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IN VIVO SCREENING OF POTATO (*SOLANUM TUBEROSUM* L.) CULTIVARS FOR SALINE WATER TOLERANCE

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ABSTRACT

In vivo salt screening of six potato varieties (Asterix, Cardinal, Desiree, Kuroda, SH-5 and Sante) was undertaken in Genetic Transformation Lab, National Institute for Genomics and Advanced Biotechnology (NIGAB), National Agricultural Research Centre (NARC), Islamabad, Pakistan during the year 2014 under greenhouse conditions. Different levels of saline solution (0, 10, 20, 40, 60, 80 and 100 mM) were applied on potato. On mean basis, variety Kuroda emerged as the most salt tolerant variety with maximum plant height (48.6 cm), number of nodes (22.26), number of shoots plant^{-1} (3.47), leaf area (657.93 cm^2); tubers plant^{-1} (5.66) and tuber weight plant^{-1} (38.65 g) almost followed by SH-5. Desiree performed as moderately salt tolerant variety under salt stress. Asterix was recorded as the most salt sensitive with minimum plant height (34.06 cm), number of nodes (18.53), number of shoots (2.93), leaf area (645.00 cm^2), number of tubers (3.93) and tuber size (19.10 mm). The results revealed that growth parameters of all varieties decreased as the salt stress level increased. The most salt sensitive varieties of this study would be selected for genetic improvement of potato against salinity and drought stresses.

KEYWORDS: *Solanum tuberosum*; potato; screering; salinity; resistance; Pakistan.

INTRODUCTION

Soil salinity is one of the most overwhelming abiotic stresses that severely retard crop productivity in the world (Shrivastava and Kumar, 2015). It has very severe and negative effect on growth and development of crop plants (Ma *et al.,* 2015 and Barnawal et al. 2014). The increased use of saline water in irrigation and huge industrialization caused more salinity and it has been reported upto 5 to 7% in the entire world (Ruiz-Lozano *et al.,* 2012). According to an estimate, 14 billion hectares of arable land and almost one billion hectare of world land are salt affected in arid and semi arid areas (Aghaei, 2009). It has been reported that approximately 6% of total arable land and about 20% irrigated land in all over the world, is salinity affected (FAO, 2008). This adverse situation of salinity is increasing rapidly and by the year 2050, over 50% of total arable acreage is expected to be salinity affected (Molla *et al.,* 2015 and Ashraf, 2004). Amel *et al.* (2015) reported greater reduction in growth and yield of potato plants under saline condition. The report of Al-Hussaini *et al.* (2015) revealed great losses in crop growth and yield of potato due to salt stress. Solh and Ginkel (2014) reported that both salinity and drought are interlinked crop yield limiting stresses that co-exist in dry regions of the world. Meloni *et al.* (2003) and Allakherdiev *et al.* (2000) observed that soil salinity decreases crop production due to several alterations in plant's biochemical, molecular and metabolic activities and also it hastens several photosynthetic enzymes that cause decomposition of cell membrane and its related structures.

Salinity also greatly disturbs rate of photosynthesis, respiration and in plants causing serious yield loses (Silva *et al.,* 2001; Zhang *et al.,* 2005; Fidalgo *et al.,* 2004). The higher amount of toxic salts of sodium in soil destroys plant roots causing water shortages, deficiency of plant nutrients by disrupting ions uptake and transport. Accumulation of Na+ and Cl-1 causes ionic stress, and interfering cellular processes like cell division and chromosomal aberration leads to reduced plant growth, development and crop yield, etc. (Munns, 2002). Zhu (2007) also reported drastic yield reduction in several crop plants due to the salinity stress.

