Progressive Agriculture
Journal homepage:http://www.banglajol.info/index.php/PA

Genetic diversity analysis of soybean genotypes using SSR markers for salinity tolerance

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Abstract

Soil salinity is a major constraint to soybean production. Five soybean genotypes were grown in pots with hydroponic culture under control and different salt stressed conditions to observe salt tolerance capacity on the basis of phenotypic screening and measure genetic diversity and relatedness among the genotypes. Minimum effects of salinity on root and shoot length was observed in Binasoyben-3, GC840 and Binasoyben-5at different salt stresses. Root dry weight and shoot dry weight of different soybean genotypes under different salt stresses were depicted. The highest reduction in root weight was noted in Binasoybean-1. The same genotypes were used to assess genetic diversity among them with simple sequence repeat (SSR) markers. A total of 33 alleles were detected among 5 soybean genotypes by using 10SSR markers. The number of alleles per locus ranged from 2 to 5, with an average of 3.33 alleles across the 10 loci. Rare alleles were observed at 10 SSR loci with an average of 2.8 alleles per locus. In this experiment, two SSR loci were found to be null alleles. The average values of null allele were 0.2. PIC values ranged from 0.27 in Satt184 to 0.77 in Satt339 with the average value of 0.56.The major allele frequency of the most common allele at each locus ranged from 0.80 in Satt184 to 0.20 in Satt339with a mean frequency of 0.48. The size of the different major alleles at different loci ranged from 173 bp for Satt509 to 407 bp for Satt339. The highest gene diversity (0.80) was observed in loci Satt339 and the lowest gene diversity (0.32) was observed in loci Satt184 with the mean diversity of 0.61. The lowest genetic distance (0.60) was observed in Asset vs Binasoybean-3 and Binasoybean-5 vs Binasoybean-3. The highest genetic distance (1.0) was observed between a numbers of genotype pairs with GC840 vs Asset. The UPGMA cluster analysis led to the grouping of the 5 genotypes into two major clusters. GC840, an advanced line identified to be salt tolerant, together with Binasoybean-5 and Binasoybean-3 clustered in the same sub group. The results from morphological and molecular study suggested that GC840 and Binasoybean-3 are moderately tolerant to salt stress.

Key words: *Glycine max*, relatedness, genetic distance, salinity

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Introduction

Identification and utilization of diverse germplasm is the critical issue in plant breeding. Thorough knowledge of the genetic diversity of the crops is necessary for parental selection that maximizes genetic improvement. More accurate and complete descriptions of genotypes and patterns of genetic diversity could

help determine future breeding strategies and facilitate introgression of diverse germplasm into the current commercial soybean genetic base. Several diversity studies in soybean have been conducted using morphological characters, pedigree information and biochemical variation (Nelson *et al*., 1988: Gizlice *et al*., 1994: Sneller, 1994). Although morphological and agronomic characters are useful in evaluating genetic diversity, collecting such data can be laborious, time consuming and the phenotypic values are often strongly influenced by the environment. Biochemical variants such as isozymes and electrophoresis patterns of storage proteins are less affected by the environment but have limited variation. DNA markers are an attractive alternative. They are nearly unlimited in numbers. Presumably selectively neutral and can be organized into linkage maps (Thormann and Osborn, 1992). Molecular markers have been proved to be valuable tools in the characterization and evaluation of genetic diversity within and between species and populations. It has been shown that different markers might reveal different classes of variation (Pogo*et al*., 1996: Rahman *et al.,* 1997). It is correlated with the genome fraction surveyed by each kind of marker, their distribution throughout the genome and the extent of the DNA target which is analyzed by each specific assay (Davila *et al.,* I999).

In Bangladesh, about twenty percent of land in the southern coastal belt is affected by various degrees of salinity (Munns *et al.,* 2006). The yield and quality of soybean are affected by salinity stress. Soybean is classified as a moderately salt tolerant crop, but the final yield of soybean will be reduced when soil salinity exceeds 8dS/m (Ashraf and Waheed,1994).Screening is most important for the identification of salt tolerant genetic resources. Salinity tolerance test in various crops have been carried out bydifferent scientists in different ways. For screening/testing salt tolerance in plants hydroponic cultivation is a quite precise and popular method. Molecular marker is the widely used approach to assess genetic diversity. Different types of molecular markers have been developed to use. Among them short oligonucleotide (1-6 base pair) repeats sequences are known as microsatellite marker. Therefore, the present work was carried out to characterize soybean genotypes at molecular level through SSR markers and to measure genetic diversity and relatedness among studied soybean genotypes in relation to salinity.

