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In vitro Screening and Molecular Genetic Markers Associated with Salt Tolerance in Potato

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Authors' contributions

This work was carried out in collaboration between all authors. Author MHSR designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Authors Md. Ashraful Haque and Md. Altaf Hossain managed the analyses of the study. Author MMI managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The main aim of this study was to screen potato germplasms for salt tolerance and to find out the suitable molecular markers associated with salt tolerance in potato lines.
Experimental Design: The present experiment was laid out in Completely Randomized Design (CRD) and replicated thrice.
Experimental Site: The experiment was conducted at the plant molecular laboratory, Department of Biotechnology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur and Tissue culture laboratory of Bangladesh Agricultural Research Institute, Gazipur, Bangladesh.
Methodology: *In vitro* screening of eight potato lines *viz.* CIP 101, CIP 102, CIP 111, CIP 112, CIP 117, CIP 124, CIP 134, CIP 139 along with two popular potato varieties namely Asterix and Diamant were used to screen for salt tolerance at different levels of NaCl concentration (0, 2, 4, 6 and 9

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dS/m). MS20 medium was used as a culture media with different concentration of NaCl where single node cuttings (SNC) from the collected plant materials were transferred accordingly. Minute observations on shoot length, root length, leaf area, shoot dry weight, and root dry weight were documented periodically.
A polymerase chain reaction (PCR) based approach, namely random amplified polymorphic DNA (RAPD) analysis was applied to the selected lines and varieties of potato in order to assess the degree of salt tolerance.
Results: Perusal of the tables revealed a significant variation among the potato lines and varieties. Shoot length, root length, leaf area, shoot dry weight, and root dry weight was decreased with the increase of salt concentration in the used medium. Banding pattern of RAPD confirmed a distinct polymorphism between salt tolerant, moderately salt tolerant and salt sensitive lines. The clustering pattern of the potato genotypes in this study suggests that, the salt tolerance and salt sensitivity of some potato lines are due to the genotypic variation and possibly not for the epigenetic adaptation under salt stress condition.
Comparing the mean value of growth parameters using ANOVA and from RAPD marker analysis, total ten potato genotypes can be classified as follows: the salt tolerant (CIP 102, CIP 112 and CIP 139), the moderately salt tolerant (CIP 111, CIP 124, CIP 134, Asterix and Diamant) and the salt sensitive lines (CIP 101, CIP 117).
Conclusion: The salt tolerant potato lines (CIP 102, CIP 112 and CIP 139) of the present experiment can be used for future breeding programs to develop salt tolerant potato variety.

Keywords: In vitro; screening; RAPD markers; salt tolerance; polymorphism.

1. INTRODUCTION

Potato (*Solanum tuberosum* L.) is a worldwide cultivated tuberous food crop. Regarding human consumption it grades the third position after rice and wheat worldwide [1]. In Bangladesh, it is a notified crop and has become an integral part of the human food supply chain because of its various forms of utilization in their daily diet [2]. Potato has also occupied a good position in processing industries as well as it can be used to produce some other diversified (by) products like alcohol, starch, glue, bio-ethanol, pharmaceutical, cosmetics etc. [3].

Now a days, potato production in Bangladesh is in increasing trend and about 8.4 million MT of potato from 460,000 ha of area has been produced with an average yield of 18 MT/ha. But the production in the coastal area are still low because of high salinity. Coastal area of Bangladesh constitutes 20% area of the country of which 53% are affected at different level of salinity [4]. Salinity problems covers more than 30% of the world's irrigated areas and this problem is a major limitation for the plant growth and productivity especially in potato. Potato has threshold salinity levels from 1.6 to 2.5 dS m-1 and considered as moderately salt sensitive likened with other crops [5]. Due to excessive ion and water shortage salinity may cause several physiological, morphological, biochemical and developmental changes in plants [6]. The most vital process that is influenced by salinity is photosynthesis [4].

Salinity is one of the major constraint for potato production in the southern belt of Bangladesh. That's why, to develop or identify a salinity tolerant potato variety is very important to ensure the food security. Selection and hybridization of salinity tolerant cultivar could be an effective solution in the saline area to ensure potato production [7].

Genetic data on potato genotype are very scarce in this country condition. In field condition, screening of large number of genotype is troublesome because of heterogeneity of soil condition and seasonal fluctuations. One of the reliable and time saving technique for selection of desirable trait under selection pressure without having any environmental influence is the potato tissue culture [8]. Molecular variation in the tissue cultured plants has been characterized at DNA level. So, to find out natural genetic variation and selection in stressed environmental condition is completed with molecular marker technology [9].

Different molecular markers *viz*., restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP), simple sequence repeat SSR and inter-simple sequence repeat (ISSR) are being utilized to characterize the genetic variation. Amongst these molecular

markers, RAPD marker requires small amount of DNA and the marker analysis is easy, simple and quick [10]. These benefit of RAPD marker justify the use and popularity of it for genetic diversity studies. Keeping in view the above-mentioned considerations, the present study set out to screen potato germplasms for salt tolerance as well as to identify molecular markers associated with salt tolerance in potato by RAPD technique.

