



## Screening of Salt Tolerant Potato Genotypes using Salt Stress and Molecular Markers

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### Authors' contributions

*This work was carried out in collaboration among all authors. Author MJM did the experiment, collected and analyzed data and wrote the first draft. The authors MAH and MMHS contributed in designing and editing the manuscript and provided laboratory supports. Author SMNI designed and monitored the experiments, edited the manuscripts and provided fund for the research. All authors read and approved the final manuscript.*

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### ABSTRACT

**Aims:** To screen potato genotypes for salt tolerance using *in vitro* salt stress and molecular markers.

**Experimental Design:** The experiment was arranged in Completely Randomized Design (CRD) with three replications.

**Place of the Study:** The experiment was conducted at the Molecular Biology and Tissue Culture Laboratories, Institute of Biotechnology and Genetic Engineering, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh.

**Methodology:** *In vitro* screening of five potato genotypes (CIP 112, CIP 117, CIP 120, CIP 127, and CIP 128) was done in an agar medium using tissue culture technique at different concentrations of salt viz. 0.0, 10, 20, 40, 60, 80, 100, 120 mM of NaCl. All genotypes were further analysed through SSR markers using three primers. The DNA bands were visualized on gel

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electrophoresis and scored for polymorphic loci, gene diversity, and genetic distance. NTSYSpc program was used for constructing unrooted neighbor-joining tree and scatter diagram.

**Results:** Potato genotypes CIP 112 and CIP 117 emerged as the most salt tolerant genotypes with the highest plant height, shoot dry weight, root length, and root dry weight at different concentrations of NaCl. CIP 127 and CIP 128 were poorly tolerant to salt stress. The most sensitive genotype CIP 120 produced minimum plant height, shoot dry weight, root length, and root dry weight at different NaCl concentrations. Results indicated that significant differences were found among cultivars. The banding pattern of microsatellite confirmed a distinct polymorphism among salt tolerant, moderately salt tolerant and salt sensitive lines. The clustering pattern of the potato genotypes suggests that variations occur due to genotypic variation and possibly not epigenetic adaptation under salt stress conditions.

**Conclusion:** The salt tolerant potato genotypes CIP 112 and CIP 117 can be used for developing salt tolerant genotypes.

*Keywords:* Salt tolerant; potato; *in vitro*; marker; SSR.

## 1. INTRODUCTION

The potato (*Solanum tuberosum* L.) is one of the most important vegetable crops in the world. Salt stress is considered a major abiotic stress that limits potato productivity globally. In Bangladesh, one million hectares of the coastal area are affected with varying degrees of soil salinity ranging from 3.63-27.67 dS m<sup>-1</sup> [1]. In the south and south-western parts of Bangladesh like Chittagong, Barisal, and Khulna divisions, potato cultivation is decreasing due to the rising of salinity. The most practical and economical approach to overcome this barrier is to screen and develop salinity tolerant potato genotypes for this salt-affected region.

Under such circumstances, there are plenty of scopes to find out genotypes that have the inherent capability of producing relatively higher yields by withstanding the moderate salinity conditions. More reliable and time saving selection techniques have been developed using tissue culture technology [2]. *In vitro* determination of salinity tolerance, utilizing nodal cuttings of tissue culture propagated (micro-propagated) plants allowed ranking of potato cultivars and wild species. Molecular characterization is an important biotechnology tool for studying genotypic variation in plant breeding programs. Microsatellites, also called SSRs (Simple Sequence Repeats), are one of the more polymorphic molecular markers available today [3]. Microsatellites also have advantages over other markers based on PCR (Polymerase Chain Reaction), such as Random Amplified Polymorphic DNA (RAPD), because they are codominant and easily reproducible and have a frequent and random distribution, allowing a wide coverage of the genome. Several studies

have used SSR markers for the characterization of potato cultivars and accessions [4,5]. In this study, five BARI potato lines were screened for their salt tolerance through *in vitro* culture and SSR markers.

## 2. MATERIALS AND METHODS

### • Potato genotypes and explants

Five potato genotypes namely, CIP 112, CIP 117, CIP 120, CIP 127, and CIP 128, developed by Tuber Crop Research Centre (TCRC), Bangladesh Agricultural Research Institute (BARI), Bangladesh, were used as experimental materials in this present investigation. Sprouts of healthy, disease free and medium sized potato were used as explants.

