Research Article

In vitro Screening of Salt Tolerance in Potato (*Solanum tuberosum* L.) Varieties

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Abstract | Salinity is one of the major abiotic constraints that severely affect the productivity of agricultural crops in arid and semiarid regions. *In vitro* screening of eight potato varieties (Asterix, Cardinal, Challenger, Desiree, Hermis, Kroda, Sh-5 and Sante) was investigated for salt tolerance at 0.0, 10, 20, 40, 60, 80, 100 mM NaCl supplemented with MS medium. Kroda emerged as the most salt tolerant variety with the highest plant height (6.5 cm), number of nodes (8.8), fresh shoot weight plant^{-1} (0.166 g), number of roots (4.6) and root length (2.5 cm) at 60 mM NaCl followed by Sh-5. Desiree and Cardinal were moderately tolerant varieties to NaCl stress. The most sensitive variety Asterix produced minimum plant height (2.7 cm), number of nodes (2.4), number of roots (2.6), root length (0.7 cm), root weight (0.021 g) and shoot weight (0.045 g) at 20 mM NaCl. The most salt sensitive potato varieties evaluated in this study would be used in *Agrobacterium*-mediated gene transformation of *AtNHX1* to improve their tolerance against salinity stress.

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Introduction

Salinity is one of the major abiotic constraints that severely affect productivity of agricultural crops especially in arid and semiarid regions. Salt stress limits yield of crops by affecting the metabolism of plants and causes important modification in different biochemical and molecular processes (Allakhverdiev et al., 2000). It can activate certain photosynthetic enzymes activity causing decomposition of membrane structures (Meloni et al., 2003). Rate of photosynthesis and respiration in crop plants is severely interfered causing reduced plant growth and low productivity at high salts (Silva et al., 2001; Zhang et al., 2005; Fidalgo, 2004). Higher level of salinity disrupts plant roots making water deficiency, nutrients imbalance by altering uptake and transport, ionic stress by higher Na^+ and Cl^- accumulation, cell membrane ineffectiveness and interfering cellular processes like cell division and genotoxicity as well resulting in reduced plant growth, development and yield (Munns, 2002). Severe yield reduction in many crops has also been reported by Zhu (2007). FAO suggested that approximately 6% of the world's total arable and 20% of irrigated land is affected by high salinity (FAO, 2008). Salinity and drought are interlinked factors occurring simultaneously in the green sector that's why the problem of salinity exists in the dry regions of the world (Solh and Ginkel, 2014).

Potato (*Solanum tuberosum* L.) is a solanaceous crop

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and it is considered as an important edible tuberous and starch rich crop. Potato is the 4th most important productive food crop globally after rice, wheat and corn and 8th most cultivated crop (FAO, 2008). It is a cheap source of vitamins, proteins, carbohydrates and minerals (Anonymous, 2014). Due to greater production of potato, is has become an essential crop to lessen food shortages. In Pakistan, its production has been enhanced radically, but still it is not at par with the world. Due to favorable agro-ecological zones, three crops can be successfully grown in plain and hilly areas of Pakistan. Among these three crops, the autumn crop contributes to 70% of the total potato area however; its production is limited by high levels of salt greater than 50 mM NaCl retarding shoot growth and tuber yield (Mahmoud et al., 2009). It is considered as moderately sensitive to salinity that adversely affects its growth and yield (Saif-Ur-Rasheed et al., 1998, Katerji et al., 2003). In previous studies, it has been reported that late tuberization, mal development of leaves, slow rate of tuber filling and small size potatoes were resulted at and above 80 mM NaCl (Zhang et al., 2005; Sanchez et al., 2003; Silva et al., 2001).

Among 21.16 million hectares of cultivated land, 6.68 million hectares have been reported as saline or saline sodic (Anonymous, 1998).There is an increase in salinity affected areas year after year due to drought (Hussain et al., 2012). Researchers have conducted various studies to resolve the problems of salinity in agricultural areas by irrigation with fresh water and preventing soil drainages by improvement of water courses. These practices are too expensive that cannot be adopted by poor and small land holders. In Pakistan it has been reported as more than 6.17 Mha are salt affected that affected agriculture production (Government of Pakistan, 2009-2010). The complex genetic makeup of potato being autotetraploid and polygenic quantitative inheritance makes it tricky to develop a variety for these abiotic stresses. *In vitro* techniques and micropropagation are very rapid and modern way for evaluation of potato cultivars for salt stress (Byun et al., 2007). Plant tissue culture techniques in assistance with conventional breeding and biotechnology have become latest approaches for making crop plants tolerant to environmental stresses especially for salt stress (Rahman et al., 2008). The study of plant salt tolerance to identify crop sensitivity seems to be a fruitful and short time approach (Zhu, 2007).