Potato (*Solanum tuberosum* L.) is an important solanaceous edible tuberous crop. It has become a cheap source of starch alongwith vitamins, proteins and minerals (Anon., 2014). Potato is the fourth most important productive food crop globally after rice, wheat and corn and eighth most cultivated crop (FAO, 2008). It is grown in about 125 countries and greater than one billion population utilizes it in its daily meal

(Mullins *et al*., 2006). It has been reported as highly salt sensitive to toxic salts of sodium and at 50 mM NaCl its growth and development reduces to 50% (Evers *et al*., 2007). Excessive accumulation of chlorine and sodium in leaves may cause toxicity and leaf burns along the margins. Potato crop is highly affected when salts are applied at the time of tuber formation (Baum and Caesar, 1990). Fidalgo *et al.* (2004) reported reduced water content, affected leaf stomatal conductance and transpiration rate in potato cultivar Desiree due to salt stress. The ultra-structure of chloroplast changed significantly that badly affected photosynthesis, consequently resulted excess starch accumulation in leaves, reduced activity of nitrate reductase and very low growth and dry matter production in tubers (Ghosh *et al.,* 2001).

The present study was conducted to screen potato varieties against salinity so that salt sensitive cultivars may be for future genetic improvement.

MATERIALS AND METHODS

This study was conducted in green house at National Institute for Genomics and Advanced Biotechnology, NARC, Islamabad, Pakistan during the year 2014. Six potato varieties i.e. Asterix, Cardinal, Challenger, Desiree, Hermis, Kuroda, Sante and SH-5 were provided by the Plant Biotechnology Program (PBP) and Horticulture Research Program (HRI) of NARC, Islamabad. Potato tubers were placed in gunny bags under a well ventilated store house at room temperature under dark and dry place for 1 to 11/2 months until sprouting occurred.

In greenhouse, ten pots for each variety with three replications were filled with peat moss, clay and sand in an equal proportion of 3:1:1 to prepare a good quality seed bed for tuber germination. The pots were saturated with equal amount of water using graduated plastic beakers. Two sprouted tubers were placed in each pot (90 cm x 30 cm) for germination under greenhouse conditions. Layout system was complete randomized design.

Salt stress was induced by using saline solution of 1M NaCl and seven salt stress levels i.e. 0, 10, 20, 40, 60, 80 and 100 mM NaCl were applied in soil mixture.
Data on various morphological growth parameters were collected and statistically analyzed in a CRD two factors (varieties and NaCl treatments) with three replications. Analysis of variance was performed using computer software Statistics 8.1 (Analytical Software 2005). The significant means of various parameters were compared by least significance difference (LSD) test.

RESULTS AND DISCUSSION

Plant height (cm)

There was a severe effect on the plant height of various potato varieties at different levels of NaCl. A negative correlation of plant height of all potato varieties with each elevated level of NaCl application was noted. ANOVA for NaCl effect on plant height of tested potato varieties revealed highly significant differences (Table 1). The influence of saline solution applied to potato pots had badly affected *in vivo* growth and development of potato varieties that responded significantly to NaCl stress. On means basis, the highest plants were produced by variety Kuroda (48.6 cm) followed by SH-5 (38.93 mm), Desiree (38.53) and Cardinal (37.66) (Table 2). Asterix stood last (34.06 cm) and was recorded as the most NaCl susceptible variety. The different levels of NaCl also produced significant effect on the plant height as no growth was recorded at 80 mM and 100 mM NaCl stress level in any potato variety within four weeks of plant cultures. It was reduced from 60.05 cm to 23.33 cm upto 60 mM NaCl i.e. maximum plant height (60.05 cm) in control and minimum (23.33 cm) in 60 mM NaCl (Table 2). Plant height in crop plants exhibits growth and development due to the proper function of photosynthesis and respiration. Their growth is retarded under any sort of environmental or induced stress. Potato being highly vulnerable to salts, this growth parameter was severely affected in present study. These findings are in strong conformity with those of Al-Hussaini *et al.* (2015) who reported reduced plant growth, increased injury to membrane stability and over all plant development in potato crop under salt stress. Similar findings were also reported by Murshed *et al.* (2015) that NaCl reduced plant height in potato plants at above 100 mM salt level. Mahmood *et. al* (2013) also reported similar effects of soil salinity on plants growth due to accumulation of Na^+ ions in leaves. Askari *et al.* (2012) further revealed reduced plant height in potato cultivar Agria due to salt stress.