Materials and Methods

The plant materials consisted of 5 soybean genotypes viz; GC840, Asset, Binasoybean-1, Binasoybean-3, Binasoybean-5 having good combination of both agronomic and quality traits were obtained from Plant Breeding Division at Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. The experiment was laid out in a randomized complete block design (RCBD) with three (3) treatments with a control and three replications. Treatments were randomly distributed within the block. There were following treatment combinations: Control (Non salinized) and Salt stress at 8 dS/m, 12 dS/m &16 dS/m. MSTATC software was used to perform data analysis on root length, shoot length, fresh weight and dry weight components for normal and salinized environments.

The genetic diversity of collected plant materials was estimated using SSR markers to identify the diverse parental lines. DNA was extractedfrom the lyophilized tissues of 15 days older soybean leaves. The quantification of the DNA was done using Spectrophotometer. Then the DNA samples were evaluated qualitatively using agarose gel electrophoresis. DNA samples were diluted to a working concentration of 10ng/µl. A set of 10 SSR primers were used for the finger printing of collect genetic materials.

The amplification of DNA samples was carried out in a thermocycler. The machine was run according to the following setup: Initial denaturation at 94°C for 3 min, 35 cycles of denaturation at 94°C for 1 min, Annealing at 55°C for 1 min, elongationat 72°C for 2 min, and final elongation at 72°C for 7 min. After completion of

cycling program, the PCR products were stored at 4°C until gel electrophoresis. PCR products were confirmed by polyacrylamide gel electrophoresis. The gel image resolution was adjusted using the camera setting.The gel was exposed to UV light in a gel documentation system and the gel image was saved as a JPEG file.

Table 1. Sequence of microsatellite markers used in the study.

Sl no.	SSR Marker	Forward	Reverse
1	Satt163	AATAGCACGAGAAAAGGAGAGA	GTGTATGTGAAGGGGAAAAACTA
2	Satt165	CACGAATAACTTGACACATT	TAAAAACAAAGCAAACATAAA
3	Satt406	GCGCGTGTGGTGGTTACATTA	GCGTTTGCAGCCATTTCCATTTAC
4	Satt194	GCGTTGTGGTCACTCTTGATAATG	GCGAGTCACGAAATAATTTTGAATAAT
5	BE806308	GCGATTTGACCCCGTTCATACAT	GCGGCAGAAATCCGCTCTCTTTA
6	Satt210	GCGAAAAACGTCAGGTCAATGACTGAAA	GCGGGGCTTAGATATAAAAAAAAAGATG
7	Satt009	CCAACTTGAAATTACTAGAGAAA	CTTACTAGCGTATTAACCCTT
8	Satt339	TAATATGCTTTAAGTGGTGTGGTTATG	GTTAAGCAGTTCCTCTCATCACG
9	Satt184	GCGCTATGTAGATTATCCAAATTACGC	GCCACTTACTGTTACTCAT
10	Satt509	GCGCTACCGTGTGGTGGTGTGCTACCT	GCGCAAGTGGCCAGCTCATCTATT

Results and Discussion

Germination, growth and development of soybean are severely affected by salinity. Soybean genotypes were screened by their phenotypic traits under salinity as and molecular level variations using SSR markers. Five genotypes of soybean seedlings were used for screening salinity tolerance. After two or three days of salinization, salt stress symptoms started. Seedlings grown in saline condition showed several symptoms of salt injury like yellowing of leaves, drying of leaves, and reduction in root growth, reduction of shoot growth and stem thickness and in many cases dying of seedlings. Some other symptoms are rolling and tip whitening. On the other hand, the seedlings in the non-salinized condition showed normal growth over the salinized condition (Figure 1). Salt tolerant seedlings were distinguished from the sensitive seedlings when grown in salinized condition.

