



IN VITRO SCREENING FOR SALT STRESS TOLERANCE OF NATIVE AND EXOTIC POTATO GENOTYPES BY MORPHOLOGICAL AND PHYSIOLOGICAL PARAMETERS

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Abstract

This study was performed on *in vitro* bioassay screening for salt tolerance of ten native and six exotic potato genotypes in Bangladesh. Single node was used to evaluate salinity tolerance especially on biomass production. Five different concentrations of NaCl (0 = control, 100, 150, 200 and 250 mM) were used in addition to MS medium and evaluated salt tolerant and sensitive genotype by various morphological and physiological parameters e.g. shoot and root length and its thickness, number of leaves and roots, fresh and dry weight of whole plant and water contents. The ANOVA, DMRT and correlation coefficient were found highly significant at $p < 0.01$ among the genotypes. With salt stress condition highly positive correlation, co-efficient were found between stem length and internodal distance, leaves number, roots number, root length, dry weight of whole plant and fresh weight of whole plant. A dendrogram based on relative values of 10 morphological and physiological parameters of growth under salt conditions were led to clustering into four distinct groups i.e. tolerant, moderately tolerant, sensitive and very sensitive. On the basis of stress tolerance trait indices (STTIs), Arun (92.78) and Ausha (80.27) showed as a highest salt tolerant, Jamal (56.33) and Chollisha (57.03) showed the most salt sensitive potato cultivars. From this finding it may be concluded that *in vitro* screening with bioassay are relatively simple, rapid and convenient and these methods can be used for further advance biotechnological research on potato improvement.

Key words: Dendrogram, *In vitro*, Salt stress, *Solanum tuberosum*, STTIs

Introduction

In terms of human consumption potato is the third most important food crop in the world after rice and wheat. More than a billion of the people worldwide eat potato reported by International Potato Center (2016). Annual production of potato is around 385 million tons and yield is 200,511 mt/ha (FAO 2016). Developing countries produced more than half of the total world potato production (Scott 2011). Bangladesh has a high agro-ecological possibility of growing potato but the average yield is 20.44 mt/ha (BBS 2018), which is very low in comparison with many other countries due to various biotic and abiotic stress factors (FAO 2016). Plants are sessile and sensitive beings that meet a different environmental stresses throughout the life cycle and its growth and productivity are negatively impressed by various environmental factors (Khan and Singh 2008, Tuteja and Sopory 2008, Yadav et al. 2012). Rengasamy (2006) reported that several parts of the world have been affected by environmental stresses like salt, drought, cold and UV, which hampers crop cultivation and yield. The world population is estimated to attain near 10 billion by 2050, which will find serious food deficits. So, tolerant crops should be developed specially for stress prone area to feed the raising world population (Gill and Tuteja 2010).

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Salinity is an important environmental stress factor that usually becomes critical constraint for crop production (Harun-Or-Rashid et al. 2017, Peethambaran et al. 2018). Moreover, increasing salinization of cultivable land is predicted to have devastating global effects, resulting in 30% land loss within next 25 years and up to 50% by the middle of 21st century (Wang et al. 2003). The United Nations Environment Program estimates that approximately 20% of agricultural land and 50% of crop land in the world is salt-stressed (Yokoi et al. 2002). Bangladesh has a coastal area of 2.5 million hectares. About 20% of the net arable land of Bangladesh coastal region is affected by different degrees of salinity (Khanom and Salehin 2012). Agricultural land use in these areas is very poor. Potato is considered as moderately salt sensitive in comparison with other crops since potato tuber yield could be adversely affected at field salinity EC levels of 2.0-3.0 dSm⁻¹ (approx. 22-33 mM NaCl) (Maas and Hoffman 1977).

Genetic variation in salt tolerance within and among plant species can be utilized to screen and select breeding candidates (Epstein and Rains 1987). Conventional field trials are not only time consuming and labour intensive, but also difficult to replicate since the whole plant response to salinity stress is complex and varies with environmental conditions (e.g. season, light, climate, and soil type) (Flowers and Yeo 1989, Siddique et al. 2014). The most effective method to cope with soil salinity problems is through the selection and use of salt tolerant crop plants (Ashraf and Wu 1994). In consideration of the environmental variations in conventional field trials, *in vitro* conditions can provide faster and more precise evaluations of plant growth under saline and drought stress (Banu et al. 2014). Rahman et al. (2008) observed that salinity effect of three Bangladeshi potato cultivars by using single node cutting. *In vitro* evaluations of NaCl or mixed salt stress effects on potato genotypes were proposed as alternatives to the costly, labour intensive and sometimes problematic field based traits (Albiski et al. 2012). Salinity stress of potato has been reported by single-node cuttings (Naik and Widholm 1993), five-node cuttings (Morpurgo 1991), root tip segment or suspension cultures were conducted by Naik and Widholm (1993) and they determined that the relative tolerance of cultivars based on a multivariate analysis of the relative means of six growth parameters of *in vitro* plantlets over a range of salinity levels. So far there is no report on *in vitro* salt tolerance screening of Bangladeshi potato genotypes. The main objective of this study was to assess the potential for *in vitro* screening of ten native and six exotic potato cultivars of Bangladesh for salt tolerance efficiency.