Number of nodes plant^{-1}

The analysis of variance showed highly significant differences in number of nodes plant-1 among the varieties and salt levels as well (Table 1). The tested varieties of potato at various salt levels of NaCl performed differently for number of nodes plant-1. The plant growth and development of potato varieties was harshly reduced and the formation of new nodes was inversely reduced by increased level of NaCl application (Table 2). Maximum number of nodes plant-1 was noted in variety Kuroda (22.26) followed by SH-5 (21.33), Desiree (20.93) and Cardinal (20.00). Asterix (18.53) and Sante (17.60) stood last. Similarly, different levels of NaCl affected all varieties of potato with reduced nodes plant-1 (Table 2). At control level of NaCl, maximum number of nodes plant-1 (32.55) were observed against minimum number of nodes at 60 mM NaCl. So number of nodes were greatly reduced by increasing level of salinity as has been also reported by Murshed *et al.* (2015). Aghaei *et al.* (2009) investigated potato varieties that exhibited very low plant growth and development due to NaCl stress. Similar results were reported by Mahmoud *et al.* (2009) and Etehadnia (2009) that nodes plant-1 alongwith tuber yield in potato was reduced at 75 mM NaCl stress and over all weak plants were developed under salt stress.

Table 1. ANOVA for the effect of various salt stress treatments on different morphological parameters of six potato varieties.

S.O.V	Mean squares							
	D. F	Plant height	Nodes plant^{-1}	Shoots plant^{-1}	Leaf area	Tubers plant^{-1}	Tuber size	Tuber weight
Varieties	5	3.62	50.04	0.68	16928	4.83	29.30	323.56
Treatments	4	3638.66	1436.69	26.12	1694643	76.91	634.32	7821.50
Variety x NaCl level	20	40.81	11.85	0.60	26389	1.04	3.55	25.84
Error	58	4.35	2.22	0.38	694	0.63	0.46	2.64
Total	89							

Number of shoots plant^{-1}

The analysis of variance showed highly significant differences for number of shoots plant^{-1} among the varieties and for salt stress levels as well (Table 1). A harsh reduction in shoot formation of potato varieties was noted under various levels of NaCl stress during this study. The elevated levels of NaCl significantly minimized the number of shoots plant^{-1}. The accumulation of Na^+ in the leaves halts photosynthesis that resulted in reduced plant shoot growth. On mean basis, variety Kuroda developed maximum number of shoots plant^{-1} (3.47) followed by Desiree and Cardinal (3.40 each). Asterix produced minimum (2.93) number of shoots plant^{-1}. Likewise, the different levels of NaCl also affected all tested varieties in the development of number of shoots plant^{-1}. Maximum (4.83) number of shoots plant^{-1} was recorded at control level while minimum (1.77) number of shoots plant^{-1} was noted at 60 mM NaCl (Table 2).

Shoot formation in crop plants is one of the vital outcomes of growth and development due to the proper functions of photosynthesis and respiration. Their formation and growth is retarded under any sort of environmental or induced stress. Potato being highly vulnerable to salts; this growth parameter was severely affected. These results agree to the findings of Qayyum and Shoaib (2013) who reported very low growth and reduced number of shoots and branches in potato under salt stress condition.