The objective of this study was to differentiate salt tolerant and salt sensitive varieties of potato to improve them for genetic improvement through transformation of salt tolerant gene and mass production through *in vitro* multiplication. The tolerance level of tested varieties was scrutinized on the basis of various growth parameters after the application of different NaCl concentrations.

Material and Methods

Eight varieties of potato namely; Asterix, Cardinal, Challenger, Desiree, Hermis, Kroda, Sante and Sh-5 were obtained from Plant Biotechnology Program (PBP) and Horticulture Research Institute (HRI), National Agricultural Research Centre (NARC), Islamabad.

Tuber germination

The seed dormancy of tubers was broken at 4°C by dipping tubers in 60 ppm GA_3 solution for 20 minutes. Then the tubers were placed under dark condition for sprouting. Sprouted tubers were placed in plastic bags filled with fertile and moist soil for germination under 35-37°C for six to eight weeks.

Explant sterilization and excision

The shoot tips with one or two leaf foliage were excised from the grown plants using surgical blades in double distilled water. They were surface sterilized by putting them in 70% ethanol for 1 minute. Then they were immersed in Clorox (1% sodium hypochlorite) for 10 minutes and washed with double distilled water (3-4 times) for 10 minutes.

Media preparation

Murashige and Skoog (1962) media was supplemented with 1 mg l^{-1} of GA_3, 8g agar, 30g sucrose and pH was adjusted at 5.8. Then the media was autoclaved at 121°C and 15 psi pressure for 15-20 minutes.

Culturing of nodal segments

The shoot tips and the nodal segments containing one or two leaves were excised by a sterilized scalpel and forceps under stereomicroscope in a laminar flow cabinet. They were inoculated in test tubes containing above mentioned MS media. These cultures were incubated in a growth room with 25±2°C under 2000 lux of fluorescent light with 16 hours photoperiod for regeneration.

Micropropagation

Micropropagation was carried out using Murashige and Skoog (MS) medium supplemented with 1mg l^{-1} of GA_3, 2mg l^{-1} IBA along with the 8 gl^{-1}agar and

30g l^{-1} sucrose while pH was adjusted at 5.8. The *in vitro* developed plants after 3-4 weeks of growth were propagated by sub culturing nodal cuttings on this media and the cultures were incubated in growth room. When the cultures reached the top of test tubes within 3-4 weeks they were multiplied for mass production of *in vitro* developed plants for further study.

In vitro screening of potato for salt tolerance

The eight potato varieties were screened for salt tolerance using *in vitro* multiplication at various concentrations of NaCl supplemented with MS media. The stem cuttings (nodal segments) were used as explants. The cultures were incubated in the growth room at 25±2°C under 2000 lux of fluorescent light with 16 hours photoperiod for 4 weeks. Each experiment was consisted of three replications and repeated two times. The following salt treatments were applied as salt stress.

Table 1: *Various salt treatments applied as salt stress for screening 8 potato genotypes*

Treatments	Composition
T_1	MS Basal Media without NaCl (control)
T_2	10 mM NaCl in MS Media
T_3	20 mM NaCl in MS Media
T_4	40 mM NaCl in MS Media
T_5	60 mM NaCl in MS Media
T_6	80 mM NaCl in MS Media
T_7	100 mM NaCl in MS Media

Growth and yield characteristics

The plant height of 10 randomly selected plants was measured from the lower part of the plantlet to the shoot tip and mean was recorded. The number of nodes plant^{-1} of 10 plants was counted as well as their mean was recorded. The 10 selected plants were taken out from the test tubes and washed to remove media, and after drying on filter paper the roots of each plant were counted and mean was recorded as well. The root length plant^{-1} of 10 plants was measured and their mean was recorded for statistical analysis and interpretation as well. The fresh roots of 10 selected plants were detached from the shoot by using scalpel and the average weight was recorded by electric balance. The fresh shoots of 10 randomly selected plants were weighed by using an electronic balance, averaged and recorded as fresh shoot weight.

