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Evaluation of Indigenous Potato Challisha (*Solanum tuberosum* L. Cv. Challisha) Somaclonals Tolerance to Salinity *In Vitro*

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ABSTRACT

Potato is one of the most important food crops in the world. It is generally sensitive to salinity and likes to grow in neutral soil. On the other hand, salinity is increasing alarmingly in the ever changing climatic conditions. Thus, the selection of salt tolerant potato cultivars is necessary to keep pace the production of potato. To select salt tolerant cultivars, here we attempt to compare the salinity level between indigenous and modern cultivars. *In vitro* selection of local and modern potato cultivars were investigated with five levels of NaCl (0, 30, 60, 90 and 120 mM). The indigenous potato Challisha and modern cultivars Diamant and Felsina were used as plant materials. Significant differences were noticed among the cultivars in response to different levels of NaCl. Plant growth and root development were gradually reduced with increased concentration of NaCl. All three cultivars were survived well with exhibiting different growth status up to 60 mM NaCl, but they performed poorly at 120 mM of NaCl. Cultivar Challisha performed better regarding shoot length, root length, the number of nodes per plantlet and the fresh weight per plant up to 90 mM of NaCl. Thus, we can conclude that local indigenous variety Challisha is salt tolerant comparing with the modern cultivated varieties.

Keywords: *Indigenous potato cultivars, salinity, tolerant*

INTRODUCTION

Plants are regularly challenged by various biotic and environmental stresses in the natural environment [1]. Environmental stresses, such as drought, salinity, coldness, high temperature and heavy metals disturb the metabolism of plants. Salt stress is affecting the metabolism of plants and causes a modification in different biochemical and molecular processes which limit yield of crops [2]. Besides, plants can sense the changes of environmental conditions and regulate their gene expression to adjust their metabolism to survive. Generally, plants are stressed in three ways in saline soils; i) low water potential of the root medium leads water deficit, ii) the toxic effects of the Na+ and Cl- and iii) nutrient imbalance by depression in uptake and/or shoot transport [3, 4]. Toxic accumulation of Na+ and Cl- in the leaves has also been correlated with stomatal closure and reduction of total chlorophyll content in leaves [5].

Cell and tissue culture techniques together with conventional breeding and genetic engineering have been considered as the potential approaches for the development of plants with increased tolerance to environmental stresses [6]. The outstanding selection of mutant lines from cultured cells and the regeneration of whole plants from such cells have stimulated many attempts for the development of salt-tolerant plants [7]. A small number of potato genotypes have been reported in salinity tolerance under outdoor, greenhouse or in vitro conditions. Field trials [8, 9, 10, 11] and greenhouse pot trials were used to examine salinity tolerance genotype under NaC1 or sodium salt irrigation solutions based on either tuber yield [12, 13], a relative reduction of foliage dry weight [14] or haulm fresh weight [15]. Conventional screening methods for the development of salt tolerant variants are laborious and time-consuming [16]. Whereas in vitro screening of potato was used successfully against various about

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stresses. For example, sensitive and tolerant varieties of potato were screened under drought stress and found that var. Maxi was tolerant from the 18 tested varieties [17]. In vitro tested of 25 potato cultivars to salinity and found that 7 cultivars were tolerant and the remaining were sensitive [18]. Kroda potato variety showed salt tolerant among the 8 varieties screened out for salt tolerant [6]. To find out the tolerant salinity among the 10 potato varieties [19] Munira et al. (2015) found that Felsina was relatively salt tolerant, while Diamant was a moderately salt tolerant [20, 18]. However, the local variety Challisha was not tested its tolerant to salinity in vitro compared with the other cultivated potato variety. For this purpose, the study was conducted to compare the salinity tolerant levels of the indigenous variety Challisha among the selected three potato cultivars.

Potato is glycophyte plant and classified as moderately salt tolerant to moderately salt sensitive compared with other crops [21]. Considerable salt stress resistances were found in the wild diploid Solanum potato relatives [7]. Despite the importance of potato, whole plant salt resistance mechanisms in diploid potato have not been extensively studied [22]. Selection of potato cultivars under different salt stress helps to develop an efficient screening technique of salinity resistant potato lines and to evaluate salt-tolerant cultivars effective for future use under salinity conditions [15]. Therefore, the main purpose of this study is to compare the salinity tolerant of the original cultivar in vitro compared with the modern potato varieties.

