A SEMINAR PAPER ON

Simple Sequence Repeat (SSR) Markers in Differentiating Salt Tolerance in Rice

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ABSTRACT

Rice production is greatly hindered by salinity stress all over the world. Sustainability of food production cannot be achieved without producing rice in the salinity affected area. Development of salt tolerant rice variety is essential for this purpose. Simple Sequence Repeat (SSR) markers assist to develop saline tolerant variety by helping in the procedure of suitable parent selection. The SSR marker RM8094 with the Polymorphic Information Content (PIC) value 0.82, Marker Index 1.92 and detect 14 alleles. The marker RM493 has the highest genetic diversity 0.8819 and Discriminatory power (D) value 0.9203. RM10772 detected 12 alleles, RM336 has a Polymorphic Information Content value of 0.84 and the Discriminatory power value for RM21 is 0.8949. Use of this markers for differentiating salt tolerant rice genotypes is reliable also. So, these markers can be used for grouping rice genotypes based on their salt tolerance.

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CHAPTER 1

INTRODUCTION

The current world population of 7.6 billion is anticipated to reach 8.6 billion in 2030, 9.8 billion in 2050. A total of 83 million people is expected to be added in every year (UN Publications, 2017). World food production must increase to 70 percent more to feed this huge sum of the population (Hunter, 2017). Expansion in the cultivable land is not attainable rather it decreases day by day. Increased population along with various environmental stresses such as a rise in temperature, drought, salinity, etc. are the main causes of the reduction of arable land. Among many environmental stresses, salinization is one of the crucial reasons which hinder global crop production (Munns, 2011). Judging the impacts of global soil salinization on plant growth and productivity and identifying approaches for mitigating salinization are subjects of global importance. Earth is a salty planet, per liter of water contains about 30g of sodium chloride (Munns and Tester, 2008). FAO Land and Plant Nutrition Management Service reported that worlds salinity affected land reached to at least 6 %. Out of the current 230 million hectares of land which are irrigated, 45 million hectares are affected by salt (19.5%), and among them, 1500 million hectares are under dry land agriculture, while 32 million are salinity affected to different degrees (Parihar *et al*., 2015). Salinity may develop either naturally or by human activity. Human intervention creates an imbalance in the natural ecosystem, makes a change in the hydrology and thus, creates the pavement of salinization in the waterways and lands. The two major human activities that accelerate salinity are irrigation and extensive removal of vegetation, which bring the groundwater along with soluble salts close to the soil surface (Hoang *et al*., 2016). When growing on salt-affected soils, crops must compete with salts in the soils for water. The crops also need to cope with ion toxification, nutritional disorders and poor physical conditions of soils to survive. Therefore, their productivity is heavily hampered (Shrivastava and Kumar, 2015).

Rice is one of the most growing cereal crops in the world feeding almost 50% population of the planet. With the accruing number of population, boost in rice production is required. International Rice Research Institute suggested that 50% more rice needs to be produced with less inputs like water, land, fertilizer, and pesticide to keep up with the average annual population growth rate of 1.9% (Ganeshan *et al*., 2016). Rice is cultivated in 114 countries of the world across the continents except for Antarctica (Virmani and Ilyas-Ahmed, 2007). In Asia, rice is the cardinal source income generation for rural people. However, rice is a crop

