shows 100% similarity with Diamant and 50% similarity with White Desiree. In the other 2 cluster Cosima have 50% similarity with Main and Kenebec again 50% similarity with Maradona. However, Kenebec is much closer to Maradona.than the other cultivars (Fig. 3).

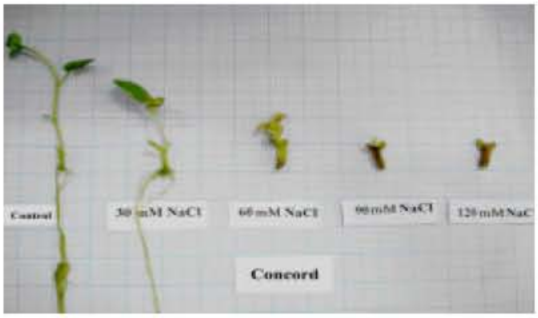

Fig. 1: Response of potato cultivar Concord (Sensitive cultivar) to different concentrations of NaCl

Fig. 2: Response of potato cultivar Kennebec (Tolerant cultivar) to different concentrations of NaCl

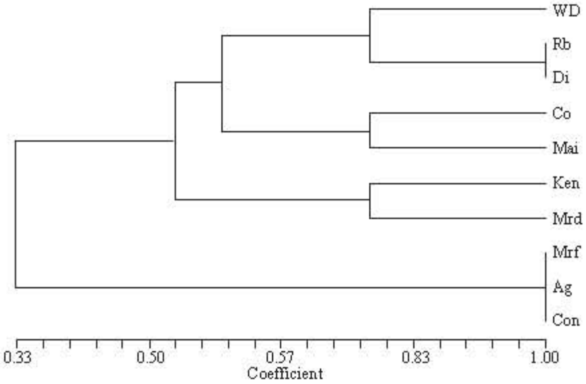

Fig. 3: Dendrogram based on dry weight of potato cultivars under salt stress.WD: White Desiree, Mrd: Maradona Rb: Russet Burbank, Di: Diamant, Co: Cosima, Mai: Main, Ken: Kennebec, Mrf: Marfona, Ag: Agria, Con: Concord

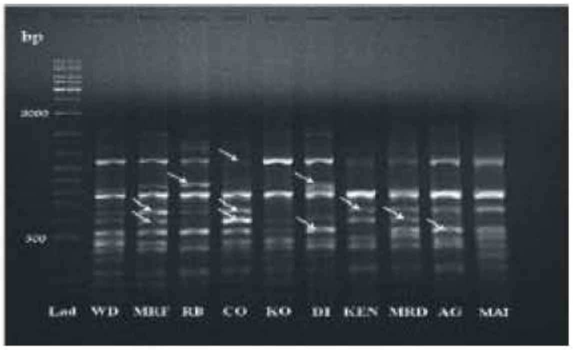

Fig. 4: Gel electrophoresis of RAPD fragments obtained with primer FPK2-19. Lad: molecular markers (1 kb DNA ladder). WD: White Desiree, MRF: Marfona, RB: Russet Burbank, CO: Cosima, KO: Concord, DI: Diamant, KEN: Kennebec, MRD: Maradona, AG: Agria and MAI: Main

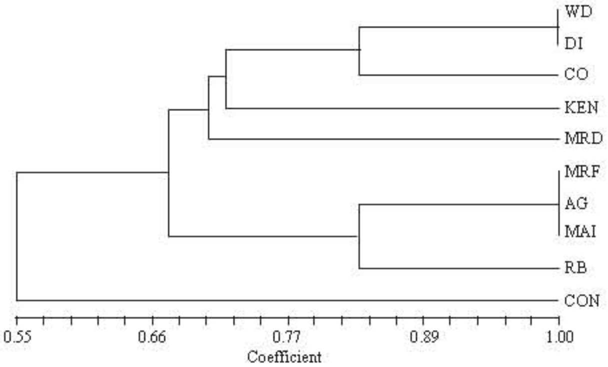

Fig. 5: Dendrogram of genetic relationship of potato cultivars based on RAPD analysis. WD: White Desiree, MRD: Maradona, RB: Russet Burbank, DI: Diamant, CO: Cosima, MAI: Main (9), KEN: Kennebec, MRF: Marfona, AG: Agria, CON: Concord

The result of DNA banding pattern using primer FPK2-19 is illustrated in Fig. 4. This primer among six random primers and four ISSR primers showed a reliable and reproducible DNA bands.

