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MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF SOYBEAN (*GLYCINE MAX* L.*)* GENOTYPES UNDRE SALT STRESS

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ABSTRACT

Three advanced lines (SB02, SB05, SB07) along with one tolerant (Lokon) and one susceptible check (Asswt) of soybean (*Glycine max* L.) were assessed for salt tolerance in terms of morpho-physiological traits and molecular markers (SSR). The experiment was conducted at seedling stage with four salinity treatments namely 0, 8, 12 and 16 dSm^{-1} following Completely Randomized design. All the genotypes displayed considerable reduction in their morphological traits, least affecting the tolerant one. None of the genotypes were survived at 12 and 16 dSm^{-1} stress condition. Among the lines tested, SB-02 and SB-05 were identified as salt tolerant at 8 dSm^{-1} based on salinity susceptibility index (SSI) scoring. These genotypes suffered less in reduction of leaf chlorophyll content (SPAD) and increase of Na^+/K^+ than the susceptible genotypes. For all the traits viz. shoot length, root length, total length, shoot fresh weiht, root fresh weight, shoot dry weight, root dry weight, total fresh weight, total dry weight, percent live leaves, chlorophyll content and Na^+/K^+ ratio, the phenotypic coefficient of variation (PCV) was higher than that of genotypic coefficient of variation (GCV). All the traits studied showed medium to high heritability ranging between 43.81% (SPAD) to 96.65% (shoot length). The genotypes were grouped into two clusters considering both Euclidian distance and Unweighted Pair Group Method with Arithmetic Mean analysis. Lokon, SB-02 and SB-05 are on the same cluster as tolerant, and SB-7 and Asset on the other as susceptible to salt stress. The molecular pattern using by SSR marker displayed an average number of 3.33 alleles per locus with PIC (Polymorphism Information Content) values ranged from 0.2688 (sat_655 and satt728) to 0.7680 (sat_210). The highest gene diversity was observed in sat_210 and satt237 and the lowest in sat_655 and satt728 with a mean diversity of 0.5733. The genotypes Lokon, SB-02 and SB-05 could be suggested as a potential germplasm source of QTL (Quantitative Trait Loci) analysis for the development of salt tolerant soybean variety.

Keywords: Soybean salt stress, molecular marker, QTL, hydroponic system.

Introduction

Soybean (*Glycine max* L.), the golden miracle bean, belonging to the family of Leguminosae (sub family Papilionoidae), is a crop widely cultivated across the world for both human consumption and animal feed purpose. It is the number one oilseed crop across the world. This crop originated in China having *Glycine ussuriensis* as probable progenitor (Vavilov, 1951; Nagata, 1960), started being popular

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at the onset of 20th century. Currently it has become the staple oilseed crop worldwide by virtue of its wide range of agro-climatic adaptability, high nutritive value and satisfying multipurpose while allowing almost 50% less seed cost compared to other pulse and oilseed crops. The oil content of soybean seeds is 42-45% as well as edible oil content is 22%.

Salinity is a major problem for most of the crops affecting over 800 million hectares of agricultural land of the world. It is a vital abiotic stress limiting the production and quality of crops (Muzaiyanah and Susanto, 2020; Hamwieh and Xu, 2008; Hamwieh *et al*., 2011; Wang *et al*., 2011). Saline soils are highly rich in Na^+, Cl^- and SO_4^{2-} ions and the harmful effect of salinity is mainly exerted by Na^+ by affecting the plant with osmotic and ionic stress (Munns and Tester, 2008; Zhang *et al*., 2010). Soybean is a glycophyte with moderate to high sensitiveness to salinity. Salinity affects during germination, growth (Abel and Mackenzie, 1964; Wang and Shannon, 1999), nodulation (Singleton and Bohlool, 1984) as well as seed yield (Parker *et al*., 1983).

