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In Vitro Shoot Bioassay of Salt Tolerant International Potato Center Bred Potato Genotypes for Assessing Their Salinity Tolerance

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ABSTRACT

The aim of the experiment is to study *in vitro* regeneration efficiency of international potato center (CIP)-bred salt tolerant potato genotypes under salt stress condition and to identify effective potato genotype(s) for saline belt areas of Bangladesh. An *in vitro* shoot bioassay of eight CIP-bred potato genotypes viz. CIP 102, CIP 106, CIP 111, CIP 117, CIP 124, BARI Alu 72 (CIP 139), and BARI Alu 73 (CIP 127) and CIP 136 were used. In this study, single node of these genotypes was cultured in MS media supplemented with 0, 80, 100, 120, 140 and 160 mM NaCl. Among the eight genotypes, BARI Alu 72 (CIP 139) showed the highest tolerance against salinity up to 160 mM NaCl (14.61 dS/m) for all studied parameters (except shoot and root initiation) with the highest plant height (9.67 cm), leaves number (13.60), nodes number (9.50), root length (6.50 cm), roots number (7.80), fresh weight of shoot (536.1 mg) and root (205.60 mg). On the other hand, CIP 106 was found the most susceptible genotype against salinity showing its highest salinity tolerance up to 120 mM NaCl (10.96 dS/m) with maximum plant height (7.17 cm), leaves number (12.50), nodes number (6.50), root length (7.50 cm), roots number (9.7), fresh weight of shoot (572.3 mg) and root (250 mg). The experiment's findings corroborated CIP's findings that they were salt tolerant, as well as recommended for their cultivation suitability in saline-affected area in Bangladesh.

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Introduction

Potato (*Solanum tuberosum* L.) belongs to the economically important family Solanaceae. It has contributed approximately 4% of global crop production as an important part of the world's food supply (FAO 2020). Potato production reached nearly 370.43 million tons in 2019, making it the world's fifth largest food crop after sugarcane, maize, rice, and wheat (FAOSTAT 2019). However, as a non-cereal food crop, it is the first and most important in the world food supply, and the United Nations has identified it as a vital crop for food security. As a part of global context, potato also plays an important role in food security of Bangladesh since 1960 (Kundu et al. 2013). Recently, Bangladesh is the 7th largest potato producing country in the world and in the Asia, it ranks 3rd (FAO 2020). Potato has become the 3rd most important cash crop after rice and wheat in Bangladesh (TCRC Annual Report 2014-15) and in terms of total crop production, it ranks 2nd position (109.179 lac MT). Among the edible vegetables, it ranks 1st (37.18 %) (MoA Annual Report 2019-20) covering nearly 34.03 % (4.646 lac Ha) of cultivated area under vegetable production with an average annual yield of about 23.50 MT/Ha (Agriculture Diary 2021). If this positive trend continues, Bangladesh is predicted to reach China, India, Russia, and the United States as one of the world's leading potato producers in a relatively short period of time. Although agriculture is considered as the backbone national economy in Bangladesh which contributes 13.35 % of national GDP engaging 40.62% of total labor force (Bangladesh Economic Review 2020). However, agriculture's contribution to GDP has been steadily declining in recent years due to several factors, the most important of which is salinity, which severely limits crop production in the country's coastal belt (Shahbaz and Ashraf 2013). According to the World Bank (2014), Bangladesh's coastal regions occupy 32 percent of the country in 19 districts, accounting for 30 percent of the country's cultivable land (Parveen et al. 2014). Approximately 1.056 million hectares have already been affected by varying degrees of soil salinity, resulting in yield losses ranging from 6 to 19 percent for each unit rise in salinity (SRDI 2010). Moreover, salinity and drought are interlinked factors which occur often simultaneously affecting almost all biochemical functions of plant (Solh and Ginkel 2014). As a result, the potential effect of salinity has become a major concern for Bangladesh's agricultural sustainability and food security to feed its rapidly growing population, as well as a critical issue for the adaptation of climate changes (Dasgupta et al. 2015). To mitigate this problem, salt tolerant potato cultivars can be chosen as they can tolerate a certain amount of salinity without compromising production or quality. Moreover, 7-8 times higher yield (ton / ha) and 5 times higher profit can be obtained from potato cultivation than the rice, wheat, jute and sugarcane cultivation from the same acreage in Bangladesh (Haque et al. 2007). Besides, easy production system, diversified use from dining table to junk restaurant and smart processing and industrial use have further increased the importance of this starch rich vegetable crop in Bangladesh. Furthermore, the potato has a high export potential. However, due to low yield of local cultivars and saline susceptibility of available high yielding varieties (HYVs) are not economically suitable for their cultivation in saline soil (Rahman et al., 2013; Uddin et al., 2010). Therefore, high yielding salt tolerant exotic potato cultivars such as CIP 102, CIP 106, CIP 111, CIP 117, CIP 120, BARI Alu 72 (CIP 139), BARI Alu 73 (CIP 127) and CIP 136 can be cultivated in saline belt areas. However, they should be tested or assessed for salt stress first.