which is grown throughout the world facing salinity stress and recently enlisted as most sensitive cereal crop with a threshold of 3 dSm^{-1} for most cultivated varieties (USDA, 2016). Therefore, it is vital to develop salinity tolerant rice variety to provide the staple food to the rice-consuming communities. Breeding for salinity tolerance is quite difficult as it a complex trait. Plant breeding approaches to maximize the genetic diversity between parental genotypes for intercrosses. Genetic divergence between parental genotypes is usually estimated through physiological and morphological differences of different quantitative and economically important traits. The disadvantages of this conventional approach are the cost of time and labor during the measurements and the influences of environmental factors. Often, these drawbacks are aggravated in salt-tolerance breeding. For example, any change in environments such as temperature, light or humidity can dramatically alter the driving forces of transpiration and, subsequently, ion uptake (Yeo *et al*., 1990). Such changes may alter salinity tolerance among genotypes. It is quite evident that morphological characters are often limited in their numbers and actual genetic relationships among genotypes may not adequately represent. Conversely, identified genetic variations based on DNA polymorphism are abundant and independent of environmental factors. Furthermore, quantitative traits measurement requires a large sample size for the evaluation of genotypes. In contrast, a small sample size can provide information for the evaluation during DNA polymorphism analysis. Thus, use of DNA markers can be an alternative to reduce time, labor and cost. Differentiation of genotypes through DNA markers are more reliable and convenient than morphological or physiological characters in the identification and characterization of genetic variation. Simple Sequence Repeat (SSR) markers have been efficiently used to identify genetic variation among rice cultivars (Garland *et al*., 1999). SSR markers are consecutively repeated sequence motifs that are distributed across the eukaryotic genome. They are easily amplified by PCR reactions using DNA nucleotide primers, the unique sequences flanking the repeat motifs. Polymorphic DNA fragments can be produced due to variation in the number of the repeat units. A number of SSR markers have already been designed for rice and their primer sequences have been published (Temnykh *et al*., 2000). SSR markers are used for DNA profiling, kinship analysis, genetic linkage analysis to locate a gene for a given trait. Researchers use SSR in population genetics and species conservation projects. SSR markers are also very useful in Marker Assisted Selection (MAS) in plant breeding. Moreover, SSR markers can discriminate different genotypes of a species based on a specific trait. Thus, use of SSR markers in grouping rice genotypes based on their salt tolerance trait can be beneficial. SSR markers have been widely applied in the genetic diversity analysis, genotypic identification and population structure

estimation in several rice genetic studies (Salgotra *et al*., 2015). So, the employment of genetic differentiation as identified by SSR markers to plant breeding programs is useful in addressing problems like salt stresses during rice production.

Considering the above-mentioned points, the review paper is aimed with following objectives:

- To highlight the most suitable SSR markers for genotypic differentiation among the salt-tolerant rice genotypes
- To review the reliability of SSR markers over other methods.

CHAPTER 2

MATERIALS AND METHODS

This paper is exclusively a review paper so that all of the information has been collected from the secondary sources. During the preparation of the review paper, various relevant books, journals publications etc. were gone through. The related topics have been reviewed with the help of library facilities of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), information from Bangladesh Institute of Nuclear Agriculture (BINA) and internet browsing. After collecting all the available information, it has been presented as per the objectives of this paper.

CHAPTER 3

RESULTS AND DISCUSSION

Simple Sequence Repeat (SSR) marker is a tract of repetitive DNA in which certain DNA motifs are repeated, usually 5- 50 times (Gulcher, 2012). To determine the utility of SSR markers in differentiating rice genotypes according to their level of salt tolerance number of allele per marker, Polymorphic Information Content (PIC), discriminating power (D value), Marker Index (MI), genetic relatedness, clustering of genotypes and development population structure are usually done. Moreover, comparing between the cluster of genotypes based on Morpho-physiological data and SSR data shows the advantages of using SSR markers over the morphological characterization for differentiating genotypes. Principal Component analysis of these data can also draw the same conclusion. For this reason, the results and discussion part of this seminar paper are divided into two sections. Firstly, emphasis is given on highlighting of the most suitable SSR markers for the differentiation among salt tolerant genotypes and secondly, the reliability of SSR markers over the morpho-physiological characterization in case of discriminating salt tolerant rice genotypes.