There are several methods which have been used for evaluating salinity in different crops. The problem with *in vivo* evaluation method is that plants become subjected to high degree of environmental variability. As a result, measurement of plant response due to solely salinity becomes very hard and even almost impossible sometimes. Laboratory or *in vitro* evaluating solves this problem by facilitating controlled, uniform and constant environmental condition. In laboratory condition, it has been carried out following different procedures such as sand culture in petri-dishes (Ansari, 1999), in plastic container and in hydroponic method (Lee *et al*., 2008; Khan *et al*., 2012) etc. In fact, *in vitro* method is a very effective method for evaluating tolerance to salinity of the plants because it can be accomplished with less space, time and precise phenotyping under controlled condition (Munns *et al*., 2006; Muzaiyanah and Susanto, 2020). It has been successfully applied in case of soybean and some other crops such as mustard, groundnut, wheat etc (Muzaiyanah and Susanto, 2020; Dasgupta *et al*., 2008; Mungala *et al*., 2008). Significant negative effects has been reported on different morphological and physiological traits upon salt stress using hydroponic system (Muzaiyanah and Susanto, 2020; Petersen *et al*., 2001; Hosseini *et al*., 2002; Essa, 2002; Khan *et al*., 2012).

Molecular markers are modern biotechnological tools that are widely used in molecular characterization of an organism, evaluating genetic variation and thus very useful in plant breeding. They have been being used as a tool to identify genes controlling complex characters like salt tolerance. Marker assisted selection (MAS) using DNA markers is a very effective way to develop new and superior cultivars (Gao *et al*., 2008). Simple sequence repeat or SSR markers, known also as microsatellite markers are advantageous over other molecular markers such as RFLP, AFLP and RAPD. SSR markers are highly abundant, polymorphic, they are co–dominant, easily detectable and have a typical fixed position in the genome or remains tightly linked with a group of genes which contains the target gene(s) or closely linked with the target gene(s); in this case, which is salt tolerance gene(s) in soybean (Moniruzzaman

et al., 2020; Hamwieh and Xu, 2008; Zhang, 2005). Based on the above discussion, the following experiment was planned to evaluate salt tolerance of soybean genotypes based on different morpho-physiological traits at seedling stage and also to characterize tolerant and susceptible genotypes using molecular markers (SSR).

Materials and Methods

Plant materials and growth condition

A total of five soybean genotypes, namely Asset (Susceptible check), Lokon (Tolerant check), SB-02, SB-05, SB-07 (Advanced lines) collected from Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, were used as plant materials in the experiment. The experiment was accomplished following Completely Randomized Design (CRD) under controlled condition at the Glass House Laboratory of Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh. The genotypes were screened at different salinity level (0, 8, 12 and 16 dSm^{-1}) at seedling stage using standard hydroponic culture with three replications for each treatment. Peter's professional nutrient solutions were used as culture media (Hemphill *et al.,* 2006). Twenty seven days after salt treatment, plants were removed carefully from styrofoams without any damage and data were collected on different growth parameters.

Determination of Sodium and Potassium (Na^+/K^+) ratio

The K^+ and Na^+ contents in plant samples were measured following standard method (Brown and Lilleland, 1946). Briefly, the harvested samples were dried and digested using 10 ml of di-acid mixture (HNO_3: $HClO_4$= 2:1) using 200°C. After cooling, the contents were taken into a 50 ml volumetric flask and the volume was made with distilled water. The digests were used for the determination of K^+ and Na^+ using flame photometer.

Calculation of Salinity Susceptibility Index (SSI)

For the measurement of salt tolerance, Salinity Susceptibility Index (SSI) of each genotype for each of the character under consideration was calculated according to the Fisher and Mauer (1978) using following formula:

Scoring of salinity susceptibility

$$SSI = \frac{\text{Value of a character in no stress condition - Value of a character in stress condition}}{\text{Value of a character in no stress condition}} \times 100$$

Scoring of salinity susceptibility is done by modified IRRI standard protocol for scoring of salinity (IRRI, 1997) as follows (Table 1).