Materials and Methods

Plant materials and culture conditions

For evaluating the response in salt stress, ten native potato lines *viz.* Ausha, Chollisha, Dohazari, Jamalu, Lalpakri, Patnai, Sadaguti, Sheelbilati, Sindurkouta and Surjamukhi and six exotic Potato *viz.* Arun, Asterix, Cardinal, Courage, Diamant and Granola were considered in this study. As primary explants, developed sprouts were measured 1.0 to 2.0 cm in length and remove them from the tubers and washed with distilled water followed by plunging in 70% alcohol for 30 seconds. Then immediately washed with distilled water and subsequently sterilization procedure were done in the laminar air flow cabinet with 0.1% aqueous solution of HgCl₂ for 4-5 minutes. Surface sterilized sprouts were washed 4-5 times with sterilized distilled water and then transferred into 15 ml of MS medium supplemented with 3% (w/v) sucrose (Merck, Germany) and 0.8% agar. Then cultures were maintained under a 16/8 h light/dark photoperiod with 2500 lux light intensity at 25±1°C. In order to assess salt tolerance, single nodes derived from sprout culture were transferred into MS (Murashige and Skoog 1962) medium containing five concentrations (0 = control, 100, 150, 200 and 250 mM) (w/v) of NaCl. In this case, four replicates were considered and each treatment consists of 15 explants. Effect of NaCl were evaluated on following parameters - i) stem length (SL), ii) stem thickness (ST), iii) leaf number (LN), iv) internodal distance (IND), v) root number (RN), vi) root length (RL), vii) root thickness (RT),

viii) fresh weight of whole plant (FWWP), ix) dry weight of whole plants (DWWP) (oven-dried at 70°C for 24 h) and x) plant water content (PWC).

Measurement of various organs

Six-week old *in vitro* grown plants from each cultivar were harvested then washed in sterilized water and morphological data were recorded. All plant organs were measured with the help of software ImageJ (public domain Java image processing program) and analyzed by the statistical software SPSS ver. 20.0. Water content (%) of whole plant and Stress Tolerance Traits Index (STTI) was estimated according to following formula.

Water content (%) of whole plant

After measuring fresh weight of whole plant and dry weight, plant water content was estimated by the following formula:

$$\text{Plant water content (\%)} = \frac{\text{Fresh weight of whole plant} - \text{Dry weight of whole plant}}{\text{Dry weight of whole plant}} \times 100$$

Stress tolerance trait index (STTI)

Major abiotic stress (salt) tolerance index (STI) was estimated as average of STTIs (Shahzad et al. 2012).

$$\text{STTI} = \frac{\text{Value of trait under stress condition}}{\text{Value of trait under no stress}} \times 100$$

Rank

Surviving genotypes at 150 mM salt stress were ranked on the basis of stress tolerance index (STIs).

Experimental design and statistical analysis

The experiment was designed as completely randomized design with four replications and each replication consist of 15 explants. Data were subjected to ANOVA using the statistical software SPSS ver. 20 and were expressed as Mean \pm standard errors. Pearson correlation analysis was achieved to examine degrees of association between characters, and to perform cluster analysis according to the lines response to salt stress based on the sum of all 10 growth parameters relative values by Ward's method (Ward 1963). A hierarchical dendrogram was constructed using PAST (PAleontological STatistics) software.

Results and Discussion

In vitro screening of potato genotypes for water stress tolerance has been offered as an alternative option to costly, labor-intensive and sometimes problematic field-based screening (Gopal and Iwama 2007, Rahman et al. 2008). Albiski et al. (2012) reported that due to spatial heterogeneity of soil chemical and physical properties and seasonal fluctuations it is difficult to screen a large number of genotypes for salt tolerance in the field. The effect of water or salinity stress on *in vitro* potato growth has been reported to be similar that observed under field conditions (Zhang and Donnelly 1997, Gopal and Iwama 2007, Khenifi et al. 2011).

In this study potato genotypes showed decreasing in morphological or physiological parameters when NaCl concentration in the MS medium increased resulting low water potential. Under extreme water stress conditions inducing with 200 mM, 250 mM NaCl all genotypes showed less shoots and in some cases did not produced any shoots and leaves. Getting genotypic difference in this case it was very difficult to measure any

morphological characters. But 100 and 150 mM NaCl, plants produced stems and leaves and no leaf necrosis was found. Differences in morphological characteristics observed only at 150 mM NaCl, while at 100 mM NaCl plant responses were generally similar to the control. Thus the proposed *in vitro* system for screening potato against salt stress only a limited level like 150 mM NaCl can be used for differentiating the genotypes. These results have similarity of findings with single-node cuttings of potato screened for salinity tolerance where use of relatively high level of NaCl failed to quantify difference among cultivars *in vitro* (Morpurgo 1991, Khenifi et al. 2011).