Statistical Analysis

Data were analysed according to analysis of variance using computer software Statistics 8.1 (Analytical Software 2005). Means were compared with the help of least significant difference (LSD) test. Various morphological parameters were presented in the ANOVA Table 2.

Results and Discussion

The plants grown on MS media containing different concentrations of NaCl (Table 1) exhibited significant differences in all studied characters of growth parameters. The growth parameters were negatively affected by different levels of NaCl in MS media. No growth was observed in any variety at 80 mM and 100 mM NaCl, however the explants remained green for more than 4 weeks without exhibiting new growth and development. The analysis of variance (ANOVA) for the studied parameters is presented in Table 2.

Plant height

Plant height was greatly affected by NaCl at different concentrations (Table 1) application *in vitro.* Highly significant differences were recorded among varieties for plant height (Table 2). Kroda showed greatest tolerance amongst the tested varieties to salt stress up to 60 mM NaCl with maximum plant height (6.5 cm) followed by Sh-5 (5.7 cm). Desiree produced 4.8 cm tall plantlets and Cardinal 4.5 cm tall plants and were among the moderately sensitive varieties for this parameter. Asterix emerged as the most salt sensitive variety with 2.7 cm tall plantlets up to 20 mM NaCl followed by Challenger with 2.9 cm plant height (Figure 1a).

Table 2: *ANOVA for the effect of various salt stress treatments on different morphological parameters of eight potato genotypes*

S.O.V.		Mean squares					
	d.f.	Plant height	Nodes plant^{-1}	Roots plant^{-1}	Root length	Shoot weight	Root weight
Varieties	7	38.123	97.2718	55.784	14.9316	0.02738	0.00564
Treatments	6	109.914	94.5163	160.942	33.5112	0.04182	0.03855
Variety x Treatment	42	4.084	5.9885	10.842	33.5112	0.00704	0.00257
Error	10	0.408	1.0187	2.877	0.4744	0.00136	0.00066
Total	**167**						

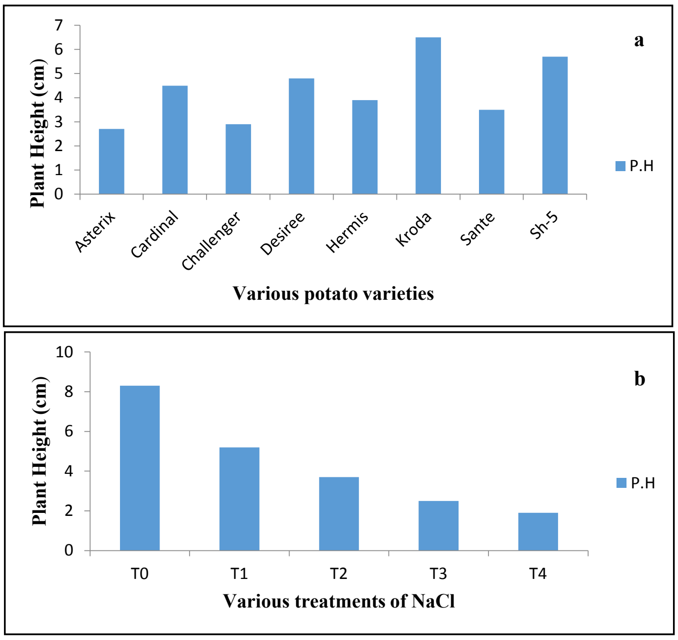

Figure 1: a. *Effect of various treatments of NaCl on plant height of in vitro potato plantlets;* **b**. *Effect of various treatments of NACL on plant height of in vitro potato plantlets*

Salt treatments expressed significant differences among the tested potato varieties. Plant height of all tested varieties was gradually reduced from 8.3 cm to 1.9 cm by increasing NaCl from 0 to 60 mM. Maximum plant height was observed at control (8.3 cm) and minimum (1.9 cm) was observed at 60 mM NaCl. Plant height was reduced to 5.2 cm at 10 mM, 3.7 cm at 20 mM and further to 2.5 cm at 40 mM NaCl stress (Figure 1b). Our findings were in strong conformity with Sudhersan et al. (2012) who reported reduced shoot growth in potato *in vitro* study due to salt stress by increasing salt concentration in MS media from 750 - 4000 ppm. The findings of Rahman et al. (2008) also reported reduced plant shoot length at 75 and 100 mM of NaCl in MS media. Aghaei et al. (2009) also reported the reduced shoot length in potato up to 90 mM and 120 mM NaCl in his two separate studies. Our results are in strong accordance with the findings of Zhang and Donnelly (1997) who found low growth and development in potato at 75 mM NaCl fortified in MS media.