Data regarding root length and shoot length (Table 2) showed that salinity stress exerted strong negative impact on shoot length and root length of soybean genotypes. However, the impact of salinity differed significantly among soybean genotypes. Minimum effects of salinity on root and shoot length was observed in soybean genotypes GC840 and Asset at 8dS/m, 12dS/m and 16dS/m salinity level. All types of soybean genotypes gradually decreased there root length and shoot length at 8dS/m, 12dS/m and 16dS/m salinity level, respectively. It was observed when compared with unstressed control. The data regarding mean root dry weight and shoot dry weight of different soybean under control, 8dS/m, 12dS/m and 16dS/m of sodium chloride stress are depicted in. Plant root and shoot dry weight reduced to varying degree among these soybean and all soybean genotypes followed the same pattern of dry weight reduction as in case of fresh weight. The highest reduction in root and shoot dry weight was noted in soybean genotypes Binasoybean-1.

The descriptive statistics showed that there was a wide range of variations among the different characters under salt stress condition revealed that soybean genotypes were much influenced by salt stress.

Mailman *et al*. (2010) also found the similar results. Plant biomass was severely affected due to increase in salinity level therefore, dry and fresh weights of plants were significantly decreased. Fresh weight of root and shoot of soybean under control and various levels of salinization (Table 2) showed that minimum reduction

in root and shoot fresh weight was exhibited by soybean genotypes GC840 and Asset at 8dS/m, 12dS/m and 16dS/m salinity level. All soybean genotypes had a gradual decrease in root biomass and shoot biomass at 8dS/m, 12dS/m and 16dS/m salinity level, respectively, when compared with unstressed control.

Figure 1. Growth of soybean on salinized and non-salinized condition. In each photograph gradual reduction of growth and yellowing of leaves with increased concentration of salinity are shown.

At the highest level of salinization (16dS/m), soybean genotypes Binasoybean-1 proved to be salt sensitive and shoot fresh weight and root fresh weight decreased up to 44 and 40%, respectively, relative to control. Dry matter accumulation of different plant parts are severely affected by high level of salinity that ultimately reduce crop yield (Chang *et al*., 1994). The relative root dry weight was less affected by salinity level than the relative shoot dry weight which is in agreement with Essa (2002). Correlation coefficients between the control and salinized conditions were positive ($p<0.01$) for shoot, root and total dry weight. Salinity susceptibility index was fully correlated with percent reduction of total dry weight. Similar associations were found by Bayuelo-Jimenez *et al.* (2002).

Initially fifteen primers were used for polymorphism survey. Out of 15 primers, 10 primers (Satto09, Satt163, Satt165, Satt184, Satt194, Satt509, Satt406, BE806308, Satt210 and Satt339) showed clear polymorphism which were used in for further analysis. The polymorphic primers were shown in Table 3.

A total of 33 alleles were detected among the soybean genotypes by using 10 SSR markers. The average number of alleles per locus was 3.3 (Table 3). The highest number of alleles was 5.0 detected for marker Satt339 whereas the lowest number of alleles was 2.0 found for marker Satt184. The number of alleles per locus ranged from 2 to 5, with an average of 3.33 alleles across the 10 loci, which agrees with earlier results (Anchal *et al*., 2015) and (Bruno *et al*., 2010). Such variation in the number of allele amplified by different primer sets is attributable to several factors including primer structure and number of annealing sites in the genome. Obviously, polymorphic bands revealing differences among genotypes would be used to examine and establish systematic relationships among genotypes as reported by Hadrys *et al*. (1992).Rare alleles were observed at 10 SSR loci (Satto09, Satt163, Satt165, Satt184, Satt194, Satt509, Satt406, BE806308, Satt210 and Satt339) with an average of 2.8 alleles per locus and a total of 33 alleles across all the loci. In general, markers detecting a greater number of alleles per locus detected more rare alleles. Locus Satt339 detected the highest number of rare allele (5) and Satt184 and Satt194 showed the lowest number of rare allele (1) (Table 3). The rare alleles are highly informative for molecular characterization of soybean variety. Similarly, the rare allele 0.68 per locus based on SSR data was observed by earlier group (Bingrui *et al*., 2013). Locus Satt339 detected the highest number of rare allele (5) and

Satt184 and Satt194 showed the minimum number of rare allele (1) (Table 3). Primer Satt406 showed in 2 null alleles (Figure 2). The average value of null allele

Table 2. Root length (cm), shoot length, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight of soybean genotypes under non-salinized (control) and salinized condition at the seeding stage after 4 weeks exposure to NaCl.