Estimation of genetic parameters

Correlation coefficients were estimated by using MINITAB®17 statistical software packages (Minitab Inc., State College, Pennsylvania, USA). Genetic parameters such as genotypic and phenotypic variance, heritability, genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV), genetic advance were estimated according to Johnson *et al*., 1955 using the following formulae:

$$\text{Heritability, } h^2_b = \frac{\sigma^2_g}{\sigma^2_p} \times 100$$

Genotypic coefficient of variations,

Table 1: Scoring of salinity susceptibility in terms of percent reduction value

Percent reduction value (here SSI)	Score	Tolerance level
10 - 20	01	Highly tolerant
21 - 35	03	Tolerant
36 - 50	05	Moderately tolerant
51 – 60	07	Susceptible
61 -100	09	Highly susceptible

$$GCV = \frac{\sqrt{\sigma^2_g}}{\bar{x}} \times 100$$

Genetic advance, $GA = h^2_b.K.\sigma_p$

Genetic advance in percentage of mean, $GA(\%) = \frac{GA}{x} \times 100$

Genotypic correlation, $rg_{1.2} = \frac{c_o V.g_{1.2}}{\sqrt{\sigma^2_{g_1} \times \sigma^2_{g_2}}}$

Phenotypic correlation, $rp_{1.2} = \frac{c_o V.p_{1.2}}{\sqrt{\sigma^2_{p_1} \times \sigma^2_{p_2}}}$

Where,

h^2_b =Heritability in broad sense

σ^2_g = Genotypic variance; and

σ^2_p = Phenotypic variance

$\bar{X}$ = Population mean

K = Selection differential, the value of which is 2.06 at 5% selection intensity

σ_p = Phenotypic standard deviation

$C_o V.g_{1.2}$ = Genotypic covariance between the trait x1 and x2.

$\sigma^2 g_1$= Genotypic variance of the trait x1.

$\sigma^2 g_2$ = Genotypic variance of the trait x2

DNA extraction, PCR, Gel electrophoresis, Allele scoring and analysis

Genomic DNA was extracted using modified CTAB mini preparation method (IRRI, 1997) and quantified spectrophotometrically using NanoDrop (ND 1000, Thermo Scientific, Madison, USA). DNA was diluted to uniform concentration of about 50 ng/µl. The list of different SSR primers are presented in supplemental Table-1. Polymerase chain reaction involved an initial denaturation at 94^0C for 4 min, followed by 35 cycles of denaturation at 94^0C for 30 sec, annealing at 2^0C below T_m of respective SSR primers for 30 sec, primer extension at 72^0C for 30 sec, followed by a final extension at 72^0C for 8 min. The 8% PAGE (Polyacrylamide Gel Electrophoresis) gel was used for visualizing banding pattern using 50bp DNA ladder for size determination. The pattern of bands obtained after amplification with the primers was scored using Alpha Viewer (Version 3.2.8) to identify the molecular weight of DNA band comparing with DNA ladder. The summary statistics including the number of alleles per locus, major allele frequency, gene diversity and Polymorphism Information Content (PIC) values were determined using Power Marker version 3.23 (Liu and Muse, 2005), a genetic analysis software. Molecular

weights for microsatellite products, in base-pairs, were estimated with Alpha viewer software. The individual fragments were assigned as alleles of the individual loci. Polymorphism Information Content (PIC) value was measured as described by Anderson, 1993; Nei *et al*., 1983. Genetic distance value for UPGMA was computed using the formula as described in the NTSYS - PC (version 2.1) software user manual.

Results

Morpho-physiological characterization and selection of tolerant genotypes

All the genotypes showed normal, healthy and vigorous growth under normal condition. Salinity suppressed the growth and development of the plant almost in all genotypes though tolerant genotypes were least affected compared to susceptible ones. In present study, as the plants of all genotypes died under 12 dSm^{-1} and 16 dSm^{-1} salt stress condition before the date (27 DAS) of data collection, their morpho-physiological characters could not be measured. So only the data taken at 8 dSm^{-1} salt stress were explained. The analyses of variance (ANOVA) of different morphological characters of soybean genotypes showed significant difference among them upon salt stress at seedling stage (supplemental Table 2). The genetic parameters *viz.,* genotypic variances, phenotypic variances, phenotypic co-efficient of variation (PCV), genotypic co-efficient of variation (GCV), heritability, genetic advance and genetic advance in percentage of mean [GA (%)] for all the studied morphological traits were estimated and presented in Table 2. In this study, all the traits showed phenotypic and genotypic variances and phenotypic co-efficient of variation (PCV) were higher than