2. MATERIALS AND METHODS

2.1 Plant Materials

Eight potato lines namely CIP 101, CIP 102, CIP 111, CIP 112, CIP 117, CIP 124, CIP 134, CIP 139 and two popular potato varieties Asterix and Diamant were used as experimental materials in the present investigation. Plant materials were collected from the Tuber Crops Research Centre (TCRC) of Bangladesh Agricultural Research Institute (BARI), Gazipur-1701, Bangladesh. Shoot length was measured from every plant in centimeters, from the base to the main stem to top of the longest leaf and average was calculated. Root length was measured from the root tip to upper portion of stem from where root started to form and average was calculated in centimeters. Leaf area (cm^2) was measured after calculating the one-sided green leaf area per unit ground surface area. Shoots and roots were separated and washed with water to remove surface salts. Then these plant parts were oven dried at 72°C for 72 hours and weighted (gm) to get dry mass.

2.2 *In vitro* Culture and Treatment

MS20 medium [11] was used as a culture media with different concentration of NaCl. Four levels of salt concentration were taken for each treatment: 25 mM/l or 2 dS/m NaCl, 50 mM/l or 4 dS/m NaCl, 75 mM/l or 6 dS/m NaCl and 100 mM/l or 9 dS/m NaCl. Single node cuttings (SNC) from the collected plant materials were transferred to culture medium treated with different level of salt and kept in a climate chamber for shoot induction in dark at 25±2°C. After three to six-week observations were carried out to note the responses.

2.3 DNA Extraction

Genomic DNA was extracted from salt treated fresh young tender leaves. Approximately 20 mg of leaf from each replication was taken into Eppendorf tube. Then, 300 µl tissue lysis buffer and 10 µl of RNAse solution was added and homogenized by homogenizer. To remove any solid particles centrifugation was done. After that 300 µl of nuclease free water was added and each plant lysate was transferred from the extraction tube to plant DNA extractor machine Maxwell 16 (origin: Promega, USA) to extract DNA [12].

2.4 Primer Selection and PCR Amplification

Ten RAPD primers (Table 1) were selected to evaluate the molecular polymorphism among the potato varieties. All the primers were selected from previous works and related to salt tolerant gene expression [13,14]. PCR reaction was performed using Promega PCR master mix kit. Master mixture is a premixed, ready-to-use solution containing *Taq* DNA polymerase, dNTPs, MgCl2 and reaction buffers at optimal concentrations for efficient amplification of DNA templates by PCR. The PCR products were resolved at 100 V for 4 hours, 1.2% agarose gel prepared in 1x Tris-borate-EDTA (TBE) buffer. Gel was photographed using Gel-Documentation system (ALPHAIMAGER TM 2200).

Table 1. List of Primers used for RAPD analysis along with their sequence

S/N	Primer	Primer sequence (5' - 3')	Number of base pairs
1	OPA 03	AGTCAGCCAC	10
2	OPA 04	AATCGGGCTG	10
3	OPB 01	GTTTCGCTCC	10
4	OPB 04	GGACTGGAGT	10
5	OPC 03	GGGGGTCGTT	10
6	OPC 04	CCGCATCTAC	10
7	OPD 01	ACCGCGAAGG	10
8	OPD 03	GTCGCCGTCA	10
9	OPD 04	TCTGGTGAGG	10
10	OPE20	AACGGTGACC	10

2.5 Data Analysis

The data for the characters under study were statistically analyzed wherever applicable. Data were analyzed using MSTAT-C statistical package developed by Gomez and Gomez [15]. The mean differences of the treatments were adjusted by least significant difference (LSD) test at 5% level of significance.

3. RESULTS AND DISCUSSION

3.1 Growth Parameter

3.1.1 Shoot length (cm)

Shoot length (cm) were significantly influenced by different salinity level (Fig. 1), decreased with the increase of salinity. CIP 139 line showed overall better performance from any other line at different salinity level. CIP 102 and CIP 112 lines were shown very similar result which is near to CIP 139 line. CIP 101 and CIP 117 lines produced small shoot length at high salinity level than any other lines of this experiment. CIP 111, CIP 134, Asterix and Diamant were shown moderate results. Egeh and Zamora [16] reported that shoot length of potato varieties decreased with the increase in salinity level. Läuchli and Epstein [17] found that salinity affects plants in different ways such as osmotic effects, specific-ion toxicity and/or nutritional disorders which have direct effect on shoot length. It has been documented that, the response of potato cultivars to salt stress is genotype dependent [18].

3.1.2 Root length (cm)

Root length is an important character in *in vitro* potato screening. Significant variation was found in length of root among the potato lines (Fig. 2). CIP 112 line produced highest root length at different salinity level than other lines. Statistically similar results were found in CIP 102 and CIP 139 lines. Very poor root length was found in CIP 101 and CIP 117 lines. High levels of soil salinity can significantly inhibit seedling growth, due to the combined effects of high osmotic potential and specific ion toxicity [19]. High concentration of salts in the root zone decreased soil water potential and the availability of water [20]. This deficiency in available water under saline condition caused dehydration at cellular level and ultimately osmotic stress occurs [21].