Morphological effects on salt stress

Due to the salt stress factors, stem length (SL) was decreased in all cultivars, ranging from 42.66% in Surjamukhi to 92.28% in Sheelbilati (Table 1). Data showed that the significant difference existed in shoot length in different genotypes of potato under salt condition (Table 3). Increases in stem thickness (ST) due to salt were also observed in all cultivars except Asterix (3.66%) and Courage (13.56%) (Table 1). It was observed that numbers of leaves were decreased due to salt stress in all cultivars ranging from 6.15% (Cardinal) to 68.67% in Sheelbilati (Table 1). Number of leaves was showed significantly differences among the genotypes (Table 3). The high range of variation (3.00-8.88) was found in total number of leaf among the cultivars (Table 3). Internodal distances were decreased in all lines because of salt stress. Such reductions ranged were found from 29.69% in Surjamukhi to 78.57% in Sindurkouta. The ranges of variation (0.17-0.70 cm) were present in the character among the studied genotypes (Table 3).

It was observed that due to salt stress number of roots (13.51%) was reduced in Patnai and 59.18% in Courage and the existence of range of variability (1.88-4.00) shown in Table 1 & 3. Root length was decreased due to salt stress in all cases; the reduction of root length ranged from 19.82% for Cardinal to 75.54% for Sadaguti. Root thickness was increased due to salt from 5.91% in Asterix to 51.55% in Arun and reduction of root thickness was observed from 5.54% in Sindurkouta to 24.52% in Courage when 150 mM NaCl was added in medium. Salt caused a decrease in fresh weight of all cultivars, with decreases ranging from 15.73% in Arun to 83.33% in Jamalu. In case of dry weight of whole plant, almost all cultivars decreased their weight due to salt (150 mM NaCl) ranging from 42% in Ausha to 86.11% in Chollisha except Arun (Table 1).

It was observed that plant water content (PWC) increases due to salt (150 mM NaCl) in the cultivars of Ausha (0.77%), Chollisha (3.69%), Dohazari (3.43%), Sadaguti (4.71%), Sindurkouta (0.60%), Cardinal (0.58%), Courage (0.60%), Diamant (0.39%) and Granola (2.97%). But PWC decreased when 150 mM NaCl added in medium for Jamalu (2.08%), Lalpakri (1.96%), Patnai (0.22%), Sheelbilati (1.89%), Surjamukhi (0.57%), Arun (2.35%) and Asterix (0.95%) (Table 1).

Analysis of variance on salt effects for different morphological characters *viz.* SL, ST, LN, IND, RN, RL, RT, FWWP, DWWP and PWC indicated highly significant difference ($p < 0.01$) among the genotypes for all characteristics (Table 2). Mean comparison of the morphological and physiological traits measured under salt stress condition using DMRT showed that cultivar Cardinal had the highest PWC (95.28%) (Table 3). The highest number of ST, FWWP and DWWP belonged to genotype Arun and highest amount of SL and IND exhibited to genotype Courage. Genotype Ausha, Patnai, Asterix and Diamant had the highest LN, RN, RL and RT, respectively. The result of morphological and physiological traits obviously revealed that culture response was greatly influenced by the potato genotypes.

Table 1. Effect of salt (150 mM NaCl) stress factors on different morphological and physiological parameters of different potato genotypes.