The dendrogram constructed using NJ method is shown in Fig. 5. The Genomic relationship among 10 potato cultivars was investigated based on the DNA banding pattern resulted in RAPD-PCR. Analysis of the DNA banding pattern using NTYSIS-PC program, showed three relatively distinct clusters (Fig. 5). White Desiree and Diamant were similar to each other (100%) while they have Approx. 50 and 35% similarity with Cosima and Kenebec respectively. Marfona, Agria (the sensitive cultivars) and Main (tolerant cultivar) showed a cluster with 100% similarity. However they illustrated about 50% similarity with Russet Burbank (moderately tolerant). Cultivar Concord as a sensitive cultivar to salt stress made a separate line with least similarity to other potato cultivars. Cultivar White Desiree and Diamant (moderately salt tolerant) made a cluster with 100% similarity where they have about 50% similarity with Cosima and Kennebec (salt tolerant). However, the clusters clearly showing the genotype dependency of salt tolerance trait of potato cultivars.

DISCUSSION

It has been documented that, the response of potato cultivars to salt stress is genotype dependent. In this study, we used dry weight as a representative of some important physiological parameters such, shoot and root length and number of roots and shoots. According to the results obtained by grouping of cultivars based on dry weight under salt stress the results of evaluation of salt tolerance in 10 potato cultivars showed they can be calcified in three groups. Relatively salt tolerant, moderately salt tolerant and salt sensitive. We found Kennebec, Cosima and Main as salt tolerant cultivars and cultivars Concord,Agria and Marfona as sensitive cultivars. Potato cultivar Kennebec has already been reported as relatively salt tolerant cultivar [4, 6, 8, 9]. We found cultivars Main and Cosima relatively salt tolerant and to some extend similar to Kennebec. In this study, Russet Burbank classified as moderately salt tolerant while, it has already been proposed as tolerant cultivars [1, 8]. They have tested the salinity tolerance of the potato cultivar under pot culture. Perhaps the growing condition of the potato plants (*in vitro* culture versus pot culture) might be a source of variation in the results.

In the present study, Concord is proposed as salt sensitive cultivar. The sensitivity of this cultivar to salt stress has been reported by other researchers for example, Collares *et al.* [5]. Cultivar Diamant has already been introduced as a salt sensitive by Rahnama and Ebrahimzadeh [9], we found this cultivar as a moderately salt tolerant and not sensitive. The difference might be due to the difference in experimental conditions or the range of salt concentrations in the medium which has been tested. One important conclusion obtained from clustering of dry weight (as an overall value for physiological parameters) is similarity of cultivar Marfona, Agria and Concord as sensitive cultivars. These cultivars are close to each other. However, the other cultivars either salt tolerant or moderately salt tolerant were more and less close to each other and they have obvious distance from the sensitive cultivars.

Interestingly, RAPD cluster "similar to dry weight" showed that, cultivar Concord is a separated genotype from other cultivars and the sensitivity of this cultivar is a genetic rather than epigenetic property. Cultivar Marfona, Agria and Main are very similar (100%) and make a close cluster, where Marfona and Agria are sensitive cultivar to salt stress, Main is a salt tolerant cultivar. Again, this result indicating that, the salt tolerance of cultivar Main might be due to physiological adaptation to salt stress while, cultivar Marfona and Agria are more likely to be genetically salt sensitive. Based on the similarity, the other potato cultivars (White Desiree, Cosima, Kennebec and Maradona), seems to be genetically salt tolerant and the tolerance in these cultivars may not be interpreted as physiological adaptation.

There is an obvious polymorphism among cultivars in both denderograms (Fig. 3 and 5). We can compare the position of some cultivars in two denderograms. Concord has separated from other cultivars in both denderograms and shows the least similarity with other cultivars. Despite of different clusters in this group Cultivar Kennebec, Cosima and Main have about 50% similarity based on dry weight.

Comparison of polymorphism Using RAPD markers and physiological parameters has been reported by other researchers [7, 12].

Seraj *et al.* [11] grouped some land races of rice based on the data obtained after salt treated of seedlings. They used plant survival, leaf area and shoot height as physiological characters for clustering. They came to the same conclusion as we found. In some cases, the salt tolerant and salt sensitive plants showed both epigenetic and/or genetic tolerance. Marconi *et al.* [6] and Ochat *et al.* [4] reported that finger printing by RAPD confirm the regenerated salt-tolerant plants. The regenerated plants have been genetically different from their parents. In another investigation Zacchini *et al.* [12] found that some maize callus lines were salt tolerant based on DNA pattern of RAPD. They suggested that, the callus response to salt stress was due to genetic differences. Similar results were reported on rice [7].

CONCLUSIONS

It can be concluded that DNA banding pattern using RAPD analysis can discriminate between salt tolerances traits originated from genetic or epigenetic (physiologic) source. In this study, we found that some potato cultivars were both genetically salt sensitive salt tolerant. These traits can be discriminated by combination analysis using physiological parameters and RAPD marker. In this way, they can be marked as genetically or epigenetically sensitive or tolerant cultivars.

AKNOWLEDGMENTS

Authors wish to thanks the Council of Graduate Study of the University of Isfahan for their collaboration and support.

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