### 2.1 *In vitro* culture

Healthy and disease-free seed tuber of potato lines were surface sterilized by 70% ethanol for 30 seconds followed by washed with 0.05% Sodium hypochlorite (NaOCl) for 20 minutes and then rinsed four times with sterilized water. After air dry, the explants were placed in the test tube containing MS agar [6] placed in the dark at 25±2°C in a growth room for shoots induction.

After 3-6 weeks of inoculation, sprouted shoots started to produce potato plants. Single node cultures (SNC) from the explants were transferred to the MS agar medium supplemented with different concentrations of NaCl viz, 10 mM L<sup>-1</sup>, 20 mM L<sup>-1</sup>, 40 mM L<sup>-1</sup>, 60 mM L<sup>-1</sup>, 80 mM L<sup>-1</sup>, 100 mM L<sup>-1</sup>, 120 mM L<sup>-1</sup>, no NaCl (control). The cultures were incubated in a temperature-controlled growth room at 25±20C

under a 16 hr light photoperiod with a light intensity of about 250-300 lux for plant growth. Day-to-day observations were carried out to note the response.

## 2.2 Recording of Data

To observe the effect of different treatments of the experiment, after four weeks, data were collected on the following parameters: shoot length (cm), root length (cm), shoot dry weight (mg), and root dry weight (mg).

## 2.3 DNA Extraction

Genomic DNA was extracted according to the modified CTAB (Cetyltrimethylammonium bromide) method [7]. Approximately 20 mg fresh young tender leaf was taken into Eppendorf 34 tube, 700 µl extraction buffer, 100 µL 20% SDS solution, 100 µl 5M NaCl, 100µl CTAB(10X) were added and incubated at 65°C for 5 min. To remove any solid particles, centrifugation was done at 12000 rpm for 5 min. Then 900 µl chloroform: isomylalcohol (24:1) was added and centrifuged at 12000 rpm for 5 min. After that 500 µl of isopropanol was added, and centrifugation was done at 13000 rpm for 30 min. The supernatant was rinsed with 70% ethanol. Then it was centrifuged again at 12000rpm for 5min. After that, ethanol was discarded, and DNA pellets were dried. At last, the pellets were suspended in 1×TE buffer.

## 2.4 SSR Marker selection and Polymerase Chain Reaction (PCR)

Three SSR primers were selected for gel run namely, STM 1106 (F: TCCAGCTGATTGGTTAGGTTG, R: ATGCGAATCTACTCGTCATGG), STM 0030 (F: AGAGATCGATGTAAACACGT, R: GTGGCATTGATGGATT) and STM 1031 (F: TGTGTTTGTCTGTAT, R: AATTCTATCCTCATCTCTA) [8].

PCR was performed in 10 µl reactions containing 3 µl DNA, 1 µl 10X reaction buffer, 2 µl 25 mM MgCl<sub>2</sub>, 0.8µl of 25mM dNTP, 0.5 µl each of 10 µM forward and reverse primers and 0.2 µl of Taq DNA polymerase. A single channel pipette was used for transferring DNA from the dilution plate to the PCR plate.

## 2.5 SSR data scoring and analysis

After electrophoresis, bands were observed in the case of the SSR marker. Three molecular

weight markers were used to estimate the size of the amplified products by comparing the distance traveled by each fragment with known sized fragments of 39 molecular weight markers. All the distinct bands or fragments (SSR markers) were there by identification numbers according to their own gel and scored visually on the basis of their presence (1) or absence (0), separately for each individual and each primer. The scores obtained using all primers in SSR analysis were then pooled to create four data matrices. This was used to estimate polymorphic loci, gene diversity, and genetic distance. NTSYSpc program was used to construct an unrooted neighbor-joining tree and a scatter diagram among the five potato cultivars. Genetic distance was computed from frequencies of polymorphic markers to estimate the genetic relationship among the five potato genotypes.

## 2.6 Statistical analysis of data

The experiment was conducted in a growth room and arranged in Completely Randomized Design (CRD) with three replications. The mean differences of the treatments were compared by the Least Significant Difference (LSD) test using Statistix 10 statistical package.

## 3. RESULTS AND DISCUSSION

In the present study, *in vitro* techniques for plant growth have been established very carefully using sprouting potato tuber shoot as an explant of five lines of potato viz., CIP 112, CIP 117, CIP 120, CIP 127, CIP 128. The effect of different concentrations of NaCl on shoot length, root length, shoot dry weight, and root dry weight were investigated. The results are elaborated based on the nature of morphogenic response of lines, salts, and salt concentrations and their effect in this investigation. The analysis of variance on different parameters was performed to investigate the superiority of cultivars regarding *in vitro* response.