Presence of excessive soluble salts such as NaCl, KCl, K_2SO_4, $MgCl_2$, $MgSO_4$ and Na_4SO_4 in the soil causes soil salinization. However, salinity stress is mainly equated with NaCl stress as it is the most dominant salt components (70%) of world salinity affected land areas. Soil salinity level is described as EC (ds/m) while *in vitro* level is expressed as mM, %, mg/L or ppm. An EC of 1 ds/m is approximately 11 mM NaCl or 640 mg NaCl/L (Lewis 1984). A plant bioassay for salinity (NaCl) tolerance quantifies the impact of NaCl on the growth process of the tested plant(s) and ranks them either *in vivo* or *in vitro*. Plant tissue culture techniques have become latest, fruitful, and short time approaches for rapid propagation and evaluation of plants against salt stress (Ahmed et al. 2020). As a result, *in vitro* evaluations of NaCl or mixed salt stress effects on potato genotypes have recently been suggested as a cost-effective, labor-intensive, and often problematic alternative to expensive, labor-intensive, and often filed-based evaluations (Al-Mahmud et al. 2018). Moreover, the effect of salinity stress on *in vitro* potato growth has been reported to be similar that observed under field conditions (Khenifi et al. 2011). Salt tolerance and sensitivity of potato cultivars are genotype dependent and may be not epigenetic adaptation under stress condition (Harun-or-Rashid et al. 2020) which make them response differently against salt stress.

Unfortunately, there are very few reports available on salt tolerant potato varieties, their micropropagation and *in vitro* bioassay studies protocol in Bangladesh. Therefore, these significant salt tolerant CIP potato genotypes have been screened to determine their salinity tolerance level for commercial cultivation in Bangladesh's saline belt areas.

Materials and Methods

Plant Materials

Eight high yielding salt tolerant potato genotypes such as CIP 102, CIP 106, CIP 111, CIP 117, CIP 124, BARI Alu 72 (CIP 139), and BARI Alu 73 (CIP 127) and CIP 136 were collected from the Tuber Crops Research Centre (TCRC), Bangladesh Agricultural Research Institute (BARI), Gazipur-1701 (24.3688° N, 88.6618° E) and used as plant materials in the experiments.

Explants

In vitro regenerated plantlets of above-mentioned genotypes were used as source material for explants. Healthy and diseases free stem segments having single node with one leaf and one lateral (axillary) bud were cut from the micro propagated plantlets and used as explants in this experiment.

Culture media

Basal MS (Murashige and skoog 1962) media supplemented with different concentrations of NaCl viz. T_0: Control (Zero NaCl); T_1: 80 mM (7.31 dS/m); T_2:100 mM (9.13 dS/m); T_3: 120 mM (10.96 dS/m); T_4: 140 mM (12.78 dS/m); T_5: 160 mM (14.61 dS/m) were used as treatments. The media were prepared according to the treatments and sterilized by autoclaving at 1.06kg/cm^2 (15 PSI) pressure with 121° C for 20 minutes.