3.1.3 Leaf area (cm^2)

Leaf area (cm^2) is the vital growth parameter of the potato plantlet. Significant variation was found in number of leaves per plantlet (Table 2). At 2 dS/m NaCl salinity level, leaf areas were ranged from 2.5 cm^2 to 3 cm^2. At 6 dS/m, highest leaf area was recorded in CIP 102 (2.4 cm^2) line and lowest in CIP 101 (1.7 cm^2). In addition to this, CIP 102 had highest leaf area in all salinity level. Munns [22] reported that

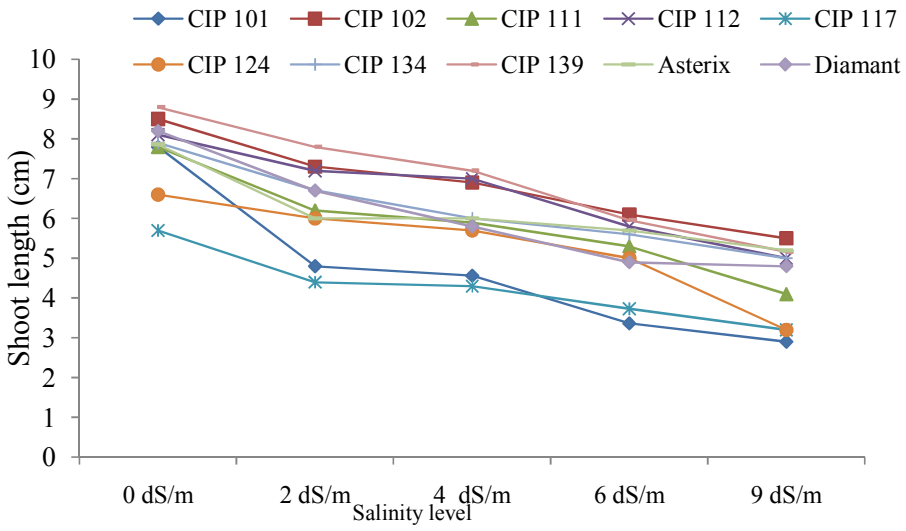

Fig. 1. Shoot length performance of potato lines at different salinity level
LSD=Least Significant Difference at 5%, CV (%)= 6.82, LSD value= 0.6520

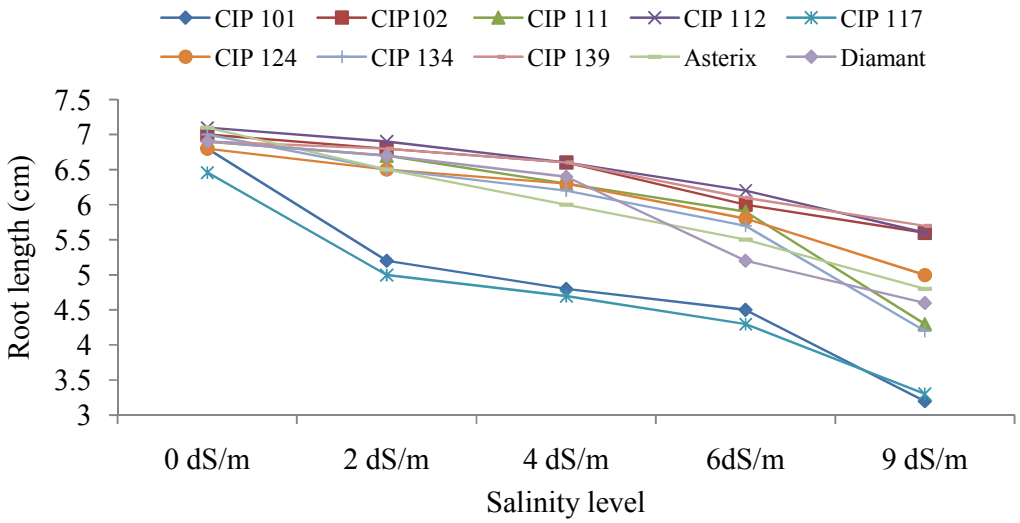

Fig. 2. Root length performance of potato lines at different salinity level
LSD=Least Significant Difference at 5%, CV (%)= 4.11, LSD value= 0.0.3935

Table 2. Interaction effect of different level of NaCl on leaf area of different potato lines

Potato lines	leaf area (cm^2) at different salinity level				
	0 dS/m	2 dS/m	4 dS/m	6 dS/m	9 dS/m
CIP 101	2.8 cde	2.5 fghe	1.9 lmn	1.7 nop	1.5 p
CIP 102	3.2 a	3 abc	2.8 cde	2.4 ghi	2.2 ijk
CIP 111	3.1 ab	2.8 cde	2.5 fgh	2.27 hijk	1.8 mno
CIP 112	3 abc	2.9 bcd	2.7 def	2.3 hi	2.1 jk
CIP 117	2.9 bcd	2.4 ghi	2.1 jkl	1.8 no	1.6 op
CIP 124	2.9 bcd	2.7 def	2.5 fgh	2.0 kl	1.9 lmn
CIP 134	3 abc	2.7 def	2.3 hij	2.1 jhijkl	2 klm
CIP 139	3.2 a	2.9 bcd	2.7 def	2.3 hi	2.0 lmn
Asterix	3.1 ab	2.77 cdef	2.4 ghi	2.0 kl	1.7 nop
Diamant	2.9 bcd	2.6 efg	2.1 jkl	1.9 lmn	1.6 op
CV (%)	6.95				

moments after salinization, cells dehydrate and shrink, but regain their original volume hours later. Despite this recovery, cell elongation and to a lesser extent cell division, are reduced leading to lower rates of leaf and root growth. Over the next days, reductions in cell division and elongation translate into slower leaf appearance and size.