MATERIALS AND METHODS

Plant material

The potato varieties were collected from the Tuber Crop Research Centre of Bangladesh Agriculture Research Institute, Gazipur. The Institute morphologically characterized that Challisha (V_1) is a less cultivated local variety and Diamant (V_2) and, Felsina (V_3) are modern cultivated variety based on their yield performance.

Tuber germination and explants preparation

We made a slight modification of [6] Zaman et al. (2015) and [25] Rahman et al. (2008) for tuber germination and explant preparation. Surface sterilization of the 3 cultivars were maintained at room temperature under total darkness for sprouting. The shoot buds were removed from the sprouted tubers using a sterile surgical knife. Then surface sterilized with 5% sodium hypochlorite with a drop of Tween 20 for 15 min. After 3-4 times washing in sterile distilled water, the shoot buds were treated with 70% ethyl alcohol for one minute and washed 3 times in sterile distilled water. The shoot meristems with a single node was isolated from the surface sterilized shoot buds under the laminar air flow cabinet.

Media preparation

Murashige and Skoog (1962) medium contained 3% sucrose and solidified with 0.8% agar and pH was adjusted at 5.8 [23]. The medium was adjusted to 5.8 and autoclaved at 121°C for 20 min. All the cultures were incubated at 25 ± 2°C with 16/8 h D/N cycle at the cool white fluorescent light [6].

Salt stress treatments

The levels of salt concentrations were selected from Murshed et al. (2015) with slight modifications [20]. Salt stress was assessed by transferring single nodes explant to medium containing five concentrations of NaCl (C0-0 mM, C1-30 mM, C2-60 mM, C3-90 mM and C4-120 mM) with four replications. The experiment ended in four weeks, and the growth response was noted on the root length, the shoot length, the number of nodes, the number of leaves, the number of root and fresh weight.

Statistical analysis

The recorded data were analyzed statistically with the help of computer following *STAT-C program*. Mean separation was done by Duncan Multiple Range Test.

RESULTS AND DISCUSSION

The *in vitro* regenerated potato cultivars were screened after four weeks at different salt concentrations. The physiological characters including: root length, shoot length, root shoot length ratio, the number of nodes, the number of leaves, the number of roots and fresh weight were measured and presented in Table 1 and Figure 1.

The results shown in Figure 1 showed that variety Challisha produced longest shoot (5.91 cm) followed by Felsina (2.97 cm) while the Diamant produced shortest shoot (2.12 cm). Variety Challisha and Felsina performed better and statistically similar regarding root length while Diamant performed inferior. Challisha produced the maximum number of nodes (7.40) followed by Felsina (5.25) and Diamant (3.48). The similar trend was found in the number of leaves and the number of roots per plantlet. Fresh weight was the

Table 1. Effect of different concentrations of NaCl on *in vitro* regeneration of three potato varieties

Levels of NaCl	*Shoot length (cm)*	*Root length (cm)*	*No. of nodes per plantlet*	*No. of leaves per plantlet*	*No. of roots per plantlet*	*Fresh weight (mg)*
C_0 - 0 mM	6.39a	8.00a	6.96a	9.01a	4.96a	122.92a
C_1 - 30 mM	4.83b	5.84b	7.08a	6.71b	3.17b	114.42a
C_2 - 60 mM	3.68c	5.65b	6.21a	5.98b	2.88b	104.00ab
C_3 - 90 mM	1.87d	4.33b	3.75b	4.42c	2.04b	87.17b
C_4 - 120mM	1.55d	4.89b	2.88b	2.46d	1.92b	57.67c

Table 2. Interaction effect of cultivars and different concentrations of NaCl on *in vitro* regeneration potato