Genotypes	SL (cm) (M ± SE)		ST (mm) (M ± SE)		LN (M ± SE)		IND (mm) (M ± SE)		RN (M ± SE)		
	Cont.	Salt (% ↓↑)	Cont.	Salt (% ↓↑)	Cont.	Salt (% ↓↑)	Cont.	Salt (% ↓↑)	Cont.	Salt (% ↓↑)	
Native	Ausha	7.00±0.29	3.98±0.19 (43.19↓)	0.70±0.03	1.24±0.06 (76.96↑)	9.87±0.44	8.88±0.44 (10.12↓)	0.72±0.04	0.45±0.02 (37.34↓)	5.75±0.25	2.88±0.15 (50.02↓)
	Chollisha	6.61±0.24	0.88±0.04 (86.62↓)	0.58±0.03	0.96±0.05 (64.37↑)	8.75±0.37	3.00±0.16 (65.73↓)	0.76±0.04	0.30±0.01 (61.10↓)	5.00±0.24	2.75±0.14 (45.01↓)
	Dohazari	8.24±0.26	0.66±0.03 (92.05↓)	0.69±0.03	1.23±0.07 (78.84↑)	11.75±0.52	3.75±0.19 (68.10↓)	0.71±0.03	0.18±0.01 (75.32↓)	3.63±0.15	2.68±0.13 (26.23↓)
	Jamalu	5.59±0.23	0.75±0.04 (86.65↓)	0.57±0.03	0.65±0.03 (14.34↑)	10.50±0.50	3.50±0.20 (66.68↓)	0.54±0.03	0.22±0.02 (60.29↓)	2.88±0.12	1.88±0.10 (34.78↓)
	Lalpakri	7.61±0.35	0.91±0.05 (88.08↓)	0.73±0.03	0.94±0.05 (30.03↑)	10.12±0.49	4.88±0.24 (51.85↓)	0.76±0.03	0.19±0.01 (75.36↓)	4.50±0.19	2.62±0.17 (41.78↓)
	Patnai	10.48±0.47	2.96±0.14 (71.77↓)	0.66±0.03	0.88±0.05 (31.98↑)	8.38±0.36	7.38±0.37 (11.95↓)	1.27±0.05	0.40±0.02 (68.09↓)	4.63±0.20	4.00±0.20 (13.51↓)
	Sadaguti	9.26±0.39	1.01±0.05 (89.12↓)	0.77±0.04	1.46±0.07 (90.25↑)	14.25±0.56	5.00±0.25 (64.92↓)	0.65±0.03	0.20±0.01 (68.42↓)	4.58±0.20	3.25±0.16 (28.96↓)
	Sheelbilati	7.09±0.32	0.55±0.03 (92.28↓)	0.55±0.02	1.07±0.06 (93.13↑)	10.38±0.39	3.25±0.18 (68.67↓)	0.69±0.03	0.17±0.01 (75.40↓)	3.63±0.13	2.13±0.11 (41.38↓)
	Sindurkouta	8.02±0.36	0.96±0.05 (87.99↓)	0.70±0.03	1.35±0.07 (93.82↑)	10.25±0.51	5.75±0.29 (43.91↓)	0.78±0.03	0.17±0.01 (78.57↓)	4.75±0.22	2.75±0.15 (42.11↓)
	Surjamukhi	6.55±0.32	3.76±0.19 (42.66↓)	1.12±0.05	1.14±0.06 (2.12↑)	10.13±0.47	8.25±0.41 (18.53↓)	0.65±0.03	0.46±0.01 (29.63↓)	4.38±0.13	3.17±0.16 (27.57↓)
Exotic	Arun	7.69±0.38	3.88±0.19 (49.60↓)	1.48±0.05	2.39±0.12 (62.20↑)	10.00±0.50	7.75±0.39 (22.50↓)	0.77±0.04	0.50±0.03 (34.84↓)	5.75±0.22	3.23±0.16 (43.91↓)
	Asterix	9.60±0.42	3.62±0.18 (62.34↓)	1.12±0.04	1.08±0.06 (3.66↓)	8.63±0.35	5.50±0.29 (36.23↓)	1.16±0.06	0.66±0.03 (42.84↓)	4.75±0.22	3.50±0.18 (26.24↓)
	Cardinal	8.81±0.36	2.61±0.13 (70.40↓)	1.27±0.04	1.45±0.07 (13.75↑)	8.13±0.39	7.63±0.38 (6.15↓)	0.61±0.03	0.36±0.01 (71.32↓)	7.75±0.24	3.32±0.19 (57.16↓)
	Courage	10.54±0.46	5.48±0.28 (48.02↓)	1.11±0.05	0.96±0.05 (13.56↓)	10.50±0.51	8.00±0.41 (23.82↓)	1.03±0.04	0.70±0.03 (32.30↓)	6.13±0.20	2.50±0.14 (59.18↓)
	Diamant	7.25±0.30	1.83±0.09 (74.77↓)	1.13±0.05	1.28±0.06 (14.04↑)	10.38±0.41	6.50±0.33 (37.35↓)	0.73±0.03	0.29±0.02 (60.85↓)	4.74±0.23	2.26±0.16 (52.26↓)
	Granola	9.44±0.30	1.80±0.10 (80.89↓)	0.93±0.04	1.01±0.05 (8.57↑)	12.13±0.47	7.55±0.40 (37.73↓)	0.79±0.03	0.24±0.01 (69.28↓)	5.50±0.19	2.75±0.15 (50.00↓)

Cont. = control, SL = stem length, ST = stem thickness, LN = leaves number, IND = internodal distance, RN = roots number, ↓= Decrease and ↑= Increase.

Contd. Table 1.