3.1.4 Shoot dry weight (mg)

Salinity effect on shoot dry weight of ten different potato line represents a significant difference (Fig. 3). Overall better performance of shoot dry weight at different salinity level was in CIP 102 line. CIP 139 and CIP 112 lines represent similar pattern in case of shoot dry weight at different salinity level. Asterix, Diamant and CIP 111 had similar shoot dry weight. On the other hand, there was a marked reduction in shoot dry weight of CIP 101 and CIP 117 with increasing level of salinity. In general, it can be said that with the increase in NaCl, 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 also reported by Zhang and Blumwald [23], Pour et al. [24].

3.1.5 Root dry weight (mg)

Salinity level at 9 dS/m had the maximum effect in reducing the root dry weight (Table 3). Control treatment (no salinity) had the maximum root dry weight. The maximum root dry weight was recorded in CIP 102, CIP 112 and CIP 139 at the

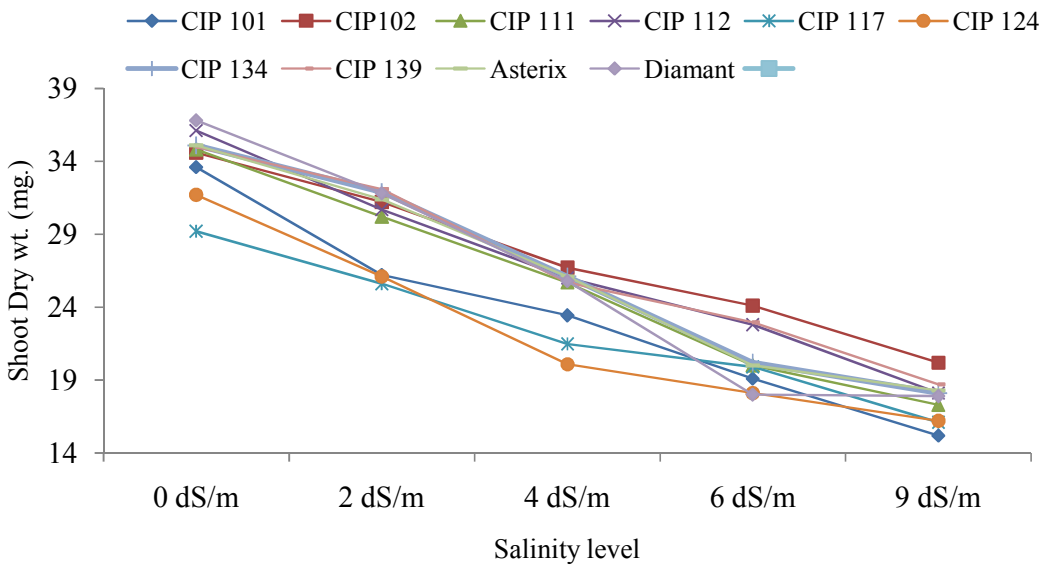

Fig. 3. Shoot dry weight performance of potato lines at different salinity level
LSD=Least Significant Difference at 5%, CV (%)= 3.55, LSD value= 1.458

different level of salinity. Significantly the least root dry weight was observed in CIP 101(3.30 mg) and CIP 117 (3.40 mg) at the salinity level of 9 dS/m. The reduction of the dry weight of root due to increased salinity may be a result of a combination of osmotic and specific ion effects of Cl- and Na+. Similar results were obtained earlier by Mohammad et al. [25] in tomato cultivars. Saline stress leads to changes in growth, morphology and physiology of the roots that will in turn change water and ion uptake revealed by Tyerman and Skerret [26].

The plants grown under high salinity (9 dS/m) fail to activate the dehydration avoidance mechanism like making root membranes impermeable for toxic ions of Na^+ and Cl^-. Plants cannot maintain stomatal conductance up to desired rate thus could not withstand high salt stress and experienced the reduction in growth observed by Abbruzzese et al. [27]. Roots are directly in contact with growth media containing toxic salts that stop the long-term root growth which indirectly affect the biomass production found by Tyerman and Skerrett [26]. Syvertsen et al. [28], Kasukabe et al. [29] found that under saline condition, CO_2 assimilation of plant become decreased and it is major energy source for the growth and development, ultimately root growth decreases. The reduction in root length caused the decrease in biomass which is commonly observed under salt stress condition [30,31]. A decrease in root length with the increments in salinity in present assessment confirms the results of Syvertsen et al. [28], Kasukabe et al. [29] and Katerji et al. [32].