### 3.1 Response of Shoot Length

The *in vitro* regenerated potato cultivars were screened after four weeks under different salt concentrations. Shoot length (cm) was significantly influenced by salinity level (Fig. 1). CIP 112 and CIP 117 lines showed better performance from any other genotypes at different salinity levels, followed by CIP 127 and 128 lines. CIP 127 and 128 showed poor performance. Overall shoot length was

decreased with the increase of salinity level. It was also previously reported that the shoot length of potato genotypes decreased with the increase in salinity level [9]. Osmotic effects, specific-ion toxicity and/or nutritional disorders have a direct effect on shoot length [10]. It has also been documented that the response of potato cultivars to salt stress is genotype-dependent [11].

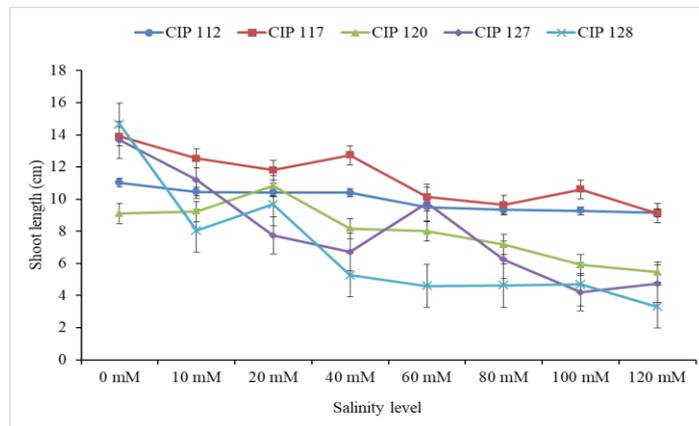
### 3.2 Response of Root Length

Statistically significant variation was found in the length of root, and root length was decreased with the increase of salinity level (Fig. 2). CIP 112 and CIP 117 lines showed the highest result at different salinity levels. Statistically poor performance was found in CIP 120, CIP 127, and CIP 128. High levels of soil salinity can significantly inhibit seedling growth due to the combined effects of high osmotic potential and specific ion toxicity [9]. High concentration of salts in the root zone decreases soil water potential and the availability of water [12]. This deficiency

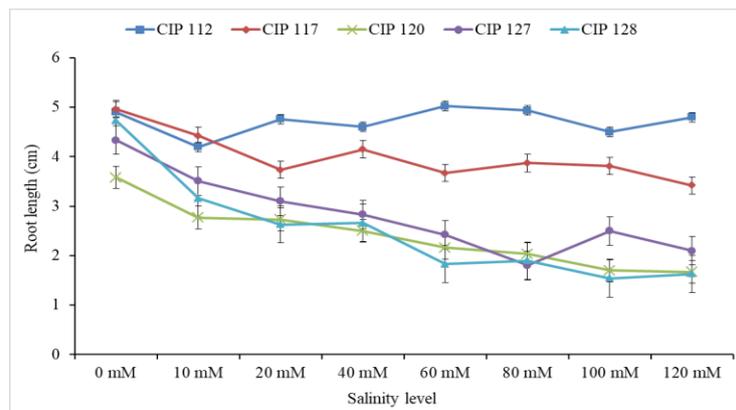
in available water under saline conditions caused dehydration at the cellular level and ultimately osmotic stress occurs [13].