Culture method of single-node cuttings for in vitro shoot bioassay

Single-node cuttings were excised from the middle of each plantlet to achieve the most consistent response to salinity. Each single-node cutting was 1-2 cm long with 1 leaf and 1 axillary bud and individually cultured in a test tube containing 10 ml media. Five node cuttings of each genotype were used at each treatment and repeated for five times. The cultures were incubated in growth room under cold fluorescent light (1500-2000 lux) for 16/8 photoperiod at a temperature of 21 ± 1°C at day and 12 ± 1°C at night. The experiment lasted for 4 weeks.

Data Calculation and Analysis

Development of plantlets was observed, and the following data were recorded for the experiment a. Days required for shoot and root initiation, b. Length of the shoot / Plant height (cm), c. Number of leaves per explant, d. Number of nodes per explant, e. Length of root (cm), f. Number of roots, g. Fresh weight of shoot and h. Fresh weight of roots. Then data on collected parameters were analyzed by MSTATE-C software. Two factor (Genotype and Treatment) factorial analysis of variance were performed at Completely Randomized Design (CRD) with 5 replications and means were compared by the Duncan's Multiple Range Test (DMRT) at 5% level of probability.

Results

Effect on Days to Shoot Initiation

The study revealed significant variation among the genotypes on their response to days required for shoot initiation (Table 1). Under salt stress, all genotypes needed more days as the salt concentration in the media increased. However, among the eight potato genotypes, BARI Alu 73 (CIP 127) needed the lowest time for shoot initiation up to 140 mL salinity level with 6.37 to 9.30 days from control to 140 mL salinity level respectively. While CIP 117 and 111 responded better up to 100 mL salinity level with 7.10 to 9.56 days and 6.53 to 9.96 days respectively followed CIP 102 and CIP 106 up to 80 mL salinity level with 7.47 to 8.17 days and 9.63 to 9.97 days respectively. On the other hand, CIP 124 took the maximum time among all genotypes from control to the highest (160 mM) salinity level with 9.50 to 12.40 days respectively followed by CIP 136 and CIP 139 with 7.87 to 12.33 days and 8.00 to 12.30 days respectively for shoot initiation.

Effect on Days to Root Initiation

Plantlets of studied genotypes showed different response to the root initiation against different degrees of salinity (Table 2). The interaction effect of genotypes against different salinity levels showed that CIP 127 reacted earlier than other genotypes for root initiation up to 160 mM salinity level with 5.46 to 10.60 days, followed by CIP 111 up to 120 mM salinity level with 7.60 to 10.60 days, and CIP 117 up to 100 mM salinity level with 6.53 to 9.80 days. Meanwhile, CIP 106 and CIP 124 responded earlier only at control and needed 10.0 to 11.60 days and 10.0 to 12.27 days respectively from 80 mM salinity level to upward. On the other hand, CIP 136 reacted most lately from control to upward with 7.60 to 14.27 days followed by CIP 139 with 6.60 to 13.00 days and CIP 102 with 6.60 to 13.60 days respectively.

Effect on Plant Height (Cm) At In Vitro

Highly significant differences were recorded among the studied genotypes for plant height, where BARI Alu 72 (CIP 139) showed the highest tolerance up to 160 mM salinity level with 9.67 cm plant height followed by CIP 102 (9.45 cm) and CIP 124 (9.43 cm) at same salinity level. On the other hand, CIP 106 emerged as the most sensitive genotype up to 100 mM salinity level with 8.50 cm tall plantlets followed by CIP 136 (9.50 cm) at same salinity level. However, BARI Alu 73 (CIP 127), CIP 111 and CIP 117 were found vigorous up to 140 mM salinity level with 9.0 cm, 8.51 cm and 8.10 cm plant height respectively (Table 3 and Plate 1).

Effect on Number of Leaves at In Vitro

The study revealed that leaves size and vigor was stunted with the increased levels of NaCl in MS media. However, all the genotypes were able to produce sufficient leaves up to 160 mM salinity level, where BARI Alu 72 (CIP 139) produced maximum leaves (13.60) in the highest salinity level followed by BARI Alu 73 (CIP 127), CIP 117, CIP 111, CIP 106, CIP 102, CIP 124 and CIP 136 with 12.50, 10.50, 10.50, 9.73, 9.73, 9.50 and 9.47 leaves respectively at same level of NaCl stress (Figure 1 and Plate 1).