Table 3. Interaction effect of different level of NaCl on root dry weight of different potato lines

Potato lines	Root dry weight (mg) at different salinity level				
	0 dS/m	2 dS/m	4 dS/m	6 dS/m	9 dS/m
CIP 101	7.1 abc	5.1 no	4.9 nop	4.6 pqr	3.3 s
CIP 102	7.3 a	6.9 bcde	6.7 cdef	6.1 hijk	5.7 klm
CIP 111	7.0 bcd	6.8 cdef	6.4 fghi	5.9 jkl	4.4 qr
CIP 112	7.3 a	7.0 bcd	6.7 cdef	6.2 kl	5.7 klm
CIP 117	7.1 abc	5.1 no	4.8 opq	4.4 qr	3.4 s
CIP 124	7.1 abc	6.6 defg	6.4 fghi	5.9 jkl	5.1 no
CIP 134	7.3 a	6.4 fghi	6.1 hijk	5.8 jkl	4.3 r
CIP 139	7.2 ab	6.9 bcde	6.7 cdef	6.2 kl	5.8 jkl
Asterix	7.3 a	6.6 defg	6.1 hijk	5.6 lm	4.9 nop
Diamant	7.2 ab	6.8 cdef	6.5 efgh	5.3 mn	4.7 opqr
CV (%)	4.21				

3.2 Molecular Characterization through RAPD Markers

3.2.1 Overall RAPD diversity

Using ten primers across ten potato lines, a total of 40 alleles were found of which OPC 03 showed the highest number of alleles (5) and OPA 03 showed the lowest number of alleles (3), with an average of 4.0 alleles across the 10 loci (Table 4). The frequency of the most common allele at each locus ranged from 30% (OPB 04, OPB 04, OPC 03, OPC 04, OPE 20) to 40% (OPA 03, OPD 01, OPD 03, OPD 04). The polymorphic information content (PIC) was ranges from 0.58 to 0.72 with an average of 0.65. The highest PIC value (0.72) was obtained for OPC 03, OPC 04, OPE 20 followed by OPB 01 and OPB 04 (0.6918) (Table 4). PIC value revealed that OPC 03, OPC 04, OPE 20 are the best marker for these ten lines. Fig. 4 showed gel pictures of amplified fragment using RAPD primers OPD-03.

Lower PIC value indicates that the genotypes under study are of closely related types, while the higher value of the PIC indicates higher diversity of the materials which is better for development of newer varieties.

The number of alleles amplified by a primer and its PIC values also depends upon the repeat number and the repeat sequence of the RAPD sequences [33,34]. Top PIC value was observed in this study for the primers OPC 03, OPC 04, OPE 20 with the repeat motif sequence GT, CT, AGC.

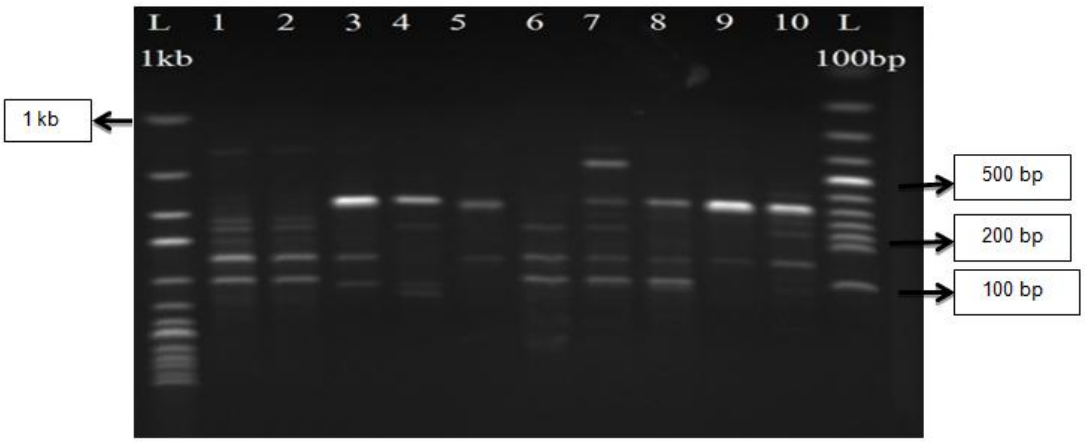

Fig. 4. RAPD profile of 10 potato lines using primer OPD-03

Lane: 1= Diamant, 2= Asterix, 3= CIP 139, 4= CIP 134, 5= CIP 124, 6= CIP 117, 7= CIP 112, 8= CIP 111, 9= CIP 102, 10= CIP 101, L= Ladder 1kb and 100 bp

Table 4. Number of alleles, allele size range, highest frequency allele and polymorphism information content (PIC) found in 10 potato lines

Marker name	Allele no.	Size range (bp)	Highest frequency allele		PIC value
			Size(bp)	Freq (%)	
OPA 03	3	63-255	193	40%	0.5862
OPA 04	4	115-220	115,181,220	33%	0.6660
OPB 01	4	117-343	178,343	30%	0.6918
OPB 04	4	179-350	223,350	30%	0.6918
OPC 03	5	229-421	323,421	30%	0.7204
OPC 04	4	184-421	184,421	30%	0.7204
OPD 01	4	234-354	276	40%	0.6454
OPD 03	4	165-330	260	40%	0.6454
OPD 04	4	184-410	189	40%	0.6454
OPE20	4	223-280	234,280	30%	0.7204

3.2.2 Genetic distance-based analysis

Cluster analysis based on UPGMA (Unweighted Pair-Group Method for Arithmetic Average) with DICE genetic distance divided the ten potato lines into two major groups, cluster I and cluster II (Figure 5). Cluster I was divided into two sub clusters, i.e. cluster IA and cluster IB. Cluster IA consist of three lines viz. Diamant, CIP 134 and CIP 101. On the other hand, cluster IB consist of CIP 117. Furthermore, Cluster II also divided into two sub clusters, i.e. cluster IIA and cluster IIB. Cluster IIA constructed by two lines viz. Asterix and CIP 111. Cluster IIB made of four lines viz. CIP 139, CIP 112, CIP 102 and CIP 124.