Charac-ters	Genotype																			
	GC840				Asset				Binasoybean-1				Binasoybean-3				Binasoybean -5			
	Control	8d S/m	12d S/m	16d S/m	Control	8d S/m	12d S/m	16d S/m	Control	8d S/m	12d S/m	16d S/m	Control	8d S/m	12d S/m	16d S/m	Control	8d S/m	12d S/m	16d S/m
Root length	10.51	5.52	5.31	5.02	10.91	4.42	4.02	3.43	10.15	5.51	5.09	4.01	10.15	6.11	6.10	6.01	10.47	7.52	6.22	5.06
Shoot length	120.0	55.0	20.21	15.11	110.0	38.0	19.81	12.0	105.0	34.0	19.0	13.0	104.0	46.0	22.0	16.20	95.0	37.0	21.40	16.0
Shoot fresh weight	8.22	5.92	0.92	0.61	11.71	5.05	0.81	0.71	10.72	4.91	0.92	0.65	9.31	3.93	1.21	0.72	10.61	5.61	1.42	0.65
Shoot dry weight	2.52	1.71	0.52	0.41	3.71	1.32	0.61	0.52	2.81	1.21	0.43	0.35	4.01	1.19	0.61	0.52	2.83	2.14	0.63	0.43
Root fresh weight	1.59	1.02	0.25	0.20	1.40	0.64	0.42	0.32	0.98	1.10	0.27	0.21	1.77	0.84	0.26	0.19	1.67	1.14	0.58	0.42
Root dry weight	0.18	0.12	0.06	0.05	0.31	0.09	0.04	0.04	0.15	0.12	0.02	0.02	0.20	0.09	0.03	0.03	0.17	0.12	0.04	0.03

Table 3. Summary statistics of soybean genotypes for selected microsatellites (SSR) markers.

Locus	**Allele size ranges (bp)**	**No. of allele**	**Rare allele**	**Null allele**	**PIC**	**Major allele**		**Gene diversity**
						Size (bp)	**Frequency**	
Satto09	170-179	3	2	0	0.4992	173	0.6000	0.5600
Satt163	217-244	4	4	0	0.6720	243	0.4000	0.7200
Satt165	254-263	3	3	0	0.5632	243	0.4000	0.6400
Satt184	176-179	2	1	0	0.2688	263	0.8000	0.3200
Satt194	225-237	3	1	0	0.4992	179	0.6000	0.5600
Satt509	225-243	3	3	0	0.5632	225	0.4000	0.6400
Satt406	238-247	3	3	2	0.5632	243	0.4000	0.6400
BE806308	350-364	3	2	0	0.4992	240	0.6000	0.5600
Satt210	233-253	4	4	0	0.6720	240	0.4000	0.7200
Satt339	407-421	5	5	0	0.7680	407	0.2000	0.8000
Mean	**-**	**3.30**	**2.8**	**0.2**	**0.5568**	**-**	**0.4800**	**0.6160**