Number of nodes plant^{-1}

In present study nodes development was severely affected by different levels of NaCl (Table 1). The varieties responded differently to nodes development and showed highly significant differences to this parameter (Table 2). Kroda produced maximum number of nodes plant^{-1} (8.8) followed by Sh-5 with 6.5 nodes plant^{-1} up to 60 mM NaCl. Likewise, Cardinal and Desiree displayed moderate level of tolerance with 5.6 and 5.2 nodes plant^{-1} respectively up to 60 mM NaCl. On the other hand, Asterix responded as most sensitive variety with 2.4 nodes up to 20 mM NaCl concentrations followed by Challenger (2.5) nodes plant^{-1} at the same level (Figure 2a).

Different levels of salt NaCl showed highly significant differences among the tested genotypes. Maximum number of nodes plant-^{1} (8.6) was studied at control and minimum number nodes plant^{-1} (2.8) was noted at 60 mM NaCl. Increasing the level of NaCl in MS media to 10 mM, number of nodes plant^{-1} was reduced from 8.6 to 6.3, whereas 4.4 nodes plant^{-1} were observed at 20 mM and 3.4 nodes plant^{-1} were studied at 40 mM NaCl (Figure 2b). Our results were in compliance with the findings of Aghaei et al. (2009) who reported white Desiree potato as moderately tolerant to salt stress, and all tested potato varieties showed overall stunted growth due to salt stress. The internodes plant^{-1} and tuber yield in potato was also reduced at 75 mM NaCl in an *in vitro* study performed by Mahmoud et al. (2009). Etehadnia (2009) study also supported our findings who reported weak plant growth and yield reduction in potato due to salt stress.

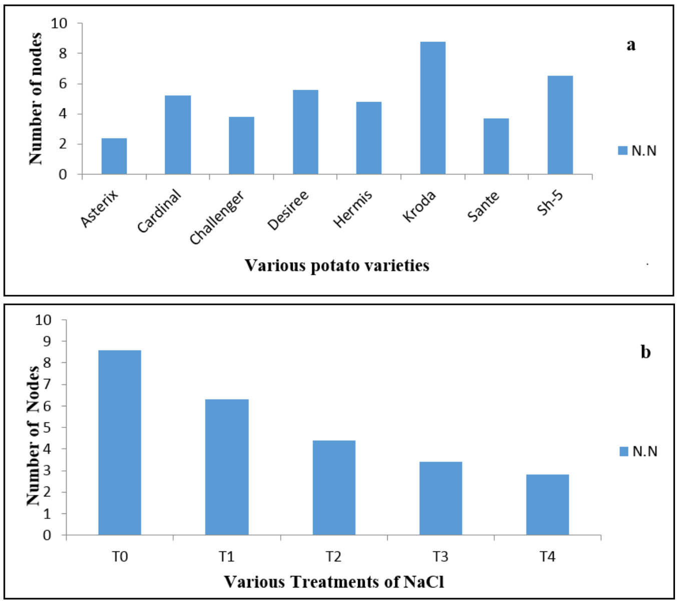

Figure 2: a. *Effect of various treatments of NaCl on the number of nodes plant^{-1} of in vitro potato plantlets;* **b**. *Effect of various salt treatment of NaCl on number of nodes plant^{-1} of in vitro potato plantlets*

Number of roots plant^{-1}

Number of roots plant^{-1} was also badly affected by NaCl stress supplemented in MS media (Table 1). The studied potato varieties also depicted highly significant to number of nodes plant^{-1} (Table 2). The highest number of roots (6.1) was noted in Sh-5 followed by Kroda (4.6). Desiree, Cardinal and Challenger developed 4.3, 4.1and 4.0 roots plant^{-1} respectively. Least mean number of roots plant^{-1} (2.6) was studied in Asterix followed by Hermis with 3.0 number of roots plant^{-1} (Figure 3a).