Genotypes	RL (cm) (M ± SE)		RT (mm) (M ± SE)		FWWP (gm) (M ± SE)		DWWP (gm) (M ± SE)		PWC (%) (M ± SE)		
	Cont.	Salt (% ↓↑)	Cont.	Salt (% ↓↑)	Cont.	Salt (% ↓↑)	Cont.	Salt (% ↓↑)	Cont.	Salt (% ↓↑)	
Native	Ausha	11.64±0.45	4.22±0.23 (63.74↓)	0.41±0.01	0.44±0.02 (6.81↑)	0.065±0.002	0.042±0.003 (35.38↓)	0.005±0.000	0.0029±0.000 (42.00↓)	92.08±1.00	92.79±2.41 (0.77↑)
	Chollisha	8.65±0.37	2.96±0.15 (65.73↓)	0.27±0.01	0.25±0.01 (8.92↓)	0.042±0.002	0.009±0.000 (78.57↓)	0.004±0.000	0.0005±0.000 (86.11↓)	90.96±0.87	94.32±1.17 (3.69↑)
	Dohazari	7.40±0.35	3.75±0.19 (49.32↓)	0.34±0.01	0.29±0.02 (15.70↓)	0.040±0.002	0.015±0.001 (62.50↓)	0.004±0.000	0.0012±0.000 (71.43↓)	88.99±0.75	92.04±0.83 (3.43↑)
	Jamalu	1.42±0.06	1.03±0.05 (27.64↓)	0.27±0.01	0.25±0.02 (8.79↓)	0.030±0.002	0.005±0.000 (83.33↓)	0.003±0.000	0.0005±0.000 (80.77↓)	91.01±0.90	89.13±2.40 (2.08↓)
	Lalpakri	6.69±0.33	1.95±0.10 (70.83↓)	0.29±0.01	0.35±0.02 (17.35↑)	0.081±0.003	0.023±0.001 (71.60↓)	0.007±0.000	0.0025±0.000 (65.75↓)	90.90±0.92	89.12±0.27 (1.96↓)
	Patnai	6.44±0.29	3.21±0.17 (50.19↓)	0.34±0.01	0.45±0.02 (31.76↑)	0.089±0.004	0.039±0.002 (56.18↓)	0.005±0.000	0.0023±0.000 (54.90↓)	94.24±0.98	94.04±0.42 (0.22↓)
	Sadaguti	7.93±0.37	1.94±0.10 (75.54↓)	0.26±0.01	0.34±0.02 (32.42↑)	0.098±0.004	0.040±0.002 (59.18↓)	0.010±0.000	0.0027±0.000 (73.79↓)	89.04±1.23	93.24±0.48 (4.71↑)
	Sheelbilati	5.79±0.27	2.58±0.13 (55.54↓)	0.32±0.01	0.27±0.02 (16.25↓)	0.010±0.000	0.003±0.000 (70.00↓)	0.001±0.000	0.0003±0.000 (72.73↓)	88.76±0.91	87.08±1.72 (1.89↓)
	Sindurkouta	7.61±0.38	3.13±0.16 (58.88↓)	0.38±0.02	0.36±0.02 (5.54↓)	0.061±0.002	0.014±0.001 (77.05↓)	0.006±0.000	0.0013±0.000 (78.33↓)	90.18±1.14	90.72±0.05 (0.60↑)
	Surjamukhi	6.67±0.31	3.42±0.18 (48.69↓)	0.45±0.02	0.41±0.02 (8.48↓)	0.177±0.006	0.049±0.003 (72.32↓)	0.008±0.000	0.0024±0.000 (68.42)	95.52±1.60	94.98±0.45 (0.57↓)
Exotic	Arun	8.30±0.38	4.59±0.23 (44.72↓)	0.49±0.02	0.74±0.04 (51.55↑)	0.178±0.006	0.150±0.009 (15.73↓)	0.008±0.000	0.0106±0.001 (27.71↑)	95.27±1.38	93.03±0.47 (2.35↓)
	Asterix	9.13±0.45	4.66±0.24 (48.95↓)	0.49±0.02	0.52±0.03 (5.91↑)	0.079±0.004	0.031±0.002 (60.76↓)	0.004±0.000	0.0023±0.000 (47.73↓)	93.38±1.08	92.50±0.23 (0.95↓)
	Cardinal	4.21±0.20	3.37±0.17 (19.82↓)	0.53±0.02	0.56±0.03 (6.25↑)	0.241±0.009	0.084±0.005 (65.15↓)	0.012±0.000	0.0039±0.000 (68.29↓)	94.73±1.16	95.28±0.33 (0.58↑)
	Courage	7.99±0.40	2.69±0.14 (66.36↓)	0.52±0.02	0.39±0.02 (24.52↓)	0.185±0.008	0.045±0.003 (75.68↓)	0.012±0.000	0.0028±0.000 (76.27↓)	93.10±0.87	93.65±0.54 (0.60↑)
	Diamant	8.00±0.40	3.10±0.16 (61.30↓)	0.49±0.02	0.59±0.03 (20.08↑)	0.139±0.006	0.045±0.002 (67.63↓)	0.007±0.000	0.0022±0.000 (69.01↓)	94.70±1.15	95.07±0.19 (0.39↑)
	Granola	8.57±0.36	4.26±0.22 (50.32↓)	0.36±0.02	0.42±0.02 (17.60↑)	0.104±0.005	0.030±0.004 (71.15↓)	0.009±0.000	0.0016±0.000 (81.18↓)	91.65±1.16	94.38±0.72 (2.97↑)

Cont. = control, RL= root length, RT= root thickness, FWWP = fresh weight of whole plant, DWWP = dry weight of whole plant and PWC = plant water content, ↓ = Decrease and ↑ = Increase.