### 3.3 Response of Shoot Dry Weight

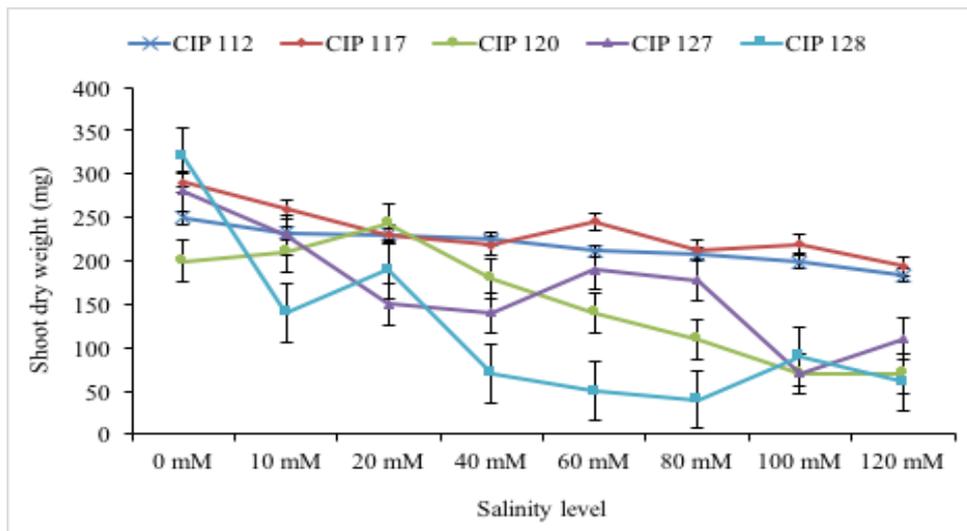
The effect of salinity on the shoot dry weight of five different potato genotypes represents a significant difference (Fig. 3). Overall better performance of shoot dry weight at different salinity levels was found in CIP 112 and CIP 117 lines. CIP 127 and 128 represent similar patterns but much poor, and CIP 120 also presented poor results. There was a marked reduction in shoot dry weight of CIP 120, CIP 127, and CIP 128 with the increasing level of salinity. In general, it was observed that with the increase of NaCl concentration, the shoot dry weight of all lines significantly decreased. Plants growing in the presence of increasing NaCl concentrations decreased their shoot and root dry weight in all potato cultivars which was reported by elsewhere [14].



**Fig. 1. Shoot length performance of potato genotypes at different salinity levels**  
(LSD=Least Significant Difference at 5%, LSD value=1.4990)



**Fig. 2. Root length performance of potato genotypes at different salinity levels**  
(LSD=Least Significant Difference at 5%, LSD value=0.7612)



**Fig. 3. Shoot dry weight performance of potato genotypes at different salinity levels**

LSD=Least Significant Difference at 5%, LSD value=53.638

### 3.4 Response of Root Dry Weight

Root dry weight decreased with the increase in the salinity levels. Salinity level 120 mM had the maximum effect in reducing the root dry weight (Fig. 4). The maximum root dry weight was recorded in CIP 112 and CIP 117 at different levels of salinity. Significantly the least root dry weight was observed in CIP 120, CIP 127, and CIP 128 at different salinity levels. The reduction of root dry weight in increased salinity levels is due to combination of osmotic and specific ion effects ( $\text{Cl}^-$  and  $\text{Na}^+$ ). Similar results were obtained earlier in tomato cultivars [15]. Saline stress leads to change in the growth, morphology, and physiology of the roots, which in turn changes the water and ion uptake. The plants grown under high salinity fail to activate the dehydration avoidance mechanism like making root membranes impermeable for toxic ions of  $\text{Na}^+$  and  $\text{Cl}^-$ . Plants cannot maintain stomatal conductance up to the desired rate thus could not withstand high salt stress and experienced a reduction in growth [16]. Roots are directly in contact with growth media containing toxic salts that stop the long-term root growth, which indirectly affects the biomass production found. Under saline condition,  $\text{CO}_2$  assimilation of the plant becomes decreased. It is a major energy source for growth and development, so, ultimately, root growth decreases. The reduction in root length caused the decrease in biomass which is commonly observed under salt stress [17].

### 3.5 Molecular Characterization of Potato Genotypes Through SSR Markers

#### 3.5.1 Overall microsatellite diversity

Among the three random primer markers, STM0030 and STM1106 identified the lowest number of alleles (02), and STM1031 identified the highest number of alleles (03) with an average of 2.33 alleles among all genotypes. It also estimated that the marker STM0030 and STM1106 showed the lowest genetic diversity (0.48), and STM1031 showed the highest genetic diversity (0.56) with an average diversity of (0.507) among all genotypes.

The range of Polymorphic information content (PIC) values was from 0.365 to 0.499 with an average 0.410. Lower PIC value indicates that the genotypes under study are not effective for specific markers in case of diversity observation. The lowest PIC value (0.365) was obtained for STM0030, and STM1106 markers and the highest PIC value (0.499) was obtained for STM1031 marker. So, PIC value revealed that STM1031 was the best marker for five different genotypes. The number of alleles amplified by a primer and its PIC values also depends upon the repeat number and the repeat sequence of the microsatellite sequences [18].