Table 1. Effect of different concentrations of salinity level on days required for shoot initiation of different CIP Potato genotypes

SI	Genotypes	Treatment (mM NaCl)					
		Control	80	100	120	140	160
01	CIP 102	7.47qr	8.17o	10.20hi	10.37g-i	11.20f	12.03 a-d
02	CIP 106	9.63 j-l	9.97i-k	10.27g-i	10.60gh	11.37ef	11.43ef
03	CIP 111	6.53s	9.27 lm	9.96i-k	10.20hi	10.27g-i	11.17f
04	CIP 117	7.10 r	8.73 n	9.56kl	11.30ef	11.40ef	11.40ef
05	CIP 124	9.50kl	10.50gh	10.70g	12.10a-c	12.10a-c	12.40a
06	CIP 127	6.37s	7.53p-r	8.00op	9.03mn	9.30lm	11.70d-f
07	CIP 136	7.87o-q	10.10h-j	10.20hi	11.73c-e	12.23a-c	12.33a
08	CIP 139	8.00 op	10.23g-i	11.77b-e	12.00a-d	12.03a-d	12.30ab
CV (%)		2.6		LSD $_{(0.05)}$		0.419	

Table 2. Effect of different concentrations of salinity level on days required for root initiation of different CIP Potato genotypes

SI	Genotypes	Treatment (mM NaCl)					
		Control	80	100	120	140	160
01	CIP 102	6.60 n	10.00ij	12.27d	12.60cd	13.00bc	13.60b
02	CIP 106	7.00mn	10.00ij	11.00gh	11.00gh	11.30g	11.60e-g
03	CIP 111	7.60m	10.00ij	10.57hi	10.60hi	11.00gh	11.57fg
04	CIP 117	6.53n	09.43jk	09.80ij	11.57fg	12.00d-f	13.00bc
05	CIP 124	8.60l	10.00ij	11.60e-g	12.00 d-f	12.23de	12.27d
06	CIP 127	5.46o	09.20kl	09.53jk	10.47 hi	10.50hi	10.60hi
07	CIP 136	7.60m	12.00d-f	12.37cd	12.53 cd	13.47b	14.27a
08	CIP 139	6.60n	11.00gh	11.43fg	12.60 cd	12.60cd	13.00bc
CV (%)		3.70		LSD $_{(0.05)}$		0.646	

Table 3. Effect of different concentrations of salinity level on plant height (cm) of different CIP Potato genotypes.

Genotypes	Plant height (cm) (mM NaCl)					
	Control	80	100	120	140	160
CIP 102	16.73a	13.20d	12.73e	12.50e	11.50g	9.45kl
CIP 106	15.00b	11.10gh	8.50m	7.17o	6.57p	5.57q
CIP 111	11.43g	10.03ij	8.90lm	8.60lm	8.51lm	4.50r
CIP 117	11.00h	9.40kl	8.50m	8.17mn	8.10mn	7.90n
CIP 124	12.50e	12.00f	10.43ij	10.17ij	9.50k	9.43kl
CIP 127	16.83a	13.50c	10.00j	9.60jk	9.00l	8.43mn
CIP 136	12.60e	11.00h	9.50k	7.67o-n	6.77p	6.43p
CIP 139	12.17ef	11.60g	10.50i	10.80ih	10.20ij	9.67jk
CV (%)	5.15		LSD $_{(0.05)}$		0.856	

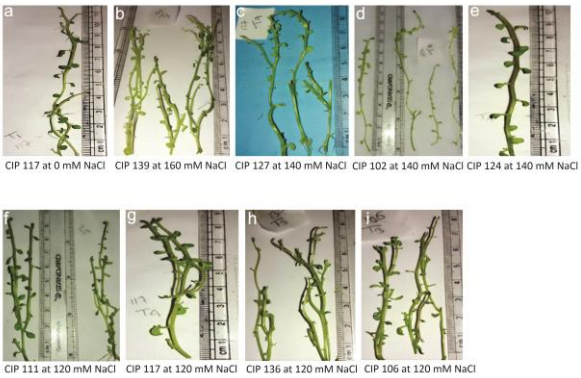

Plate no. 1 (a-i): Effect of different concentrations of salinity level on shoot length (cm), leaves no., node no. and fresh weight (mg) of shoot of different CIP Potato genotypes.