Additionally, ten lines were classified into four clusters when similarity coefficient was considered as 25% (Table 5). It was also obtained from Cluster analysis based on UPGMA that the pair of lines viz. Diamant and CIP 134 which belongs to Cluster-I and CIP 102, CIP 112 and CIP 139 was in cluster IV. The result indicated that the genotypes viz. Diamant and CIP 134 (Cluster I), CIP 102, CIP 112 and CIP 139 (Cluster II) might be same genetic background which could be verified using more markers.

A dendrogram based on Nei's [35] genetic distance using unmeasured pair group method of arithmetic mean (UPGMA) was established with 10 popular potato lines (Table 6). The result indicated that, low and high level genetic distance exists between the genotypes. The line CIP 102, CIP 139, and CIP 112 showed highest level of genetic diversity from Asterix, Diamant, CIP 134, CIP 111, and CIP 117 which can be used for further potato breeding program, and in between them they show lower genetic diversity.

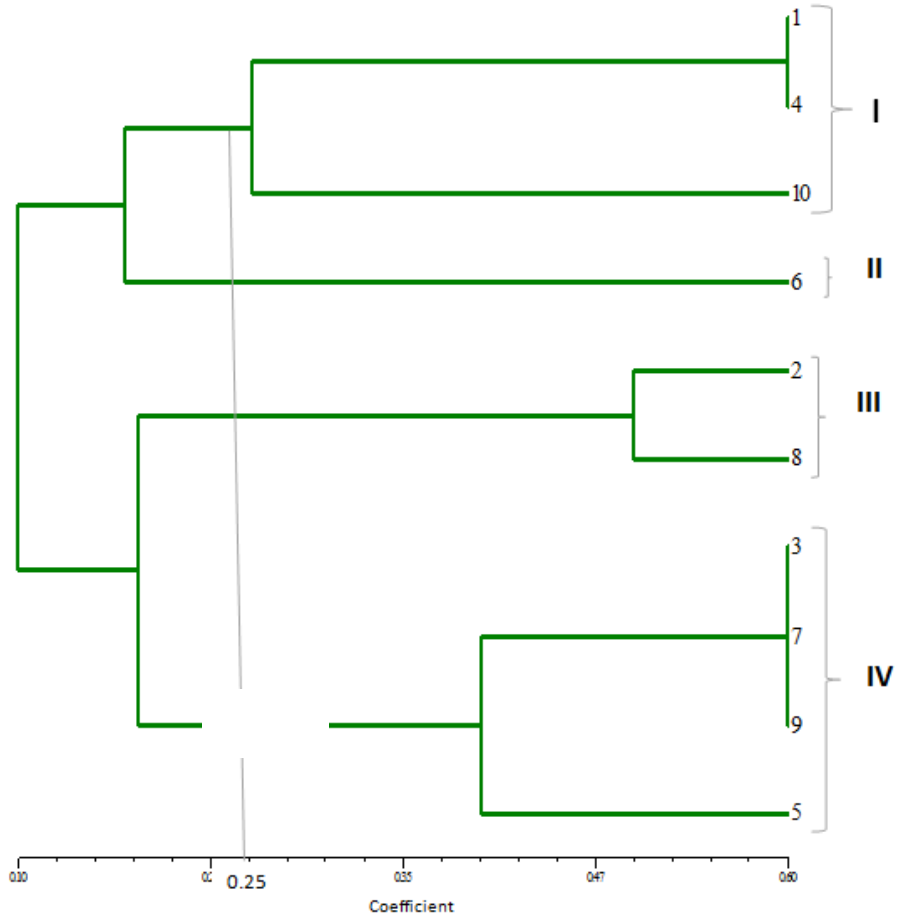

Fig. 5. UPGMA dendrogram based on Nei's (1972) genetic distance, between 10 potato lines according to RAPD analysis

Legend- Cluster I: Diamant (1), CIP 134 (4), CIP 101 (10); Cluster II: CIP 117 (6); Cluster III: Asterix (2), CIP 111 (8); Cluster IV: CIP 139 (3), CIP 112 (7), CIP 102 (9), CIP 124 (5)

Table 5. Distribution of 10 potato lines into 4 clusters

Cluster	Number of genotypes	Genotypes
I	3	Diamant, CIP 134, CIP 101
II	1	CIP 117
III	2	Asterix, CIP 111
IV	4	CIP 139, CIP 112, CIP 102, CIP 124

Table 6. Genetic identity (above diagonal) and genetic distance (below diagonal) values among the ten potato genotypes