Table 2. Estimation of genetic parameters for different morpho-physiological traits of soybean

Characters	Phenotypic variance (σ^2_p)	Genotypic variance (σ^2_g)	GCV (%)	PCV (%)	Heritability (%)	GA	GA (%)
Shoot length	451.37	436.27	27.65	28.12	96.65	42.30	56.00
Shoot fresh wt.	3.30	2.80	22.63	24.59	84.68	3.17	42.90
Shoot dry wt.	0.18	0.16	29.40	31.43	87.50	0.77	56.64
Root length	0.74	0.45	8.04	10.30	60.89	1.08	12.92
Root fresh wt.	0.02	0.01	11.56	15.29	57.14	0.18	18.00
Root dry wt.	0.01	0.01	60.96	63.49	92.18	0.23	120.57
Total length	438.13	422.43	24.51	24.96	96.42	41.57	49.57
Total fresh wt.	3.31	2.77	19.84	21.70	83.65	3.14	37.39
Total dry wt.	0.13	0.10	20.82	23.65	77.50	0.58	37.76
Percent live leaves	1.14	0.32	1.59	1.71	86.92	2.78	3.06
SPAD	17.42	7.63	9.70	14.66	43.81	3.77	13.23
Na^+/K^+	0.11	0.08	30.29	35.33	73.53	0.51	53.51

genotypic coefficient of variation (GCV) for all the traits. Among the traits, root dry weight exhibited high estimates of PCV (60.96%) and GCV (63.49%) where the lowest PCV and GCV were observed in percent live leaves (1.59% and 1.71%). The estimates of heritability were classified as Johnson *et al*., (1955) into low (below 30%), medium (30-60%) and high (above 60%). All the traits studied in this experiment exhibited high to medium heritability ranging from 96.65% to 43.81%. The highest heritability was found in shoot length (96.65%) and the lowest was found in SPAD (43.81%); the highest GA (%) was found in root dry weight (120.57) and the lowest e was in percent live leaves (3.06).

The performances of soybean genotypes for all twelve morpho-physiological parameters exhibited distinguishable difference in different salt stress. Exposure to salt stress reduced growth parameters in all genotypes but affected the least in most cases in tolerant genotypes (Table 3). In the present study, salinity stress stunted the shoot length, root length, total plant length, shoot fresh weight, root fresh weight and total fresh weight as well as their respective dry weights in all genotypes. Percent reduction in shoot fresh weight (55-72%) was more than root fresh weight (38-54%), percent reduction in shoot length (42-61%) was higher than that of root length (29-48%) also but percent reduction in shoot dry weight (33-63%) was less than root dry weight (51-66%) (Table 3). This might be an indication that although the shoots accumulated more dry matter relatively than the roots, exposure to salt stress reduced the water content in the shoots more than the roots. Considering a whole plant, the percent reduction in the total plant length (41.75%), total fresh weight (55.61%) and total dry weight (45.67%) were the lowest in tolerant genotype, Lokon followed by tested genotype SB-02 and SB-05 and higher percent reduction was displayed by susceptible check Asset (55.33%, 70.65%, 60.60%) and tested genotype SB-07 (60.76%, 68.35%, 63.25%) (Table 3). The leaf chlorophyll content (SPAD) of the soybean genotypes were depleted with the increment of the salinity

Table 3. Mean performance of the soybean genotypes for morpho-physiological growth parameters