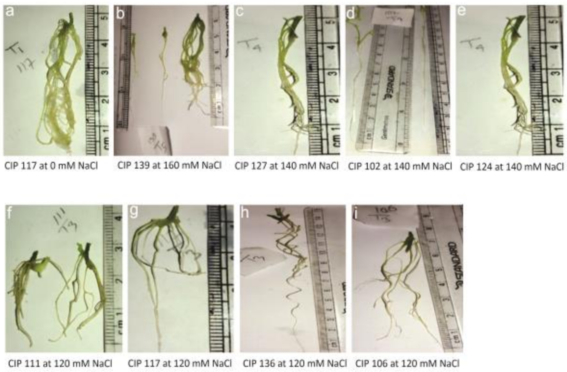

Plate no. 2 (a-i): Effect of different concentrations of salinity level on root length (cm), root no. and fresh weight (mg) of root of different CIP Potato genotypes.

Table 4. Effect of different concentrations of salinity level on node number of different CIP Potato genotypes

Genotypes	Number of nodes per plant (mM NaCl)					
	Control	80	100	120	140	160
CIP 102	12.50b-d	12.00c-f	11.50d-g	11.00 f-h	10.50g-i	9.43i-l
CIP 106	12.50b-d	11.60d-f	9.37j-l	6.50n	6.50n	6.43n
CIP 111	11.50d-g	11.00f-h	10.50g-i	10.43 g-j	9.43i-l	8.43lm
CIP 117	12.20c-e	11.00f-h	10.50g-i	10.50 g-j	10.17h-k	9.43i-l
CIP 124	12.50b-d	11.27e-g	11.17e-h	11.17 e-h	11.10f-h	9.40j-l
CIP 127	15.33a	12.83bc	11.13e-h	10.50 g-j	10.50g-j	8.50lm
CIP 136	12.50b-d	9.50i- l	9.33kl	8.50 lm	7.60 m	7.50mn
CIP 139	13.37b	11.50d-g	11.50d-g	11.50 d-g	10.50g-i	9.50i-l
CV (%)	6.26		LSD $_{(0.05\%)}$		1.068	

Effect on number of nodes per plant at in vitro

In terms of nodes number, all the genotypes developed their maximum nodes number at salt free MS media ranging from 12.20 to 15.33 and with the increase level of salinity on MS media nodes development was retarded. However, BARI Alu 72 (CIP 139), CIP 102, CIP 117, CIP 124, BARI Alu 73 (CIP 127) and CIP 111 developed sufficient nodes up to 160 mM salinity level with 9.50, 9.43, 9.43, 9.40, 8.50 and 8.43 respectively (Table 4). On the other hand, CIP 106 emerged as most salinity sensitive genotype showing its tolerance up to 100 mM salinity level with 9.37 nodes and produced the lowest nodes number (6.43) at 160 mM NaCl stress among all interactions. Whereas CIP 136 was found tolerant up to 120 mM salinity level with 8.50 nodes.

Effect on length of root (cm) at in vitro

Remarkable variation was observed among the genotypes in terms of root length per explant (Table 5 and Plate 2**)** where tested potato genotypes can be grouped into two broad categories. First one comprised of CIP 117, CIP 111 and CIP 124 and MS media with NaCl caused drastic effect on their root length revealing their highest tolerance up to 100 mM salinity level with 4.50, 4.30 and 3.50 cm root length respectively. But the second group comprised of CIP 102, CIP 136, BARI Alu 72 (CIP 139), BARI Alu 73 (CIP 127) and CIP 106 showed their better performance up to 160 mM salinity level with 9.50, 9.30, 6.50, 6.50 and 5.00 cm root length respectively at this highest level of salt stress.

Effect on Number of Roots at In Vitro

Different levels of salinity in culture media expressed highly significant difference for root number. Combined effect of genotypes and salinity level showed that all the genotypes were able to produce root up to 160 mM salinity level but performed better up to 120 mM salinity level. Considering both primary and secondary root, BARI Alu 72 (CIP 139) and CIP 106 were found tolerant up to 160 mM salinity level with 7.80 and 7.30 total root number respectively. CIP 111, CIP 124, CIP 117 and BARI Alu 73 (CIP 127) showed well tolerance up to 140 mM salinity level with 9.0, 9.0, 8.0 and 7.0 total root number respectively. On the other hand, CIP 136 and CIP 102 performed better up to 120 mM salinity level with 8.0 and 6.30 total root number respectively (Figure 2 and Plate 2).