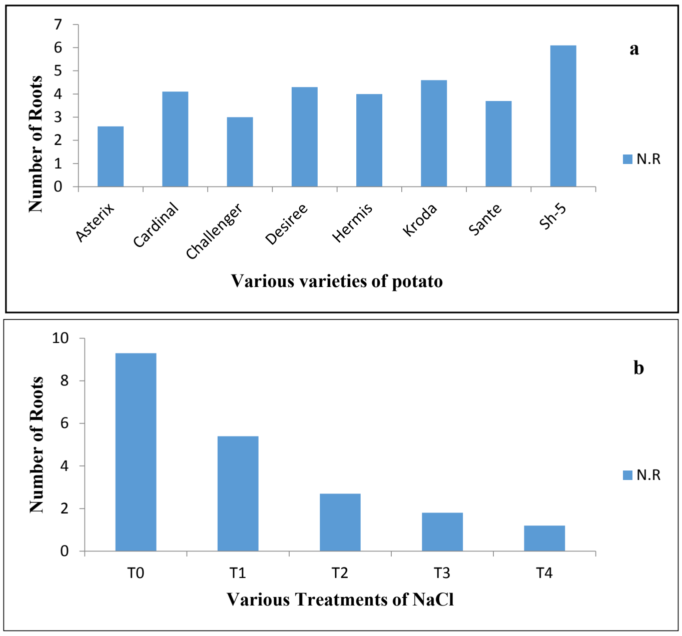

Figure 3: a. *Comparison for the effect of various treatments of NaCl on the number of roots plant^{-1} of in vitro potato plantlets;* **b**. *Effect of various treatments of NaCl on number of roots plant^{-1} of in vitro potato plantlets*

Highly significant differences were found for number of roots plant^{-1} at different levels of NaCl (Table 1). The maximum number of roots plant^{-1} (8.3) was recorded at control and minimum number of roots plant^{-1} (1.9) at 60 mM NaCl. The *in vitro* plantlets produced 5.2 roots plant^{-1} at 10 mM NaCl, likewise at 20 mM number of roots plant^{-1} were 3.7 and at 40 mM only 2.5 number of roots plant^{-1} were recorded (Figure 3b). Study of Sudhersan et al. (2012) is in strong compliance of our findings who reported reduced roots plant^{-1} in potato varieties by increasing salt in MS media from 750 - 4000 ppm. Farhatullah et al. (2002) reported that the studied potato varieties failed to develop roots even at 1% NaCl added in MS media. Parakash et al. (1993) findings were in agreement of our results also reported poor root development and inhibited plant growth in potato at and above 100 mM NaCl. Ewers et al. (1999) also studied depressed rooting plant^{-1} in potato due to salt stress applied in the MS media.

Root length

The NaCl in MS media caused drastic effect on root length of different potato cultivars. The maximum root length (3.1 cm) was observed in Sh-5 followed by Kroda (2.5 cm) up to 60 mM NaCl. In Desiree and Cardinal 2.3 cm and 2.1cm root length was studied respectively. Asterix emerged as one of the most salt stress vulnerable potato variety with 0.7 cm root length followed by Challenger (1.4 cm) as presented in Figure 4a. Highly significant results were studied in this character (Table 2).

Highly significant differences were observed at different levels of NaCl for root length. Maximum root length (3.6 cm) was observed at control and minimum (0.9 cm) was studied at 60 mM NaCl. Here root length was found the most sensitive to salt stress from 10 to 40 mM NaCl (Figure 4b). Our findings were in strong conformity with the assessments of Parakash et al. (1993) who described severely reduced root length in all tested potato cultivars by increasing salt treatments up to 75 mM NaCl. The investigation of Rahman et al. (2008) was similar to our study who reported reduced plant root length at 75 and 100 mM NaCl. Sudhersen et al. (2008) also envisaged reduced rooting in potato due to NaCl addition in MS media from 750 - 4000 ppm.