Genotypes	Diamant	Asterix	CIP 139	CIP 134	CIP 124	CIP 117	CIP 112	CIP 111	CIP 102	CIP 101
Diamant	0.00	0.90	1.00	0.40	0.90	1.00	1.00	0.90	1.00	0.70
Asterix	0.90	0.00	0.80	0.80	0.90	0.80	0.80	0.50	1.00	0.80
CIP 139	1.00	0.80	0.00	1.00	0.70	1.00	0.40	0.70	0.40	0.70
CIP 134	0.40	0.80	1.00	0.00	1.00	0.70	0.90	1.00	1.00	0.80
CIP 124	0.90	0.90	0.70	1.00	0.00	0.90	0.60	0.60	0.50	1.00
CIP 117	1.00	0.80	1.00	0.70	0.90	0.00	0.90	1.00	0.80	0.80
CIP 112	1.00	0.80	0.40	0.90	0.60	0.90	0.00	0.80	0.40	0.80
CIP 111	0.90	0.50	0.70	1.00	0.60	1.00	0.80	0.00	1.00	0.80
CIP 102	1.00	1.00	0.40	1.00	0.50	0.80	0.40	1.00	0.00	0.80
CIP 101	0.70	0.80	0.70	0.80	1.00	0.80	0.80	0.80	0.80	0.00

Sawy et al. [36] reported that, RAPD technique can be successfully applied to determine the genetic fidelity of potato plant. Mondal et al. [37] reported that an understanding of the nature and magnitude of variability among the genetic stock is of prime importance to the breeders. Hence, it is important to analyze the genetic variability of parental materials. Molecular based analysis of present finding can provide information on actual genetic diversity among the potato cultivars.

4. CONCLUSION

Cluster analysis based on UPGMA (Unweighted Pair-Group Method for Arithmetic Average) with DICE genetic distance, CIP 102, CIP 112 and CIP 139 lines are genetically similar and diversified from other genotypes of this experiment. These genotypes were found salt tolerant compared with others. The better performed lines can be used as a tool for the selection of salt tolerant line for field transplantation and for designing breeding programs.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Haverkort AJ, Struik PC, Visser RG, Jacobsen E. Applied biotechnology to combat late blight in potato caused by *Phytophthora infestans.* Potato Research. 2009;52(3):249-64. Available:https://doi.org/10.1007/s11540-009-9136-3
2. Saha KM, Hossain MS. An analysis of present production and marketing situation in Bangladesh. Hortex Foundation Newsl. 2011;11:1-8.
3. Li Y, de Vries R, Slaghek T, Timmermans J, Cohen Stuart MA, Norde W. Preparation and characterization of oxidized starch polymer microgels for encapsulation and controlled release of functional ingredients. Biomacromolecules. 2009;10(7):1931-8. Available:https://doi.org/10.1021/bm900337n
4. Munira S, Hossain MM, Zakaria M, Ahmed JU, Islam MM. Evaluation of potato varieties against salinity stress in Bangladesh. International Journal of Plant and Soil Science. 2015;6(2):73-81. Available:https://doi.org/10.9734/IJPSS/2015/15879
5. Murshed R, Najla S, Albiski F, Kassem I, Jbour M, Al-Said H. Using growth parameters for *in-vitro* screening of potato varieties tolerant to salt stress. Journal of Agricultural Science and Technology. 2015 Mar 1;17(2):483-494.
6. Gomes MA, Pestana IA, Santa-Catarina C, Hauser-Davis RA, Suzuki MS. Salinity effects on photosynthetic pigments,

proline, biomass and nitric oxide in *Salvinia auriculata* Aubl. Acta Limnologica Brasiliensia. 2017;29.