Table 2. Analysis of variance for salt stress effects on various characteristics of potato

SOV	df	MS									
		SL	ST	LN	IND	RN	RL	RT	FWWP	DWWP	PWC
Genotype in salt	15	143.04**	9.03**	232.03**	1.78**	17.71**	60.76**	1.1**	0.078**	0.00**	349.12**
Error	48	3.436	0.781	19.938	0.069	4.662	5.525	0.11	0.002	0.00	221.88

SOV: source of variance, **significant at 1% level of probability. SL = Stem length, ST = stem thickness, LN = leaves number, IND = internodal distance, RN = roots number, RL = root length, RT = root thickness, FWWP = fresh weight of whole plant, DWWP = dry wt of whole plant and PWC = plant water content as foot note.

Table 3. Mean comparison for morphological data under salt stress condition using DMRT

Genotypes	SL	ST	LN	IND	RN	RL	RT	FWWP	DWWP	PWC
Ausha	3.98e	1.24def	8.88f	0.45ef	2.88defg	4.22gh	0.44f	0.04h	0.0029e	92.79cd
Chollisha	0.88ab	0.96bc	3.00a	0.30c	2.75cdef	2.96cde	0.25a	0.01abc	0.0005a	94.32d
Dohazari	0.66ab	1.23def	3.75ac	0.18a	2.68cde	3.75fg	0.29abc	0.02cd	0.0012ab	92.04bcd
Jamalu	0.75a	0.65a	3.50a	0.22ab	1.88a	1.03a	0.25a	0.01ab	0.0005a	89.13ab
Lalpakri	0.91ab	0.94bc	4.88b	0.19ab	2.62cd	1.95b	0.35cd	0.02fde	0.0025de	89.12ab
Patnai	2.96d	0.88b	7.38de	0.40de	4.00i	3.21de	0.45f	0.04fgh	0.0023cde	94.04cd
Sadaguti	1.01b	1.46g	5.00b	0.20ab	3.25fgh	1.94b	0.34bcd	0.04gh	0.0027e	93.24cd
Sheelbilati	0.55a	1.07bcd	3.25a	0.17a	2.13ab	2.58c	0.27ab	0.003a	0.0003a	87.08a
Sindurkouta	0.96ab	1.35fg	5.75bc	0.17a	2.75cdef	3.13de	0.38cde	0.01bcd	0.0013abc	90.72bc
Surjamukhi	3.76e	1.14cde	8.25ef	0.46ef	3.17efgh	3.42ef	0.41def	0.05h	0.0024de	94.98d
Arun	3.88e	2.39f	7.75e	0.50f	3.23fgh	4.59h	0.74h	0.15j	0.0106g	93.03cd
Asterix	3.62e	1.08bcd	5.50b	0.66g	3.50h	4.66h	0.52g	0.03efg	0.0023cde	92.50bcd
Cardinal	2.61d	1.45g	7.63e	0.36d	3.32gh	3.37ef	0.56efg	0.08i	0.0039f	95.28d
Courage	5.48f	0.96bc	8.00e	0.70g	2.50bcd	2.69cd	0.39def	0.05h	0.0028e	93.65cd
Diamant	1.83c	1.28efg	6.50cd	0.29c	2.26abc	3.10cde	0.59g	0.05h	0.0022cde	95.07d
Granola	1.80c	1.01bc	7.55e	0.24bc	2.75cdef	4.26gh	0.42ef	0.03ef	0.0016bcd	94.38d

Mean values followed by the same letter are not significantly different according to Duncan's Multiple Range Test.

SL = stem length, ST = stem thickness, LN = leaves number, IND = internodal distance, RN = roots number, RL = root length, RT = root thickness, FWWP = fresh weight of whole plant, DWWP = dry weight of whole plant and PWC = plant water content as foot note.

Stress Tolerance Trait Index (STTI)

Plant growth was determined by number of plants, leaves, root and shoot length and fresh and dry weights. It was observed that 150 mM NaCl stress showed STTI of shoot length ranged from 7.72% (Sheelbilati) to 57.34% (Surjamukhi) (Table 4). Maximum (193.82%) STTI for shoot thickness was recorded in Sindurkouta and minimum (86.44%) in Courage. Table 4 represents data on salt tolerance trait indices of leaf number ranged from 31.33% (Sheelbilati) to 93.85% (Cardinal). Maximum (70.37%) STTI for internodal distance was gained by Surjamukhi and minimum (21.43%) by Sindurkouta. Under NaCl (150 mM) stress root plays a vital role for plant survival and growth in adverse environmental conditions. STTI of root number varied from 40.82% (Courage) to 86.49% (Patnai) (Table 4). Generally tolerant cultivars revealed better root growth than susceptible ones. Another important salt tolerance trait index of root length ranged from 24.46% (Sadaguti) to 80.18% (Cardinal). Similar to shoot thickness, STTI of root thickness was also increased. It was ranged from 75.48% (Courage) to 151.55% (Arun). Fresh and dry weights of whole plants were affected by 150mM salinity stress. The STTI values of fresh weight of whole plant ranged from 16.67% (Jamalu) to 84.27% (Arun). STTI values of dry weight of whole plant varied from 13.89% (Chollisha) to 127.71% (Arun). The stress tolerance trait index in salt of plant water content ranged from 97.65% (Arun) to 103.69% (Chollisha). According to the stress tolerant indexes (STIs) ranking, studied genotypes are arranged as below:

Arun>Ausha>Patnai>Cardinal>Surjamukhi>Asterix>Sadaguti>Sindurkouta>Dohazari>Diamant>Sheelbilati>Granola >Courage >Lalpakri>Chollisha>Jamalu.