Effect on Fresh Weight of Shoot (FWS) (Mg) at In Vitro

Pronounced variation was observed among the genotypes in terms of fresh weight of shoot. The study revealed that all the genotypes were able to survive up to 160 mM salt stress with enough shoot development. But with increased level of salt stress in media decreased shoot growth was observed. However, the highest FWS (783.5 mg) was measured in BARI Alu 73 (CIP 127) at simple MS media free from salt stress and the minimum (492.5 mg) in CIP 117 at 160 mM salinity level (Table 6).

Effect on Fresh Weight (Mg) of Root at In Vitro

Application of salt stress in MS media revealed that fresh weight of root of *in vitro* produced plant was severely reduced with the increase of salinity (Figure 3). Highest FWR (370.0 mg) was measured in BARI Alu 72 (CIP 139) at MS media free from salt which was statistically similar with BARI Alu 73 (CIP 127) (370.0 mg) at same treatment. On the other hand, lowest FWR (200.0 mg) was measured in CIP 117 at 160 mM salt stress which was statistically similar with CIP 111 (205.0 mg) and CIP 106 (205.6 mg) at same treatment.

Table 5. Effect of different concentrations of salinity level on root length (cm) of different CIP Potato genotypes

Genotypes		Root length (cm) / plant (mM NaCl)					
		Control	80	100	120	140	160
01	CIP 102	12.60a	12.50a	11.57b	11.40bc	11.00c	9.50h
02	CIP 106	10.10ef	9.60gh	8.66ij	7.50k	5.10m	5.00n
03	CIP 111	4.50l-n	3.70p	3.50pq	3.46pq	2.70r	2.50r
04	CIP 117	5.20m	5.13mn	4.50o	4.30 m-o	3.50pq	1.50s
05	CIP 124	5.00n	4.36op	4.30op	3.50pq	3.50pq	3.30q
06	CIP 127	10.50e	10.00f	9.70g	8.10j	7.50k	6.50l
07	CIP 136	10.50e	10.50e	10.33ef	10.00f	9.30hi	9.00i
08	CIP 139	11.50b	10.77d	10.00 f	9.77 g	9.30hi	6.50l
CV (%)		7.11		LSD (0.05%)		0.86	

Table 6. Effect of different concentrations of salinity level on fresh weight (mg) of root of different CIP Potato genotypes

Genotypes		Fresh weight (mm) of shoot / plant (mM NaCl)					
		Control	80	100	120	140	160
01	CIP 102	593.3m	566.2q	553.0r	550.0s	536.1u	511.3y
02	CIP 106	626.8h	583.2n	579.7o	572.3p	549.0s	519.0y
03	CIP 111	732.8c	616.5j	610.0k	582.9no	519.3xy	500.3z
04	CIP 117	542.0t	534.7u	525.0 w	522.7wx	512.7z	492.5z
05	CIP 124	599.7l	580.0no	569.7pq	541.3t	529.7v	509.0y
06	CIP 127	783.5a	715.8d	632.3 g	631.5g	572.3p	529.9v
07	CIP 136	637.3f	637.2f	622.0i	542.8t	532.8uv	501.0z
08	CIP 139	751.3b	641.0e	622.7i	599.7l	550.3rs	536.1u
CV (%)		0.37		LSD (0.05%)		3.516	

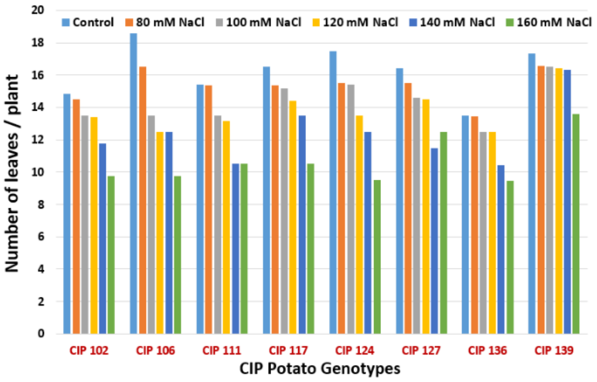

Figure 1. Effect of different concentrations of salinity level on leaf number of different CIP Potato genotypes