Interaction of Cultivars and NaCl (Treatment Combination)	*Shoot length (cm)*	*Root length (cm)*	*No. of nodes per plantlet*	*No. of leaves per plantlet*	*No. of roots per plantlet*	*Fresh weight (mg)*
V_1C_0	9.48a	7.56abc	9.25a	10.63a	7.13a	144.25a
V_1C_1	8.69a	5.59bc	9.38a	8.50abc	3.75bc	138.25ab
V_1C_2	6.41b	8.65ab	8.25ab	8.00abc	4.00bc	131.00abc
V_1C_3	3.25de	7.51abc	6.68abc	6.38cde	3.50bcd	107.25abcde
V_1C_4	1.71efg	6.38bc	3.50cde	3.50fg	3.13cde	79.75def
V_2C_0	5.59bc	8.08ab	6.38abc	7.13bcd	4.88ab	101.00abcde
V_2C_1	1.74efg	1.70de	4.00cde	4.50def	2.25bcde	93.75cde
V_2C_2	1.73efg	0.43e	3.75cde	3.63efg	1.38cde	86.25def
V_2C_3	1.11fg	0.98e	2.25de	2.38fg	0.88de	73.75ef
V_2C_4	0.45cg	0.80e	1.00e	1.00g	0.63e	47.50f
V_3C_0	4.11cd	8.38ab	5.25bcd	9.28ab	2.88bcde	123.50abcd
V_3C_1	4.08cd	10.24a	7.88ab	7.13bcd	3.50bcd	111.25abcde
V_3C_2	2.90df	7.88abc	6.63abc	6.31cde	3.25bcde	94.75bcde
V_3C_3	1.25efg	4.48cd	2.37de	4.50def	1.75cde	80.50def
V_3C_4	2.50defg	7.49abc	4.13cde	2.88fg	3.00bcde	45.75f

highest (120.10 mg) in Challisha, which was statistically superior to Felsina (91.15 mg) followed by Diamant (80.45 mg). The appearance of the variety also showed that Challisha and Felsina performed better compare to Diamant (Figure 2). Our results were in compliance with the findings of [6] Zaman et al. (2015) and [24] Aghaei et al. (2009) they reported that white potato is moderately salt tolerant and other tested varieties were salt sensitive. In vitro study also showed that internodes and tuber yield in potato were reduced at higher salt levels by Mahmoud et al. (2009) [25]. Etehadnia (2009) also studied details of potato performance under salt stress and found that salt stress affects plant growth and reduced yield which also supported our findings [16].

Different levels of NaCl had a significant effect on all growth parameters of potato (Figure 1). Most of the parameters decreased gradually with the increase of NaCl concentrations. Potato plant produced longest shoot (6.39 cm) at control treatment (absence of NaCl) followed by 30 mM NaCl (4.83 cm) and 60 mM NaCl (3.68 cm). Shoot length drastically reduced to 1.87 cm at 90 mM NaCl and 1.55 cm at 120 mM NaCl. The findings of [18] Sudhersan et al. (2012) were in strong conformity with our results they reported reduced shoot growth in potato *in vitro* due to salt stress by increasing salt concentration in MS media. Root length, the number of nodes per plantlet, the number of leaves per plantlet, the number of roots per plantlet and fresh weight were the highest (8.00 cm, 6.96, 9.01, 4.96 and 122.92 mg respectively) in control treatment closely followed by 30 mM and 60 mM NaCl levels. Most of the parameters reduced about 50% at 90 mM of NaCl and were least at 120 mM NaCl level. Farhatullah et al. (2002) reported that even at 1% NaCl in the media suppressed the root growth of the tested potato vari