Table 4. STTIs of 10 morphological traits under 150 mM NaCl stress on sixteen genotypes

Genotype	SL %	ST %	LN %	IND %	RN %	RL %	RT %	FWWP %	DWWP %	PWC %	STI %	Rank
Ausha	56.81	176.96	89.88	62.66	49.98	36.26	106.81	64.62	58.00	100.77	80.27	2
Chollisha	13.38	164.37	34.27	38.90	54.99	34.27	91.08	21.43	13.89	103.69	57.03	15
Dohazari	7.95	178.84	31.90	24.68	73.77	50.68	84.30	37.50	28.57	103.43	62.16	9
Jamalu	13.35	114.34	33.32	39.71	65.22	72.36	91.21	16.67	19.23	97.92	56.33	16
Lalpakri	11.92	130.03	48.15	24.64	58.22	29.17	117.35	28.40	34.25	98.04	58.02	14
Patnai	28.23	131.98	88.05	31.91	86.49	49.81	131.76	43.82	45.10	99.78	73.69	3
Sadaguti	10.88	190.25	35.08	31.58	71.04	24.46	132.42	40.82	26.21	104.71	66.74	7
Sheelbilati	7.72	193.13	31.33	24.60	58.62	44.46	83.75	30.00	27.27	98.11	59.90	11
Sindurkouta	12.01	193.82	56.09	21.43	57.89	41.12	94.46	22.95	21.67	100.60	62.20	8
Surjamukhi	57.34	102.15	81.47	70.37	72.43	51.31	91.52	27.68	31.58	99.43	68.53	5
Arun	50.40	162.20	77.50	65.16	56.09	55.28	151.55	84.27	127.71	97.65	92.78	1
Asterix	37.66	96.34	63.77	57.16	73.76	51.05	105.91	39.24	52.27	99.05	67.62	6
Cardinal	29.60	113.75	93.85	58.68	42.84	80.18	106.25	34.85	31.71	100.58	69.23	4
Courage	51.98	86.44	76.18	67.70	40.82	33.64	75.48	24.32	23.73	100.60	58.09	13
Diamant	25.23	114.04	62.65	39.15	47.74	38.70	120.08	32.37	30.99	100.39	61.14	10
Granola	19.11	108.57	62.27	30.72	50.00	49.68	117.60	28.85	18.82	102.97	58.86	12
Average	27.10	141.07	60.36	43.06	59.99	46.40	106.35	36.11	36.94	100.48	65.79	

SL = stem length, ST = stem thickness, LN = leaves number, IND = internodal distance, RN = roots number, RL = root length, RT = root thickness, FWWP = fresh weight of whole plant, DWWP = dry weight of whole plant and PWC = plant water content as foot note.

Correlations co-efficient between morphological parameters due to salt stress conditions were found highly significant for most of the parameters considered under this study (Table 5). For example, shoot length appeared as a function of internodal distance ($R^2 = 0.93$), leaf number ($R^2 = 0.83$), root number ($R^2 = 0.80$), root length ($R^2 = 0.78$), fresh weight of whole plant ($R^2 = 0.61$) and dry weight of whole plant ($R^2 = 0.71$), while leaf number appeared as a function of internodal distance ($R^2 = 0.66$), root number ($R^2 = 0.59$), root length ($R^2 = 0.69$) and dry weight of whole plant ($R^2 = 0.63$). Root number appeared as a function of fresh weight of whole plant ($R^2 = 0.78$) and dry weight of whole plant ($R^2 = 0.79$). Fresh weight of whole plant appeared as a function of dry weight of whole plant ($R^2 = 0.92$).