Genotype	Shoot length (cm)	Root length (cm)	Total length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Total fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Total dry weight (g)	Percent Live leaves	SPAD	Na^+/K^+ ratio
Asset	86.3b	8.317bc	94.62b	8.618b	0.885b	9.507a	1.63a	0.139c	1.766a	88.58b	27.38b	1.096a
Lokon	96.01a	8.008bc	104a	5.952c	0.972b	6.93b	1.118b	0.323a	1.445b	96.52a	32.45a	0.6803b
SB-02	65.28cd	7.785c	73.05cd	6.212c	1.007ab	7.22b	0.997b	0.195b	1.186c	94.45a	28.67ab	0.7903b
SB-05	68.53c	8.72ab	77.15cd	8.008b	1.138a	9.138a	1.555a	0.153bc	1.707a	91.25b	27.02b	0.879b
SB-07	61.68d	9.083a	70.76d	13.15a	0.997b	9.148a	1.538a	0.123c	1.66ab	84.5c	26.77b	1.182a
Level of significance	**	**	**	**	**	**	**	**	**	**	**	**

** = Significant at 1% level of probability

stress. Tolerant genotypes displayed higher chlorophyll content (32.45 & 28.67) than the susceptible genotypes (26.77 & 27.02). The genotype asset showed the highest Na^+/K^+ ratio (1.096) whereas the lowest in Lokon (0.6803) followed by SB-02 (0.790) and SB-05(0.879) under stressful condition (Table 3). Phenotypic correlation coefficient among different traits under salt stress condition of those genotypes were estimated and presented in Table 4, which clearly showed the positive and significant correlation among the traits studied. Salinity susceptibility index for the genotypes of different traits were calculated and showed in Table 5. Based on the scoring, a part from susceptible check Asset (7.4), SB-07 (7.4) was found to be a salinity susceptible genotype. Advanced lines SB-02 (5.6) and SB-05 (5.8) were found to be moderately tolerant besides tolerant check genotype Lokon (5.2) (Table 5).

Molecular characterization of tolerant and susceptible genotypes

Nine Simple Sequence Repeat (SSR) markers were used to study the polymorphism initially, among which six were selected as they showed clear polymorphism. The selected markers are satt237, sat_358, sat_655, satt702, satt728 and sat_210. Banding patterns of the soybean genotypes for molecular analysis using six polymorphic SSR markers are presented in Fig 1. A total of twenty alleles were detected by using six SSR markers (satt237, sat_358, sat_655, satt702, satt728 and sat_210) in five soybean genotypes. The detailed information obtained after analyzing through fingerprinting is presented in Table 6. The frequency of the most common allele at each locus ranged from 20% (sat_210 and satt237) to 80% (sat_655 and satt728) with a mean frequency of 50. Using six SSR markers, a total of 20 alleles were detected among

Table 4. Phenotypic correlation coefficient among different traits of soybean

Characters	SL	SFW	SDW	RL	RFW	RDW	TL	TFW	TDW
SFW	0.795***								
SDW	0.720***	0.968***							
RL	0.799***	0.952***	0.890***						
RFW	0.746***	0.835***	0.761***	0.895***					
RDW	0.797***	0.460**	0.342**	0.572***	0.703***				
TL	0.999***	0.819***	0.744***	0.827***	0.769***	0.791***			
TFW	0.801***	0.999***	0.963***	0.959***	0.860***	0.487**	0.825***		
TDW	0.807***	0.977***	0.986***	0.925***	0.828***	0.491***	0.828***	0.977***	
%LL	0.679***	0.844***	0.797***	0.821***	0.825***	0.483***	0.673***	0.853***	0.822***
SPAD	0.825***	0.705***	0.658***	0.736***	0.756***	0.701***	0.830***	0.718***	0.718***

Here, *** = Significant at 0.1% level of probability, ** = Significant at 1% level of probability, * = Significant at 5% level of probability.

Here, SL = Shoot length, SFW = Shoot fresh weight, SDW = Shoot dry weight, RL = Root length, RFW = Root fresh weight, RDW = Root dry weight, TL = Total length, TFW = Total fresh weight, TDW = Total dry weight, %LL = Percent live leaves and SPAD = Leaf chlorophyll content.