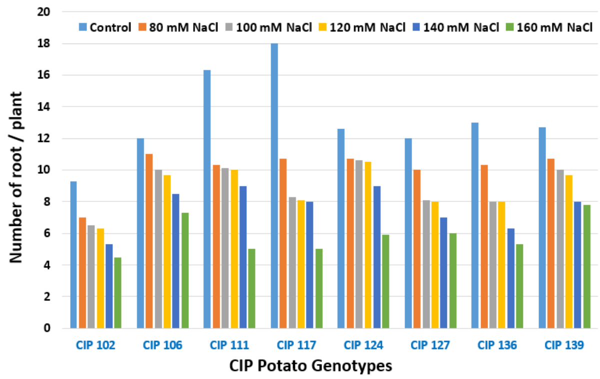

Figure 2. Effect of different concentrations of salinity level on root number of different CIP Potato genotypes

Discussion

Tested potato genotypes reacted differently to different growth parameters in our current experiment. For shoot initiation and development, days required from 6.37 to 9.63 days while, for control, it needs 11.17 to 12.40 days at 160 mM NaCl (Table 1). It revealed that with the increase level of salinity on media, days required for shoot initiation and development was affected and delayed by salt stress which is consistent with previous reports (Sudhersan et al. 2012). No toxic material restricts plant growth more than salt, which affects plant metabolism and induces significant changes in various biochemical and molecular processes, limiting plant growth and development everywhere (Zhu 2007). However, salt tolerant potato cultivars can tolerate salinity at some certain level according to their potentiality. Such as, in our experiment although days required for root initiation was increased but all the genotypes were able to develop root up to 160 mM salinity level ranging from 5.46 to 8.60 days at control to 10.60 to 14.27 days at 160 mM salinity level respectively which testified their salt tolerance capacity at this certain level (Table 2). In vitro root initiation and growth of potato cultivar Serrana was discovered in MS liquid medium containing 150 mM NaCl in a previous study (Morpurgo 1991).

However, another study found drastic effect of salinity on shoot length of potato genotypes from 75 mM to upward concentration (Rahman et al. 2008). While researchers reported the reduced shoot length in potato from to 90 mM and 120 mM NaCl to upward in their two separate studies (Aghaei et al. 2009). Plantlets of tested potato genotypes were found to be resistant up to 100 mM NaCl salinity level with 8.50 to 12.73 cm shoot length, regardless of plant height (Table 3 and Plate 1). The plant height was reduced to 7.67 to 12.50 cm at 120 mM to 4.50 to 9.67 cm at 160 mM salinity level. Contrary to that, the previous report found maximum plant height (6.5 cm) in potato cultivar Kroda at 60 mM NaCl stress even no growth in potato cultivar Asterix, Cardinal, Challenger, Desiree, Hermis, Kroda, Sh-5 and Sante at 80 mM and 100 mM NaCl (Zaman et al. 2015).

In terms of leaves development, it was found significant differences on leaves number from 100 to 200 mM salinity level where Daimant and Loane were able to produce 7.67 and 4.67 leaves at 160 mM NaCl stress as well as Sylvana and Amarin showed their tolerance up to 200 mM salinity level with 3.67 leaves for each (Murshed et al. 2015). These findings are in line with a study that found leaves' development was unaffected by various salt stress conditions, indicating that they are salt tolerant. Even all the studied genotypes were able to produce enough leaves up to 160 mM salinity level with 9.5 to 13.6 leaves (Figure 1 and Plate 1).