Leaf area

In leaf area of potato varieties, great reduction was also recorded due to increased levels of NaCl stress. Formation of leaves was inversely affected by NaCl application at certain concentrations in the pots. According to mean data, maximum leaf area was recorded in (693.40 cm^2) followed by Desiree (664.93 cm^2) and Kurela (657.93 cm^2) (Table 2). Minimum leaf area was noted in Sante (595.47 cm^2) followed by Cardinal (627.2 cm^2). Different levels of NaCl salt showed highly significant differences for all tested varieties. Maximum leaf area (1046.3 cm^2) was recorded in control against minimum (300.5 cm^2) at 60 mM NaCl (Table 2). These results are in strong agreement of Murshed *et al.* (2015) and Askari *et al.* (2012) who reported reduced leaf area in potato cultivars under salt stress. Also Etehadnia (2009) studied effect of NaCl stress on potato genotypes and reported severe reduction in leaf area alongwith yield and growth parameters as well. Arvin and Donnelly (2008) also reported similar findings of leaf area reduction in potato cultivars under NaCl stress application.

Number of tubers plant^{-1}

The different levels of NaCl caused significant variations in this parameter. Based on mean values, Kuroda produced maximum number of tubers plant-1 (5.6) (Table 2). Desiree stood (5.2) second followed by SH-5 (5.0). Variety Asterix developed minimum (3.9) mean number of tubers plant^{-1}. Different levels of NaCl also significantly affected the process of tuber formation in tested potato varieties. Maximum tubers plant-1 (8.11) were noted at zero salt level and minimum (2.43) at 60 mM NaCl. Basically, roots elongated to low depth in the salinity affected soil for nutrients availability and absorption of water that results in the ultimate reduction of plant growth and low tuber formation. With increasing salinity, rooting was decreased in all cultivars and it was completely inhibited at 80 and 100 mM NaCl. This observation could be due to ion accumulation of Na^+ in the roots as well. In crops the roots were reported to be amongst the first organs that are affected by salt stress. The present results confirm the findings of Munira *et al.* (2015) and Ghosh *et al.* (2001) who reported reduced tuber yield in potato under salt stress. Kirk *et al.* (2006) reported drastic loss in yield and tuber formation in potato due to salt stress application.

Table 2. Effect of NaCl concentrations on different parameters of potato grown under *in vivo* conditions.