3.1 Highlighting the most suitable SSR markers for genotypic differentiation among salt tolerant genotypes

3.1.1 DNA banding pattern

DNA banding pattern in agarose gel or polyacrylamide gel produces the data for the analysis of SSR marker. 54 rice genotypes collected from the IRRI, Philippines and Indian Institute of Rice Research (IIRR), India were used for screening salt tolerance at seedling stages by 14 SSR markers. Amplified fragments of different sizes were considered as different alleles. DNA bands that were amplified by a given primer were scored as present (1) or absent (0) for all the samples under study. The banding pattern of DNA of 54 genotypes of rice by primer RM8094 was shown in Figure 1. The genotypes had their band in different position (base pair) on the gel (Chowdhury *et al*., 2016). The marker identified seven different alleles among the 54 genotypes (Figure 1). Different SSR markers show band in different positions as each marker is designed for a particular locus.

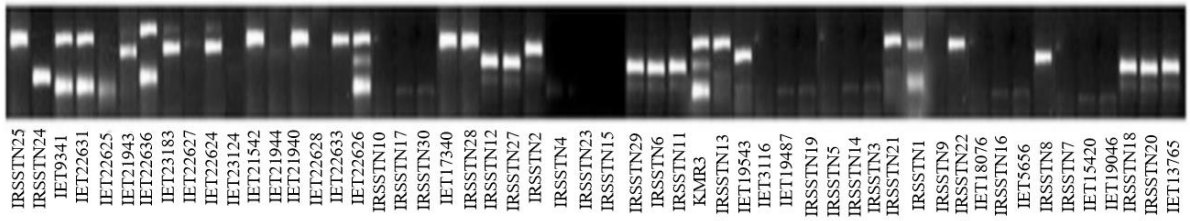

Figure 1: SSR profiles of 54 rice genotypes generated by primer RM8094 (Chowdhury *et al*., 2016).

In Figure 2 Gel image shows the DNA banding pattern of a mutant rice line M4-62 developed in Advanced Seed Research and Biotech Centre, Bangladesh using the 20 SSR markers as the parent linked with saline tolerance (Raihan *et al*., 2016).

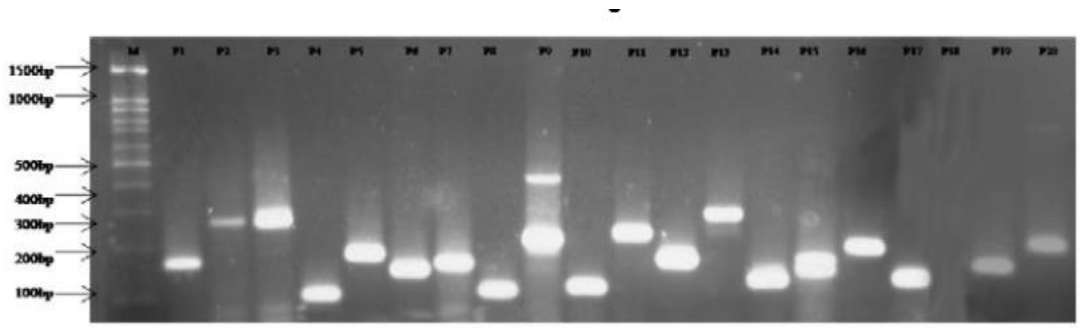

Figure 2: Banding pattern of mutant rice line M4- 62 with 20 SSR markers. Lane M is the 100 bp (base pair) molecular weight marker DNA or DNA ladder. A banding pattern of 19 SSR markers which include primers 1, 2, 3, 4, 5, 6,7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 19 and 20. The markers have been indicated as P1 to 20 (Source: Raihan *et al*., 2016)

3.1.2 Allele number

The number of alleles detected by a SSR marker indicates the usefulness of that marker. The marker which can identify more number of alleles are usually considered as useful marker for any genetic study. Eight tightly linked SSR markers to salt tolerance used for the evaluation of salt tolerance among 30 rice genotypes. All the eight SSR markers amplified polymorphic bands using 30 rice genotypes (Table 1). The lowest amplicon size belonged to RM10796 (131 bp), and the highest amplicon size belonged to RM10772 (386 bp). The number of alleles of used markers ranged from 5 to 14. RM8094 produced the highest numbers of alleles (14) followed by RM10772 (12 alleles) while RM140 produced the lowest (5) followed by RM10745 which produced six alleles (Mohammadi- Nejad *et al*., 2012). The results showed that the marker RM 8094 and RM10772 are the most powerful marker in identifying differences among these 30 rice genotypes while RM140 and RM10745 were the least powerful markers.