Table 5. Correlation of the morphological parameters for salt stress on 32 soma clones (control and stresses)

Chart.	SL	ST	LN	IND	RN	RL	RT	FWWP	DWWP
ST	-0.288 ^{ns}								
LN	0.833**	-0.250 ^{ns}							
IND	0.927**	-0.271 ^{ns}	0.658**						
RN	0.796**	-0.071 ^{ns}	0.585**	0.673**					
RL	0.777**	-0.196 ^{ns}	0.689**	0.744**	0.725**				
RT	0.103 ^{ns}	0.724**	0.097 ^{ns}	0.148 ^{ns}	0.232 ^{ns}	0.113 ^{ns}			
FWWP	0.614**	0.377*	0.479**	0.487**	0.777**	0.443*	0.558**		
DWWP	0.714**	0.270 ^{ns}	0.634**	0.545**	0.788**	0.549**	0.433*	0.916**	
PWC	0.057 ^{ns}	0.444*	-0.003 ^{ns}	0.145 ^{ns}	0.239 ^{ns}	0.029 ^{ns}	0.596**	0.480**	0.222 ^{ns}

* and **significant at 5% and 1% level of probability respectively, ns: non-significant

SL = Stem length, ST = Stem thickness, LN = Leaves number, IND = Internodal distance, RN = Roots number, RL = Root length, RT = Root thickness, FWWP = Fresh weight of whole plant and DWWP = Dry weight of whole plant as foot note.

Cluster analysis

For salt stress hierarchical dendrogram showed that the 16 potato genotypes were categorized into four main clusters: (1) Cluster-A salt tolerant group contained four genotypes, i.e., Arun, Ausha, Courage and Asterix. (2) Cluster-B moderately tolerant group consisting of five potato genotypes i.e., Granola, Diamant, Cardinal, Surjamukhi and Patnai. (3) Cluster-C salt sensitive group included four genotypes, i.e. Sadaguti, Chollisha, Dohazari and Sindurkouta and (4) Cluster-D very sensitive group consist of three potato genotypes i.e. Lalpakri, Jamal and Sheelbilati (Fig. 1). It has been reported that Diamant and Cardinal has salt sensitive cultivars (Khrais et al. 1998, Rahnama and Ebrahimzadeh 2005). In this case Cardinal and Diamant showed as a moderately salt tolerant cultivar. Evaluation to salt stress conditions similar types of results found by Aghaei et al. (2008). This difference might be due to the variation in experimental conditions or the range of salt concentrations in the culture medium which has been tested.

So far as we know till a very little work has been done on abiotic stress screening in Bangladeshi indigenous potato genotypes. An indigenous potato cultivar Sheelbilati has been investigated as salt tolerant by Rahman et al. (2008). Khatun et al. (2010, 2011) reported that in their experiment that some local varieties namely Sadaguti and Zaubilati have the potentially to perform better *in vivo* salt stress condition. In this case results

obtained on 10 indigenous potato cultivars of Bangladesh and among these indigenous cultivars Sheelbilati showed very salt sensitive. This dissimilarity might be due to the different range of salt concentration in the medium which has been examined.

In comparison with other species, potato is moderately salt sensitive (Ochatt et al. 1999, Aghaei et al. 2008). Based on the relative values of shoot length, fresh and dry weight as well as root length, fresh and dry weight Zhang and Donnelly (1997) screened *in vitro* genotypes of potato for salinity tolerance. Physiological parameters were used by Aghaei et al. (2008), with random amplification of polymorphic DNA screening to confirm the reliability of screening using physiological parameters. Ranalli et al. (1996) showed that differences in canopy temperature between irrigated and stressed treatments can be used for screening for drought tolerance among potato genotypes.

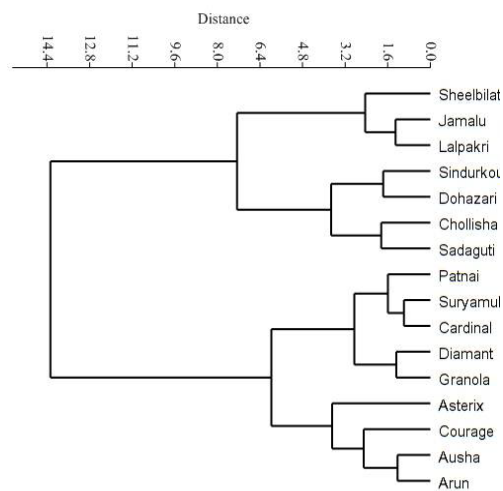


Fig. 1. Dendrogram based on relative values of ten morphological and physiological parameters of growth of potato lines under salt stress. Cluster-A (salt tolerant group): Arun, Ausha, Courage and Asterix. (2) Cluster-B (moderately tolerant group): Granola, Diamant, Cardinal, Surjamukhi and Patnai. (3) Cluster-C (salt sensitive group): Sadaguti, Chollisha, Dohazari and Sindurkouta, and (4) Cluster-D (very sensitive group): Lalpakri, Jamalu and Sheelbilati.



Fig. 2. A. Salt tolerant (Arun), and B. Salt sensitive (Sheelbilati) genotype.

Conclusion

Screening practice of the potato cultivars suggested that the salt tolerance and sensitivity of some potato cultivars are genotype dependent and maybe not epigenetic adaptation under stress condition. This is also clear from these experiments that *in vitro* screening with bioassay is relatively simple, rapid, convenient and repeatable. They can be used to substitute for field trials to screen and select salt tolerant potato genotype for further potato improvement in Bangladesh through biotechnological and breeding methods.

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