Table 5. Scoring of salinity susceptibility for different parameters

Genotype	SL	SFW	SDW	RL	RFW	RDW	TL	TFW	TDW	%LL	SPAD	SSI (mean score)	Tolerance level
Asset	7	9	7	7	7	9	7	9	9	3	3	7.4	Susceptible
Lokon	5	7	3	3	7	9	5	7	5	1	1	5.2	Tolerant
SB-02	5	7	5	5	7	9	5	7	5	1	3	5.6	Moderately tolerant
SB-05	5	9	7	3	5	7	5	9	7	1	3	5.8	Moderately tolerant
SB-07	9	9	9	5	5	9	7	9	9	3	3	7.4	Susceptible

SL = Shoot length (cm), SFW = Shoot fresh weight (g), SDW = Shoot dry weight (g), RL = Root length (cm), RFW= Root fresh weight (g), RDW = Root dry weight (g), TL = Total length (cm), TFW = Total fresh weight (g), TDW = Total dry weight (g), %LL = Percent live leaves and SPAD = Leaf chlorophyll content

the five soybean genotypes. The average number of allele per locus was 3.33 with a range of 2.00 (sat_655, satt702 and satt728) to 5.00 (sat_210 and satt237). According to the measure of the informative nature of microsatellites, the PIC values ranged from a low value of 0.26 (sat_655 and satt728) to a high value of 0.76 (sat_210, satt237 and sat_358) with an average value of 0.51. Low PIC score indicates the marker possesses low value of genetic diversity and high PIC score indicates high value of genetic diversity.

A Dendrogram constructed based on Nei'S (1973) genetic distance using Unweighted Pair Group Method of Arithmetic Means (UPGMA) indicated differentiation of the five soybean genotypes by six markers (Fig 2). All the soybean genotypes were easily distinguished. The UPGMA cluster analysis led to the grouping of the five genotypes into

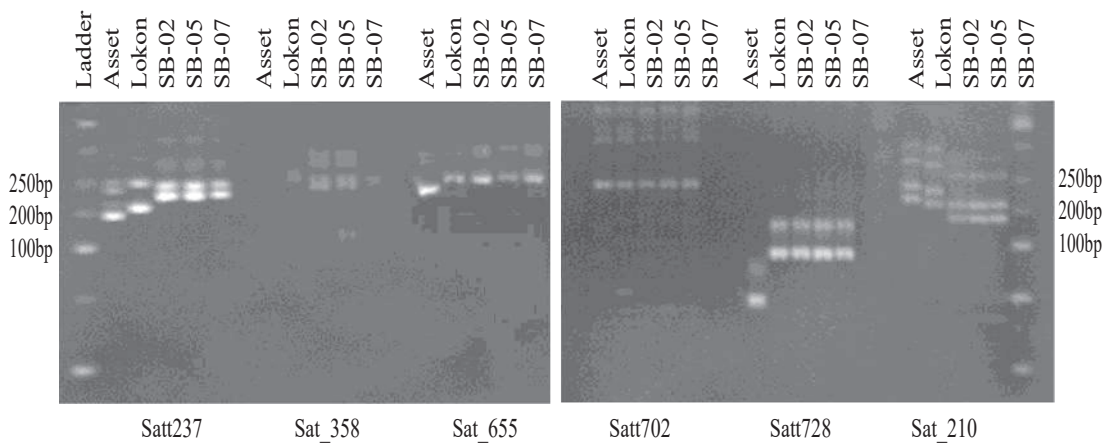

Fig 1. Banding pattern of five soybean genotypes using six different SSR markers

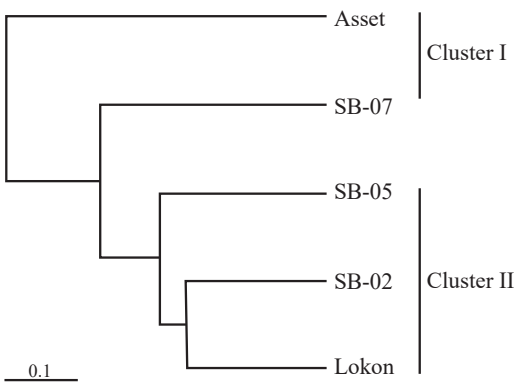

Fig. 2. UPGMA dendrogram based on Nei's (1973) genetic distance showing the differentiation of five soybean genotypes using six markers

two major clusters. Cluster I included SB-02, SB-05 and Lokon (tolerant check) and cluster II contained Asset (susceptible check) and SB-07.