7. Ashraf M, Shahbaz M. Assessment of genotypic variation in salt tolerance of early CIMMYT hexaploid wheat germplasm using photosynthetic capacity and water relations as selection criteria. Photo-synthetica. 2003;41(2):273-80.
8. Balkrishna RA, Shankarrao SS. *In vitro* screening and molecular genetic markers associated with salt tolerance in maize. African Journal of Biotechnology. 2013; 12(27):4251-5.
9. Govindaraj M, Vetriventhan M, Srinivasan M. Importance of genetic diversity assessment in crop plants and its recent advances: an overview of its analytical perspectives. Genetics Research International; 2015.
10. Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research. 1990;18(22): 6531-5.
11. Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum. 1962;15(3):473-97.
12. Inga G, Tamara K, Marina K, Nelly D. Application of PCR-based methods for rapid detection of corn ingredients in processed foods. Int J Nutr Food Sci. 2014;3:199–202.
13. Chakrabarti SK, Birhman RK, Pattanayak D. Genetic similarity analysis and identification of Indian potato cultivars by random amplified polymorphic DNAs; 1999.
14. Hasan SM, Shafie MS, Shah RM. Analysis of random amplified polymorphic DNA (RAPD) of Artemisia capillaris (Wormwood capillary) in East Coast of Peninsular Malaysia. World Appl. Sci. J. 2009;6(7): 976-86.
15. Gomez KA, Gomez KA, Gomez AA. Statistical procedures for agricultural research. John Wiley & Sons; 1984;17.
16. Egeh AO, Zamora OB. Growth and nutrient content of mungbean (*Vigna radiata* L.), cowpea (*Vigna unguiculata* L.) and soybean (*Glycine max* L.) under different levels of salinity. Philippine Journal of crop science. 1992;17(2):75-83.
17. Läuchli A, Epstein E. Plant responses to saline and sodic conditions. Agricultural salinity assessment and management. 1990;71:113-37.
18. Aghaei KE, Ehsanpour AA, Balali GH, Mostajeran AK. In vitro screening of potato (*Solanum tuberosum* L.) cultivars for salt tolerance using physiological parameters and RAPD analysis. American-Eurasian J Agric and Environ Sci. 2008;3(2):159-64.
19. Moisander PH, McClinton EI, Paerl HW. Salinity effects on growth, photosynthetic parameters, and nitrogenase activity in estuarine planktonic cyanobacteria. Microbial Ecology. 2002;43(4):432-42. Available:https://doi.org/10.1007/s00248-001-1044-2
20. Lloyd J, Kriedemann PE, Aspinall D. Comparative sensitivity of 'Prior Lisbon' lemon and 'Valencia' orange trees to foliar sodium and chloride concentrations. Plant, Cell & Environment. 1989;12(5):529-40. Available:https://doi.org/10.1111/j.1365-3040.1989.tb02126.x
21. Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ. Plant cellular and molecular responses to high salinity. Annual Review of Plant Biology. 2000;51(1):463-99. Available:https://doi.org/10.1146/annurev.arplant.51.1.463
22. Munns R. Comparative physiology of salt and water stress. Plant, Cell & Environment. 2002;25(2):239-50. Available:https://doi.org/10.1046/j.0016-8025.2001.00808.x
23. Zhang HX, Blumwald E. Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit. Nature Biotechnology. 2001;19(8):765.
24. Pour MS, Omidi M, Majidi I, Davoodi D, Tehrani PA. In-vitro plantlet propagation and microtuberization of meristem culture in some wild and commercial potato cultivars as affected by NaCl. African Journal of Agricultural Research. 2010;18; 5(4):268-74.
25. Mohammad M, Shibli R, Ajlouni M, Nimri L. Tomato root and shoot responses to salt stress under different levels of phosphorus nutrition. Journal of Plant Nutrition. 1998;21(8):1667-80. Available:https://doi.org/10.1080/01904169809365512
26. Tyerman SD, Skerrett IM. Root ion channels and salinity. Scientia Horticulturae. 1998;78(1-4):175-235. Available:https://doi.org/10.1016/S0304-4238(98)00194-0

27. Abbruzzese G, Beritognolo I, Muleo R, Piazzai M, Sabatti M, Mugnozza GS, Kuzminsky E. Leaf morphological plasticity and stomatal conductance in three *Populus alba* L. genotypes subjected to salt stress. Environmental and Experimental Botany. 2009;66(3):381-8. Available:https://doi.org/10.1016/j.envexpbot.2009.04.008
28. Syvertsen JP, Lee LS, Grosser JW. Limitations on growth and net gas exchange of diploid and tetraploid Citrus rootstock cultivars grown at elevated CO2. Journal of the American Society for Horticultural Science. 2000;125(2):228-34.
29. Kasukabe Y, He L, Nada K, Misawa S, Ihara I, Tachibana S. Overexpression of spermidine synthase enhances tolerance to multiple environmental stresses and up-regulates the expression of various stress-regulated genes in transgenic *Arabidopsis thaliana.* Plant and Cell Physiology. 2004;45(6):712-22. Available:https://doi.org/10.1093/pcp/pch083
30. Vasquez EA, Glenn EP, Guntenspergen GR, Brown JJ, Nelson SG. Salt tolerance and osmotic adjustment of *Spartina alterniflora* (Poaceae) and the invasive M haplotype of *Phragmites australis* (Poaceae) along a salinity gradient. American Journal of Botany. 2006;93(12): 1784-90. Available:https://doi.org/10.3732/ajb.93.12.1784
31. Keutgen AJ, Pawelzik E. Quality and nutritional value of strawberry fruit under long term salt stress. Food Chemistry. 2008;107(4):1413-20. Available:https://doi.org/10.1016/j.foodchem.2007.09.071
32. Katerji N, Van Hoorn JW, Hamdy A, Mastrorilli M. Salinity effect on crop development and yield, analysis of salt tolerance according to several classification methods. Agricultural water management. 2003;62(1):37-66. Available:https://doi.org/10.1016/S0378-3774(03)00005-2
33. Temnykh S, Park WD, Ayres N, Cartinhour S, Hauck N, Lipovich L, Cho YG, Ishii T, McCOUCH SR. Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). Theoretical and applied genetics. 2000;100(5):697-712.
34. Yu SB, Xu WJ, Vijayakumar CH, Ali J, Fu BY, Xu JL, Jiang YZ, Marghirang R, Domingo J, Aquino C, Virmani SS. Molecular diversity and multilocus organization of the parental lines used in the International Rice Molecular Breeding Program. Theoretical and Applied Genetics. 2003;108(1):131-40.
35. Ni J, Colowit PM, Mackill DJ. Evaluation of genetic diversity in rice subspecies using microsatellite markers. Crop Science. 2002;42(2):601-7.
36. El-Sawy AD, Bekheet S, Aly UI. Morphological and molecular characterization of potato microtubers production on coumarin inducing medium. J Agri Biol. 2007;9(5):675-80.
37. Mondal MA, Hossain MM, Rasul MG, Uddin MS. Genetic diversity in potato (*Solanum tuberosum* L.). Bangladesh Journal of Botany. 2007;36(2):121-5. Available:https://doi.org/10.3329/bjb.v36i2.1499

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