Variety	NaCl concentrations (mM)					
	0	10	20	40	60	Mean
(a) Plant height (cm)						
Asterix	61.3 ±2.08b	36.0 ±2.6 hi	32.0 ±2.0 lmn	25.0 ±1.5pqr	16.0±2.6s	34.06 D
Cardinal	53.6 ±4.04d	45.0 ±2.0ef	38.3 ±2.08hij	28.0 ±1.0op	23.3 ±1.5r	37.66 BC
Desiree	54.0 ±2.64d	45.3 ±2.08ef	39.6 ± 2.08ghi	30.3 ±1.52mno	23.3 ±2.51r	38.53 BC
Koruda	67.3 ±0.57a	53.6 ±2.08 d	48.0 ±1.0ef	41.0 ±1.0 gh	33.0 ±2.0 klm	48.6 A
Sante	66.3 ±1.52a	42.3 ±1.52fg	37.3 ±0.57ij	33.0 ±1.0ijk	17.0 ±1.0s	37.40 C
SH-5	57.6 ±2.51c	45.3 ±2.52f	35.0 ±2.0jkl	29.3 ±3.51no	27.3 ±2.08opq	38.93 B
Mean	60.05A	44.61B	38.38C	29.61D	23.33E	
(b) No. of nodes/plant						
Asterix	34.0 ±1.0ab	23.3 ±1.0ef	17.6 ±1.52hi	12.3 ±1.0lmn	8.0 o	18.53 C
Cardinal	31.3 ±1.52c	27.0 ±1.0d	21.0 ±1.0fg	14.6 ±1.52 jkl	8.0 ±1.52o	20.00 B
Desiree	30.3 ±2.08c	21.3 ±2.08fg	18.0 ±hi	14.6 jkl	10.6 n	20.93 B
Kuroda	36.0 a	31.6 bc	18.0 hi	15.0 jk	10.6 n	22.26 A
Sante	31.3 c	21.0 fg	15.6 ijk	12.0 mn	8.0 o	17.60 CD
SH-5	32.3 bc	24.0 e	2.0 gh	16.6 ij	13.6 klm	21.33 B
Mean	32.55A	24.72B	18.38C	14.22D	9.83E	
(c) No. of shoots/plant						
Asterix	5.3 a	3.6 cdef	2.3 ghij	2.0 hij	1.3 j	2.933 B
Cardinal	5.3 a	4.3 abcd	3.6 cdef	2.3 ghij	1.3 j	3.400 A
Desiree	5.0 ab	3.6 cdef	3.3 defg	2.6 fghi	2.3 ghij	3.400 A
Kuroda	5.0 ab	4.6 abc	3.6 cdef	2.3 ghij	1.6 ij	3.466 A
Sante	4.3 abcd	3.6 cdef	3.0 efgh	2.3 ghij	2.0 hij	3.066 AB
SH-5	4.0 bcde	3.6 cdef	3.3 defg	3.0 efgh	2.0 hij	3.200 AB
Mean	4.83A	3.94B	3.22C	2.44D	1.77E	
(d) Leaf area (cm^2)						
Asterix	1010.7 c	885.7 f	621.3 k	433.7 o	273.7 u	645.00 D
Cardinal	006.3 c	808.3 h	584.0 m	421.3 p	316.3 s	627.27 E
Desiree	1032.0 b	886.0 f	622.3 k	435.7 o	348.7 r	664.93 B
Kuroda	1180.7 a	953.3 e	640.3 j	307.7 t	207.7 v	657.93 C
Sante	866.0g	616.3 k	595.0 l	520.0 n	380.0 q	595.47 F
SH-5	1186.0 a	971.7 d	652.0 i	380.7 q	276.7 u	693.40 A
Mean	1046.9 a	853.6 b	619.2 c	416.5 d	300.5 e	
(e) No of tubers/plant						
Asterix	8.0 ab	4.0 ghij	3.3 ijk	2.6 klm	1.6 m	3.93 C
Cardinal	8.0 ab	5.0 defg	4.6 efgh	4.3 fghi	2.6 klm	4.93 B
Desiree	8.3 a	5.0 defg	4.6 efgh	4.3 fghi	3.6 hijk	5.20 AB
Kuroda	8.3 a	7.0 bc	5.3 def	4.6 efgh	3.0 jkl	5.66 A
Sante	8.0 ab	6.0 cd	4.6 efgh	3.3 ijk	2.6 klm	4.93 B
SH-5	8.0 ab	6.0 cd	5.6 de	3.3 ijk	2.0 lm	5.00 B
Mean	8.11 a	5.50 b	4.66 c	3.83 d	2.61 e	
(f) Tuber size/plant (mm)						
Asterix	34.0 a	21.2 f	13.9 no	14.6 lmn	11.7 p	19.10 C
Cardinal	31.8 b	22.0 f	17.2 ij	15.5 kl	14.3 lmno	20.20 B
Desiree	30.8 b	24.0 e	18.6 gh	17.1 ij	15.4 klm	21.23 A
Kuroda	31.1 b	23.7 e	18.5 gh	15.2 lm	13.1 o	20.39 B
Sante	25.8 d	19.6 g	17.7 hij	14.7 lmn	14.2 mno	18.43 D
SH-5	28.3 c	22.2 f	18.0 hi	16.6 jk	14.7 lmm	19.99 B
Mean	30.34 a	22.17 b	17.35 c	15.64 d	13.95 e	
(g) Tuber weight/plant (g)						
Asterix	69.33b	37.66f	33.66h	22.00jk	15.66 m	35.067 B
Cardinal	63.66c	34.00 g	29.517 ij	23.50 jk	13.48 mn	31.247 C
Desiree	70.00b	40.98 e	32.00 gh	26.00i	22.00 jk	38.197 A
Kuroda	74.66 a	49.00 d	27.00 i	22.00jk	20.61kl	38.655 A
Sante	62.00 c	38.33 ef	23.33j	21.33jkl	11.237n	27.247 E
SH-5	63.66 c	34.00 g	22.00 jk	18.68l	11.363 n	29.943 D
Mean	67.22 A	38.99 B	24.75 C	20.58 D	15.402 E	