Discussion

Soybean is a mildly salt tolerant crop. It`s growth parameters and yield tend to decrease when soil salinity exceeds 5 dSm^{-1} (Ashraf and Wu, 1994). The seedling stage is more susceptible to salt stress than the adult stage (Hosseini *et al*., 2002). In this experiment, three advanced lines of soybean were analyzed for salt stress tolerance. Exposure to salt stress affected their agronomic traits severely which is also similar to the results of the experiment conducted by Abel and Mackenzie, 1964; Chang *et al*., 1994. The analysis of variance (ANOVA) revealed that genotypes, treatment (salinity levels) and interaction between the genotypes and the salinity levels for all the characters were highly significant suggesting the presence of considerable variation among the genotypes as well as the salinity levels (Table 2). Salinity has significant effects on soybean phenotypes and growth parameters (Singh *et al.,* 2020) like shoot fresh as well as dry weight (Shereen *et al*., 2001), seed germination rate and seedling growth (Hosseini *et al*., 2002; Essa 2002; Datta *et al*., 2003), shoot-root dry weight, plant height and plant biomass (Essa, 2002; Kamal *et al*., 2003), petiole dry weight (Mannan *et al*., 2010), root volume, leaf SPAD and leaf area (Khan *et al*., 2012) etc, which has uniformity to the findings of the present study also.

The estimations of genetic parameters like genotypic and phenotypic coefficient of variation, genetic advance, heritability etc. have a prime role in genetic breeding programs as they help to make decisions about the suitable strategy to handle the population and select the traits to be considered during the initial and final steps of the breeding program. It helps the breeder to choose the best breeding strategy (Hamawaki *et al*., 2012). The coefficient of variation i.e. GCV and PCV give information about the nature and magnitude of variation. Higher difference between PCV and GCV indicates more environmental effect on the variations (Ali *et al*., 2016). The difference between the PCV and GCV values were higher in root length, root fresh weight, root dry weight, total dry weight, and leaf chlorophyll content (SPAD) and Na^+ /K^+ ratio (Table 3). The result indicated high environmental effect on these characters expression (Ali *et al*., 2016). As a result, adapting the genotypes to the environment or creating a favorable environment might be suitable for their best expression. The remaining traits having less PCV to GCV distance were less affected by the environment and mostly by additive effects of gene. So their expression is contributed mostly by their genetic base and selection

based on phenotypic performance might be chosen as a very suitable tool for their further improvements (Aditya *et al*., 2011). Heritability is an important genetic parameter which provides the proportion of the total phenotypic variance that is attributed to genetic causes (Hamawaki *et al*., 2012). Traits having higher heritability increase the chances of achieving superior progenies by selection (Hamawaki *et al*., 2012). In the present study, root fresh weight and leaf SPAD had moderate heritability while the all other traits displayed high heritability (Table 3). The traits having high heritability are easy to improve by selection (Ali *et al*., 2016). Genetic advance (GA) and genetic advance as percentage of mean (%GA) gives information about the expected gain in a trait due to selection (Ali *et al*., 2016). Although heritability itself denotes to the success in selection process, according to Johnson *et al*. (1995) heritability along with genetic gain would be more fruitful in forecasting the effect of selection process to find out the best individual. In this study, root fresh weight and leaf SPAD was coupled with low heritability and low GA (%) indicating that it would be less reliable if selection is performed upon them. The other traits had high heritability associated with high to moderate GA (%) which indicated that those traits can be improved through selection and this may be due to additive gene action (Panse, 1957) and thus, adapting selection without progeny testing could improve the traits.