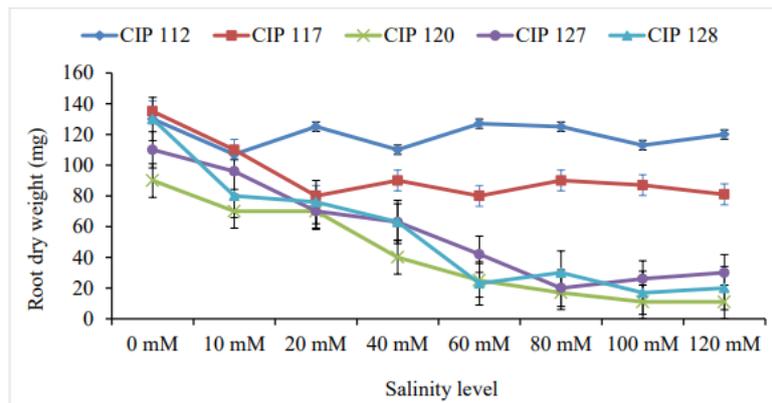
#### 3.5.2 Genetic distance-based analysis

Cluster analysis based on UPGMA (Unweighted Pair-Group Method for Arithmetic Average) with

Nei's genetic distance divided five potato genotypes into three major groups viz. cluster I, cluster II and cluster III (Fig. 5). Cluster I had two genotypes viz. CIP 112 and CIP 117. Cluster II also had two genotypes (CIP 127 and CIP 128). Cluster III had only one genotype, CIP 120.

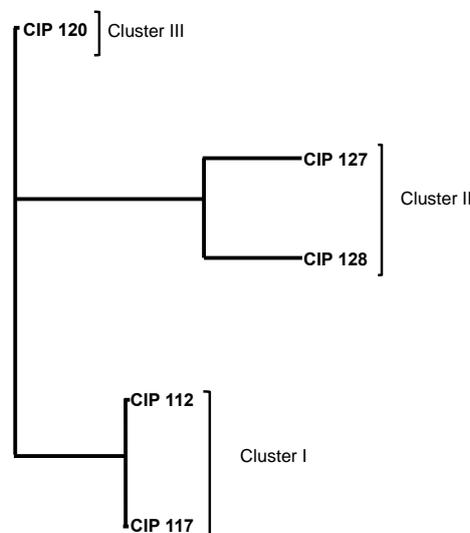
The result indicated that the genotypes CIP 112 and 117 may have a similar genetic background which may be verified through using more markers. Like that genotype CIP 127 and CIP 128 might have the same genetic background. Only CIP 120 showed a totally different genotype. So, similar genotypes residing in the same cluster could be verified using more markers.

The dissimilarity matrix showed that minimum dissimilarity (0%) was in between CIP 112 and 117 genotypes (Table 1). On the other hand, the genotype CIP 120 showed 33% dissimilarity with CIP 112 and 117 genotypes. The genotype CIP 127 showed 100% dissimilarity with CIP 112 and 117 and 66% dissimilarity with CIP 120 genotype. At last the CIP 128 genotype showed 100% dissimilarity with CIP 112 and 117 genotypes, 66% dissimilarity with CIP 120 genotype and 33% dissimilarity with CIP 127 genotype. So, it was estimated that that there was no genetic dissimilarity in between CIP 112 and 117 genotypes and these lines could be suggested for further analysis with more markers.



**Fig. 4. Root dry weight performance of potato genotypes at different salinity levels**

LSD=Least Significant Difference at 5%, LSD value=34.581



**Fig. 5. UPGMA dendrogram based on Nei's (1972) genetic distance between 5 potato genotypes according to SSR analysis**

Legend: Cluster I: (CIP 112, CIP 117), Cluster II: (CIP 127, CIP 128), Cluster III: (CIP 120)

**Table 1. Dissimilarity matrix analysis among the potato genotypes**

Gynotypes	CIP112	CIP117	CIP120	CIP127	CIP128
CIP112	0				
CIP117	0	0			
CIP120	0.3333	0.3333	0		
CIP127	1	1	0.6667	0	
CIP128	1	1	0.6667	0.3333	0

#### 4. CONCLUSION

The salt tolerance of a plant is often defined as the degree to which the plant can withstand, without significant adverse effects, moderate or high concentrations of salt in the water on its leaves or in the soil within reach of its roots. Among all the genotypes, CIP 112 was the best performer, while CIP 120 contributed the lowest result. CIP 112 and CIP 117 were also found to be promising and performed comparatively better than the other genotypes. On the contrary, CIP 127 and CIP 128 performed similarly poorly than the other two better performer genotypes. Finally, CIP 120 showed the poorest result than other genotypes, which indicates that these plants have difficulties taking up water in their leaves resulting in lower growth and development of plants. This research provided baseline information for salt tolerant potato genotypes. Further screening with additional markers and field study are needed for potato breeding program for salt area.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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