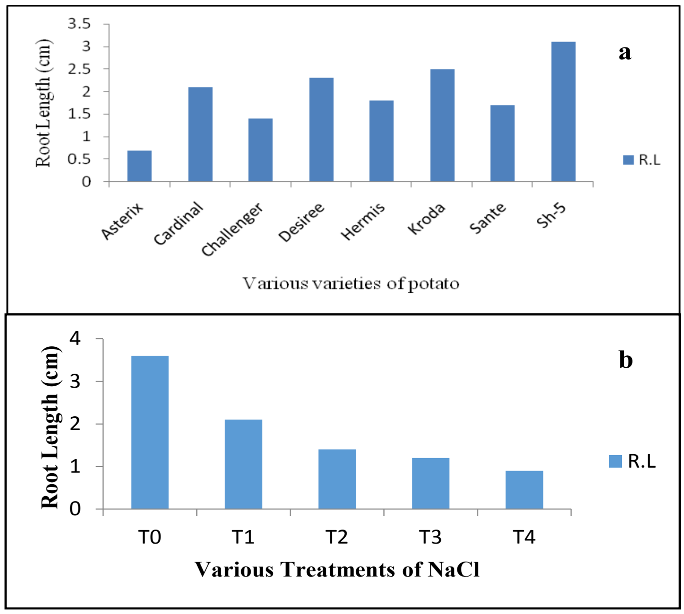

Figure 4: a. *Comparision for the effect of various salt concentrations on shoot weight plant^{-1} of in vitro potato plantlets;* **b**. *Comparison for the effect of various treatments of NaCl on root length of in vitro potato plantlets*

Fresh root weight plant^{-1}

Fresh root weight of *in vitro* plants was affected by various levels of NaCl in MS media (Table 1) for regeneration of nodal cuttings. The investigated varieties produced highly significant differences at 0.05 % level of probability (Table 2). Sh-5 produced the maximum fresh root weight (0.051 g), whereas Kroda displayed the 2nd highest fresh root weight (0.046 g). Being moderately sensitive to salt stress Desiree produced fresh root weight plant^{-1} (0.039 g), whereas Cardinal and Sante both expressed same fresh root weight (0.025 g). Asterix showed minimum fresh root weight (0.016 g) again performed as the most salt stress vulnerable potato variety (Figure 5a).

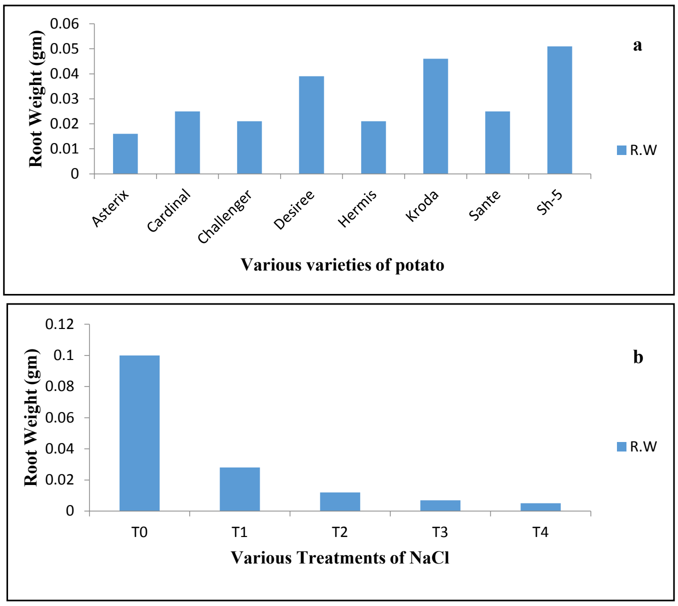

Figure 5: a. *Comparison for the effect of various salt concentrations on root weight of in vitro plantlets;* **b**. *Comparison for the effect of various treatments of NaCl on root weight plant^{-1} of in vitro potato plantlets*

Highly significant differences were observed at different levels of NaCl. Maximum fresh root weight plant^{-1} (0.10 g) was observed at control and minimum (0.005 g) was recorded at 60 mM NaCl. Fresh root weight plant^{-1} was severely reduced by increment of NaCl stress level in MS media. At 10 mM NaCl, 0.028 g fresh root weight was recorded, likewise at 20 mM NaCl (0.012 g) and at 40 mM NaCl fresh root weight plant^{-1} (0.007 g) was observed (Figure 5b). Our results were in strong acquiescence with findings of Parakash et al. (1993) who revealed limited rate of root weight plant^{-1} due to increased salt level of NaCl in MS media. Significantly reduced root mass was reported in potato at 75 mM and 100 mM NaCl in MS media by Rahman et al. (2008). Also severe loss of root weight in potato cv. Agria was reported by increasing NaCl stress (Table 1) in MS media from 50 to 150 mM by Askari et al. (2012).