The knowledge on correlation between characters is very important in plant breeding when the selection of a specific trait is harder because of its low heritability and identification difficulties (Cruz *et al*., 2004). In the present study, correlation among all the traits was positively significant (Table 4). The significant and positive association between the traits supported additive genetic model, thereby, gets less affected by environment. Strong positive correlation was observed between shoot length and all other characters. Positive and significant correlation between morphological characters and yield has also been reported by Singh *et al.* (2020); Singh and Singh, (1999); Rajanna *et al*. (2000); Bangar *et al*. (2003); and Moniruzzaman *et al.* (2019).

Development of salt tolerant soybean varieties has been a quite problematic task for plant breeders due to failure to evaluate salt tolerant breeding lines during selection process which is high likely to be solved by use of molecular markers which are tightly associated or linked with the target salt tolerant locus or loci. High polymorphism of SSR loci has been reported for both genetic diversity and number of alleles per locus (Moniruzzaman *et al.,* 2019; Clark *et al*., 2007, Lam *et al*., 2010) in soybean. In the present study, a set of six SSR markers identified a total of 20 alleles among the five soybean genotypes with average alleles per locus 3.33 (Table 6). Markers which detected higher number of major allele frequency (%) often showed lower gene diversity and which detected lower number of major allele frequency (%) given higher gene diversity. Hossen *et al*., 2017 and Dhar *et al*., 2012 also found a similar result where gene diversity was lower when number of major allele frequency (%) was high and higher gene diversity in case of low number of major allele frequency (%). PIC values enable to extinguish between the soybean progenies with SSR markers (Diwan and Cregan, 1997; Garcia *et al*., 2004; Risliawati *et al*., 2016). In the present

Table 6. Major allele frequency, Polymorphism Information Content (PIC) and gene diversity among five soybean genotypes

Locus	Major allele Frequency (%)	No. of alleles	PIC	Gene Diversity
Sat_210	20	5.0000	0.7680	0.8000
Satt237	20	5.0000	0.7680	0.8000
Sat_358	40	4.0000	0.6720	0.7200
Sat_655	80	2.0000	0.2688	0.3200
Satt702	60	2.0000	0.3648	0.4800
Satt728	80	2.0000	0.2688	0.3200
Mean	50	3.3333	0.5184	0.5733

study, the average PIC value obtained is 0.573 which is very similar to the study conducted by Chaerani *et al*., 2011. According to Botsein *et al*., 1980, sat_210, satt237 and sat_358 markers are highly informative and very useful in studying the efficiency of selecting salinity tolerant soybean genotypes. The UPGMA cluster analysis splitted the soybean genotypes into two groups (Fig. 2). The first group consisted of genotypes Lokon, SB-02 and SB-05 whereas the second group consisted of genotypes Asset and SB-07. The clustering indicated genetic similarity among the same group members and dissimilarity between the members of the two clusters for salt tolerance based on the six SSR marker analyses in this study. Advanced lines SB-02 and SB-05 were clustered in the same group with check tolerant genotype Lokon and therefore identified as tolerant genotypes. Advanced line SB-07 was identified as salt susceptible being grouped with check susceptible genotype Asset.

Conclusions

Among the advanced soybean lines tested, SB-02 and SB-05 were identified as salt tolerant at 8 dSm^{-1} based on salinity susceptibility index (SSI) scoring. These genotypes suffered less reduction in leaf chlorophyll content (SPAD) and increase of Na^+/K^+ ratio was less. The genotypes were grouped into two clusters considering both Euclidian distance and UPGMA analysis. Lokon, SB-02 and SB-05 are on the same cluster as tolerant, and SB-7 and Asset on the other as susceptible to salt stress. Genotype Lokon, SB-02 and SB-05 could be suggested as suitable cultivars for cultivation in salt-affected areas, and also can be used as a potential germplasm source of QTL (Quantitative Trait Loci) analysis for the development of salt tolerant soybean variety.

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