In respect of node development, it was reported that reduced number of nodes at 75 mM NaCl stress in an in vitro study of potato (Etehadnia, M., 2009). On the other hand, Zaman et al. (2015) found maximum number of nodes plant-1 (8.8) up to 60 mM NaCl in their study for potato cultivar Kroda. These results are almost similar to this experiment, where it was observed the reduced number of nodes from 80 mM NaCl stress to onwards with 9.50 to 12.83 at 80 mM NaCl stress to 6.43 to 9.50 at 160 mM NaCl salt stress (Table 4). Pour et al. (2010) reported decreased root length of potato growing in the increasing NaCl concentrations. In this study, it was also noticed MS media with NaCl caused negative effect on root length of experimented genotypes from 80 mM salinity level (3.70 to 12.50 cm) to 160 mM NaCl salinity level (1.50 to 9.50 cm) (Table 5 and Plate 2). The investigation of Rahman et al. (2008) was similar to this study who reported reduced plant root length of his studies genotypes from 75 to 100 mM NaCl.

In an in vitro analysis, it was discovered that MS media supplemented with more than 60 mL of NaCl had a negative impact on the root formation (1.9 roots per plant) of potato cultivars. (Zaman et al. 2015). However, in this study, it was found that gradual decreasing in root number from 80 mL salt stress to onwards (Figure 02). On the other hand, it was reported reduced number of roots per plant in potato varieties by increasing salt in MS media from 750 - 4000 ppm (Sudhersan et al. 2012).

In this study, it was noticed that fresh weight of shoot was not significantly affected up to 160 mM salinity level that ranged 492.50 to 536.10 mg at this highest salinity level (Table 6). Under salt stress condition, relatively salt tolerant potato cultivars can accumulate more fresh weight than salt sensitive cultivars (Rahnama and Ebrahimzadeh, 2004).

Unlike fresh weight of shoot application of salt stress in MS media revealed negative effect on fresh weight of root of studied genotypes. For all the genotypes, maximum fresh weight of root was measured at control which was statistically significant from all other treatments and minimum at 160 mM salinity level which was also statistically significant from rest of the treatments (Figure 3). Increased NaCl in MS media from 50 to 150 mM resulted in a significant loss of fresh root weight in the potato cultivar Agria (Askari et al. 2012).

Salt tolerant CIP potato clones can accumulate more proline than the susceptible clones which make them tolerant at certain level against salt tress (Al- Mahmud et al. 2018). On the other hand, due to lack of this mechanism the addition of NaCl to the culture media adversely affected the growth of most of the potato cultivars even they were high yielding (Khenifi et al. 2011). However, considering the stress susceptibility index, proline accumulation and yield performance found that potato clone CIP-139 (CIP 396311.1) and CIP-102 (301029.18) were best suited for 8 to 10 dS/m of NaCl stress in pot trial and 10-12 dS/m^2 of salinity in three studied saline location (Al- Mahmud et al. 2018). In this study, it also found that experimental genotypes can tolerant up to120 mM NaCl (10.96 dS/m) even though survived at 160 mM (14.61 dS/m) salinity level with limited growth parameters. Besides, revealing their salt tolerance level at certain level it also testified the strong correlation between the in vitro tissue culture technique and field experiment cum pot trial against salt stress.

Conclusion

All the experimented genotypes were found tolerant up to 120 mM NaCl without significant effect on growth parameters even all of them survived up to 160 mM NaCl in MS media. It revealed them salt tolerant as stated by

International Potato Centre (CIP). However, among the eight genotypes BARI Alu 72 (CIP 139) was found as the most salt tolerant genotype showing excellent performance up to 160 mM (14.61 dS/m) salinity level at *in vitro* study. Besides, BARI Alu 72 (CIP 139), BARI Alu 73 (CIP 127), CIP 102 and CIP 124 performed better up to 140 mM (12.78 dS/m) salinity level at *in vitro* condition. On the contrary, CIP 106 showed minimum salinity tolerance up to120 mM NaCl (10.96 dS/m) followed by CIP 136, CIP 117 and CIP 111 at the same salinity level. Considering the overall results of *in vitro* shoot bioassay, BARI Alu 72 (CIP 139) can be considered as the most effective potato genotype for saline belt areas of Bangladesh as well as BARI Alu 73 (CIP 127), CIP 102 and CIP 124 can also be chosen for the same purpose. It is expected that using this protocol a new chapter will be opened for salt tolerant potato cultivars identification and their commercial cultivation in saline affected areas of Bangladesh.

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