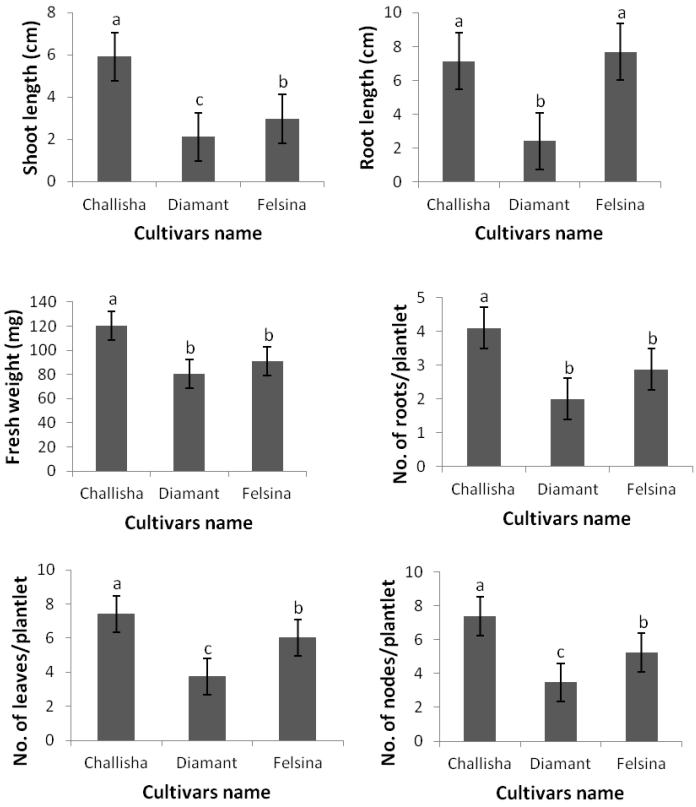

Figure 1. The performance of Challisha is better than other cultivars in salt stress. Sprouted healthy tubers were selected as the primary source of explants and four weeks old in vitro plantlets of two nodal segments were transferred to MS media supplemented with various concentration of NaCl. At the four weeks of culture, data were collected and analyzed statistically with the help of computer following STAT-C program. Different letters denote significant differences at the 95% confidence level using Duncan's Multiple Range Test with four replications.

(a) (b) (c)

Figure 2. Response of three potato cultivars, Diamant (a), Felsina (b), and Challisha (c), under NaCl salt stress. The appearance of the variety also showed that performance of Challisha and Felsina are better than Diamant

eties [26]. NaCl at 100 mM inhibited root growth in potato which was also agreement with our findings [15].

The growth of the potato cultivars in different parameters was significantly influenced by the interaction of cultivars and concentrations of NaCl (Table 2). The Longest shoot (9.48 cm) was found in V_1C_0, which was statistically similar to V_1C_1 (8.69 cm) followed by V_1C_2 (6.41 cm) and V_2C_0 (5.59 cm). The treatment combinationV_3C_1 produced longest root (10.24 cm) which performed statistically similar to V_1C_2, V_3C_0, V_2C_0, V_3C_2, V_1C_0, V_1C_3 and V_3C_4. The number of nodes per plantlet was maximum in V1C1 (9.38) closely followed by V_1C_0 (9.25), V_1C_2 (8.25) and V_3C_1 (7.88). The number of leaves per plantlet was the highest (10.63) in V1C0 which was statistically identical to

V_3C_0 (9.28), V_1C_1 (8.50) and V_1C_2 (8.00). The treatment combination V_1C_0 produced the maximum number of roots per plantlet (7.13) which performed statistically identical to V_2C_0 (4.88) while V_2C_4 performed least. Fresh weight was the highest (144.25 mg) in V_1C_0 which was statistically identical to V_1C_1 (138.25 mg), V_1C_2 (131.00 mg), V_1C_3 (107.25 mg), V_2C_0 (101.00 mg), V_3C_0 (123.50 mg) and V_3C_1 (111.25 mg) while it was the lowest in V_2C_4 (47.50 mg). It was clear that the variety Cahallisha performed better up to 90 mM NaCl in most of the parameters studied.

The physiological growth data were used to predict cultivars tolerance at higher salinity levels in vitro. [7] Zhang and Donnelly (1997), and [24] Aghaei et al. (2009) reported that the effect of salt stress on in vitro potato growth has been similar to that observed under field conditions. A significant correlation was found between in vitro growth and field performance. [27] Morpurgo (1991) also suggested *in vitro* screening of potato parental material for tolerance to salinity. Therefore, it appeared that salinity tolerance of potato genotypes could be successfully evaluated in vitro as a promising substitute for conventional field evaluations. There is merit in evaluation at a range of salinity levels since different genes are apparently expressed at various stress levels *in vitro* as observed in vivo in potato [7, 28]. However, it is recommended to take more potato varieties for *in vitro* and *in vivo* micropropagation scheme. It could be used as a tool for the selection of broad tolerant to salt cultivars for field transplantation and also for designing breeding program.

CONCLUSION

Challisha, Diamant, and Felsina are survived well with exhibiting different growth status up to 60 mM NaCl, but they performed poorly at 120 mM of NaCl. Cultivar Challisha performed better regarding shoot length, root length, the number of nodes per plantlet and the fresh weight per plant up to 90 mM of NaCl. Thus, we can conclude that local indigenous variety Challisha is salt tolerant comparing with the modern cultivated varieties.

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