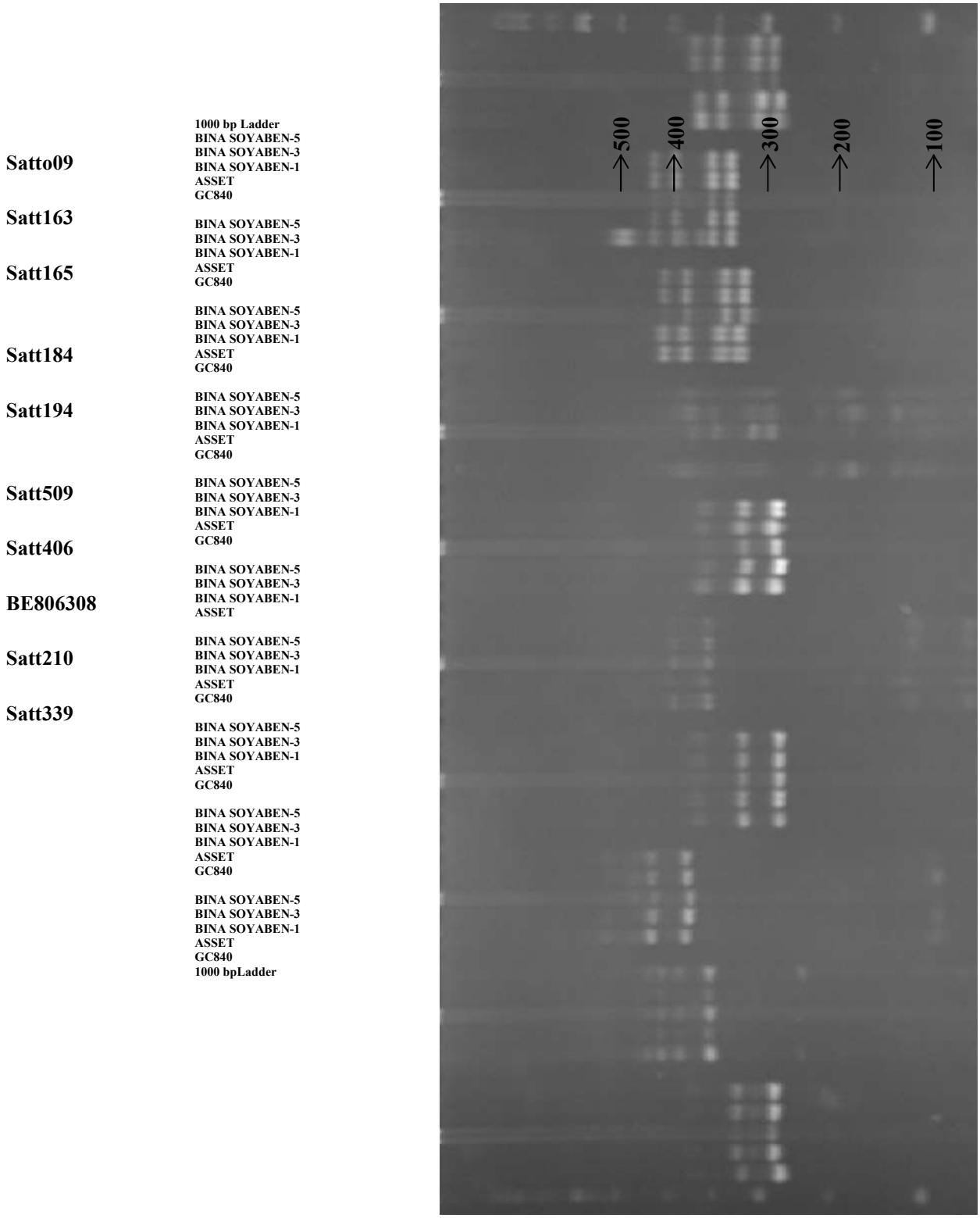

Figure 2. Banding pattern of 10 SSR primers in 5 soybean genotypes.

is 0.0.2 (Table 3). The frequency of the most common allele at each locus ranged from 0.80 in Satt184 to 0.20 in Satt339with a mean frequency of 0.48 (Table 3). The size of the different major alleles at different loci ranged from 173 bp for Satto09 to 407 bp for Satt339 (Table 3).The highest gene diversity (0.80) was observed in loci Satt339 and the lowest gene diversity (0.32) was observed in loci Satt184 with the mean diversity of 0.61 as estimated following the formula of Nei *et al*. (1983) (Table 3). It was observed that markers detecting the lower number of alleles showed lower gene diversity than those detecting the higher number of alleles reveled higher gene diversity. In this study, polymorphic information content (PIC) values ranged from 0.2688 in Satt184 to 0.7680 in Satt339 with the average value of 0.5568. The results of this study are significantly higher than the PIC values reported from other studies (Anchal *et al*., 2015). The SSR markers used in this study were highly informative because PIC values higher than 0.50 indicate high polymorphism. Vaiman *et al*. (1994) and Allard *et al*. (1984) also supported observed markers with PIC values of 0.5 or higher are highly informative for genetic studies. The highest gene diversity (0.80) was observed in loci Satt339 and the lowest gene diversity (0.32) was observed in loci Satt184 with the mean diversity of 0.61 as estimated following the formula of Nei's, (1983) (Table 3). It was observed that markers detecting the lower number of alleles showed lower gene diversity than those detecting the higher number of alleles reveled higher gene diversity. The lowest genetic distance (0.60) was observed in Asset vs Binasoybean-3 and Binasoybean-5 vs Binasoybean-3. The highest genetic distance (1.00) was observed between a numbers of genotypes pair with GC840 vs Asset (Table 4). In another study, Brown-Guedira*et al*. (2000) found the genetic distances ranged from 0.08-0.76 when they studied the genetic relationship among 105 soybean genotypes.

Table 4. Genetic distance of soybean genotypes based on selected microsatellite alleles.