Tuber size (mm)

The ANOVA depicted highly significant differences for tuber size in the envisaged potato varieties under NaCl stress (Table 1). Tuber size was also significantly reduced in all tested varieties by increasing the stress level of NaCl from 10-60 mM NaCl. The tuber size reduction in the tested potato varieties showed negative impact of NaCl at higher concentrations

(Table 2). Based on mean, Desiree produced maximum tuber size plant^{-1} (21.23 mm) followed by Kuroda (20.39mm) and Cardinal (20.20mm) (Table 2). SH-5 also developed (19.99 mm) tubers upto the same level. Asterix and Sante produced minimum (19.10 and 18.43 mm) tuber size (Table 2). There was a highly significant effect of different levels of NaCl on tuber size. Tuber size was markedly reduced from 30.34 mm to 13.95 mm at control and 60 mM NaCl concentration (Table 2). Moreover, significant differences were deserved among the NaCl levels.

The output of optimum growth and development is the proper and normal size of tubers. Tuber size is mainly influenced by the growth related factors like photosynthesis, respiration rate, transpiration and movement of nutrients in the plant. The normal supply of nutrients is retarded under salt stress condition. The plant cannot get even available nutrients from soil if the amount of NaCl is more in soil. Present results are in strong conformity with Shateriana *et al.* (2005) who found 42% reduction in tuber size and over all tuber yields by NaCl application at 150 mM. Also Patella *et al.* (2001) and Tan *et al.* (2000) unanimously reported severe loss in tuber size of potato under salinity stress.

Tuber weight (g)

The analysis of variance (ANOVA) displayed highly significant results for tuber weight in the assessed potato varieties under NaCl stress (Table 1). The application of NaCl at various concentrations developed a negative impact as presented in Table 2. Tuber weight of all investigated potato varieties was drastically affected due to application of saline water to induce salt tolerance. At each elevated stress level of NaCl a highly significant deduction in tuber weight of potato varieties was recorded. On the basis of mean, Kuroda and Desiree produced maximum tuber weight plant^{-1} (38.65g and 38.19 g) followed by Cardinal (35.06 g). Asterix showed the least tolerance to NaCl application and developed 27.24 g mean tuber weight. Higher NaCl levels reduced the tuber weight significantly in all investigated potato varieties. The potato plants at control level produced maximum (67.22 g) tuber weight plant^{-1} whereas, more than 50% reduction was recorded at 10 mM and 20 mM NaCl level. At 40 mM NaCl further reduction of 69% was noted and at 60 mM NaCl 78% reduction was recorded in tuber weight. These findings agree to those of of Ghosh *et al.* (2001) who reported harsh potato yield reduction and reduced tubers plant^{-1}due to salt stress.

CONCLUSION

The i*n vivo* produced potato plants in the plastic pots were very easy to asses against salinity stress. Kuroda emerged as most salt tolerant variety among evaluated potato varieties. SH-5 and Desiree performed as moderately NaCl tolerant varieties. Asterix and Sante evolved as most NaCl vulnerable potato varieties in this investigation.

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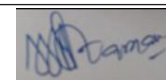
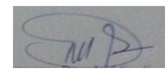
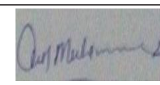
COUNTRIBUTION OF AUTHORS

S. No.	Name of author	Contribution	Signatures
1.	Muhammad Shah Zaman	Major author, conceived the idea, supervised the research work	
2.	Ghulam Muhammad Ali	Provided technical inputs in describing the results	
3.	Aish Muhammad	Prepared research proposal and designed salt stress levels	
4.	Iqbal Hussain	Statistically analysed the data