Fresh shoot weight plant^{-1} (g)

In our study, fresh shoot weight of *in vitro* produced plants was reduced severely by application of NaCl stress in MS media for regeneration of nodal cuttings. Due to the effect of salt highly significant results were recorded (Table 2). The maximum shoot weight plant^{-1} (0.166 g) was recorded in Kroda up to 60 mM NaCl. Sh-5 displayed the 2nd highest shoot weight plant^{-1} (0.109 g). Being moderately sensitive to NaCl stress, Desiree and Cardinal produced shoot weight plant^{-1} (0.081 and 0.080 g) respectively. Asterix exhibited shoot weight plant^{-1} (0.045 g), while Sante produced shoot weight plant^{-1} (0.032 g) and Challenger with shoot weight plant^{-1} (0.024 g) emerged as the most NaCl stress vulnerable potato varieties (Figure 6a).

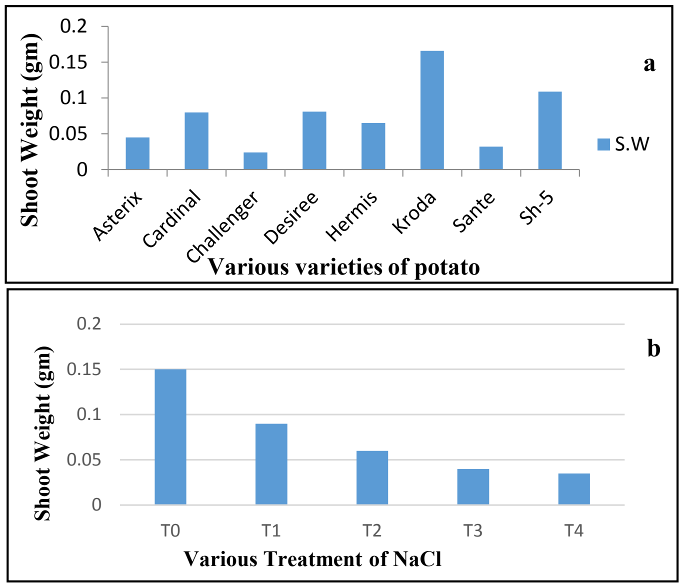

Figure 6: a. *Comparison for the effect of various salt concentrations on shoot weight plant^{-1} of in vitro potato plantlets;* **b**. *Effect of various treatments of NaCl on shoot weight plant^{-1} of in vitro potato plantlets*

The different levels of NaCl (Table 1) also expressed highly significant differences for fresh shoot weight plant^{-1}. Maximum shoot weight plant^{-1} (0.142 g) was observed at control, while minimum (0.041 g) was observed at 60 mM NaCl. Shoot weight plant^{-1} was reduced to 0.087 g at 10 mM NaCl, whereas fresh shoot weight plant^{-1} (0.059 g) was found at 20 mM NaCl and fresh shoot weight plant^{-1} (0.045 g) was measured at 40 mM NaCl (Figure 6b). Similar findings were reported by Rahman et al. (2008) who reported reduced shoot mass of potato varieties Shepody, Atlanta and Shibilaty at 75 mM and 100 mM NaCl in MS media. A reduced fresh shoot weight plant^{-1} and dry shoot weight plant^{-1} was revealed in different potato varieties at 90 - 120 mM NaCl by Aghaei et al. (2009).

The findings of Pour et al. (2010) also supported our results who studied fresh shoot weight reduction in the tested potato cultivars by increasing NaCl level from 25 to 100 mM. An adverse affect on fresh shoot weight plant^{-1} of *in vitro* potato was envisaged by Farhatullah et al. (2002). Similar findings were furnished by Askari et al. (2012) who recorded reduced fresh shoot weight in potato cultivar Agria by increasing NaCl in MS media from 50 to 150 mM.

Conclusions

In vitro screening was concluded as more suitable and short time practice that could be carried out in the laboratory round the year. Single nodes from *in vitro* grown plantlets were found as cheap explant source than that of tubers for salt screening. Kroda evaluated as salt tolerant variety followed by Sh-5. Desiree and the Cardinal were assessed as the moderately tolerant to salt stress of NaCl. Asterix was recorded as the most salt susceptible potato variety followed by Challenger and Sante.

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