OTU	**GC840**	**Asset**	**Binasoybean-1**	**Binasoybean-3**	**Binasoybean-5**
GC840	***				
Asset	1.0000	***			
Binasoybean-1	0.9000	0.8000	***		
Binasoybean-3	0.8000	0.7000	0.7000	***	
Binasoybean-5	0.8000	0.6000	0.8000	0.6000	***

A dendrogram was constructed based on the Nei's (1973) genetic distance calculated from those SSR alleles generated from these soybean genotypes. UPGMA grouping showed two clusters. Cluster-I consisted with Asset and Binasoyabean-1 (Figure 3). The findings of the phenotypic and genotypic data reveal that GC840 together with other two (Binasoybean-3 and Binasoybean-5) genotypes that were grouped in Cluster-II. GC840 isan advance line that was previously found to be saline tolerant in trials at saline prone areas and having potential for recommendation as a tolerant variety. Grouping of

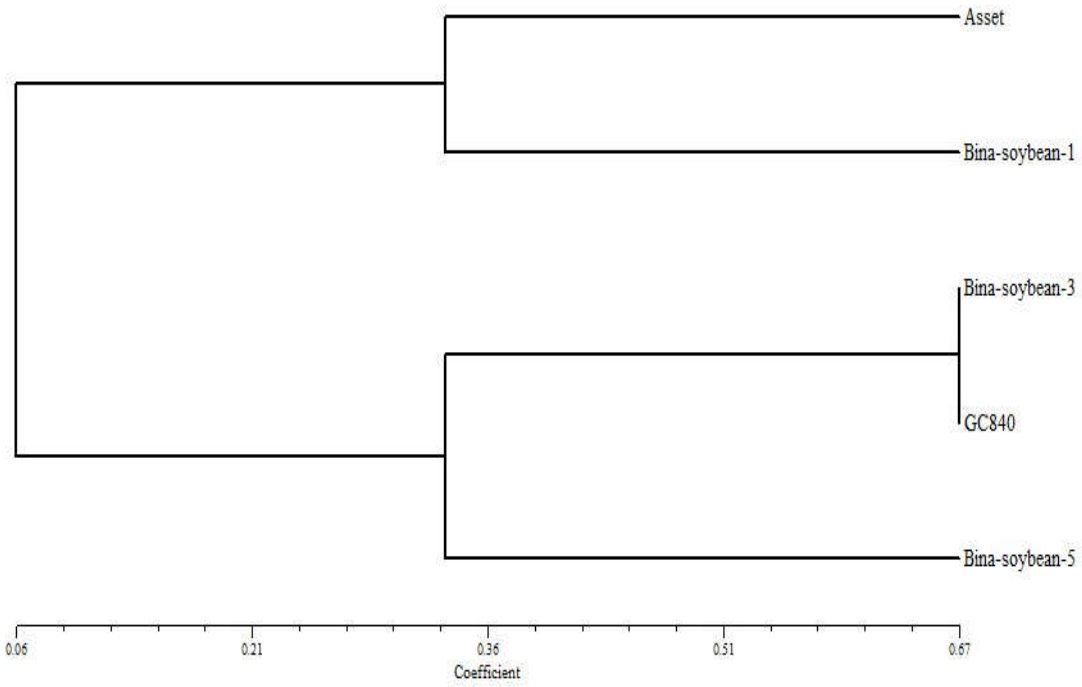

Figure 3. UPGMA for 5 soybean genotypes showing the genetic similarity.

these three genotypes in the same cluster. The clustering of GC840, Binasoybean-5 and Binasoybean-3 in the same sub group is very significant and indicates that Binasoybean-3 and Binasoybean-5 might be tolerant and can have further trials in the field condition followed by screening with more SSR markers linked to salinity for the confirmation as salt tolerant genotypes.

Conclusion

Minimum effects of salinity on shoot length and root length; shoot fresh and dry weight; root fresh and dry weight were observed in soybean genotypes GC840, Binasoybean-3 and Binasoybean-5 at different salinity levels. These genotypes might be tolerant on the basis of phenotypic screening. Previous studies at BINA showed that GC840proved potential saline tolerant in trials at saline prone areas and can be recommended as a tolerant variety. UPGMA grouping according to SSR markers have resulted in two clusters, of which cluster-II contained GC840with other two genotypes Binasoybean-3 and Binasoybean-5thatperformed well under salt stress condition also. Therefore, these three can be considered as saline tolerant by both phenotypic and molecular study.

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