Table 1: Number of alleles and amplicon size of SSR markers for 30 rice genotypes

Marker	Allele Number	Amplicon Size range (bp)
RM140	5	248-264
RM493	9	193-253
RM1287	11	147-192
RM3412	11	225-260
RM8094	14	166-220
RM10745	6	182-201
RM10764	8	131-171
RM10772	12	321-386

(Source: Mohammadi- Nejad *et al*., 2012)

3.1.3 Polymorphic Information Content (PIC) and Marker Index (MI)

Polymorphic Information Content (PIC) value is a reflection of allelic diversity and frequency among the varieties. PIC value of each marker was evaluated by the number of alleles, and it varied greatly for all the SSR loci tested. The allelic diversity as well as the level of polymorphism among 33 rice genotypes was evaluated using 10 SSR loci and showed variability among markers (Figure 3).

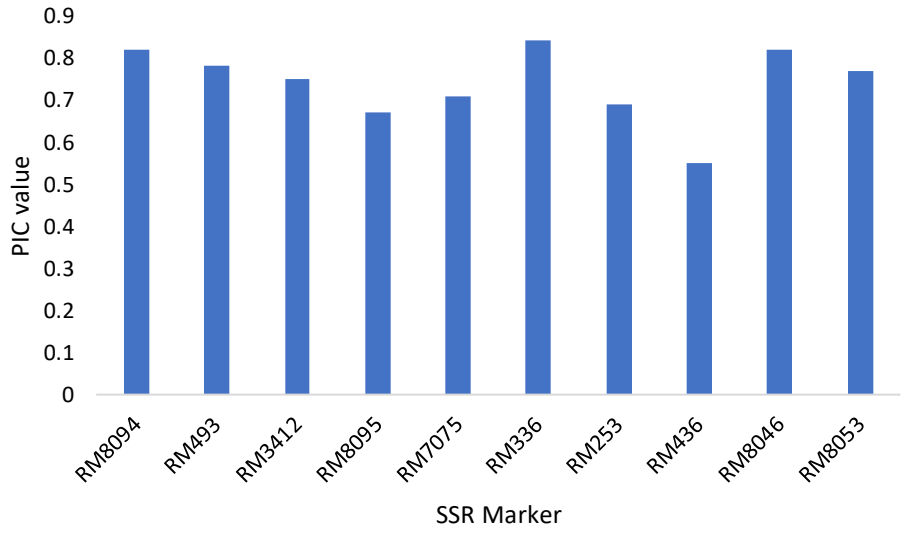

Figure 3: Polymorphic Information content of 10 SSR markers for 33 rice landraces used for screening salinity tolerance (Source: Ali *et al*., 2014)

PIC value varied from 0.55 to 0.84, the highest value belonged to RM336 followed by RM8094 (0.82) and RM8046 (0.82), while RM 436 showed the lowest PIC value followed by RM8095

(0.67) and RM253 (0.69) (Ali *et al*., 2014). The PIC value higher than 0.5 for a marker indicates that the marker is highly polymorphic and suitable for any genetic study. However, the results showed that the marker RM336 is the most powerful marker among these 10 SSR markers for analyzing these 33 rice genotypes.

Marker Index (MI) along with PIC value can detect capable markers for distinguishing among genotypes. In Figure 4, the Marker Index(MI) was lowest in RM 436 (0.77), and it was highest in RM 8094 (1.92) (Ali *et al*., 2014).

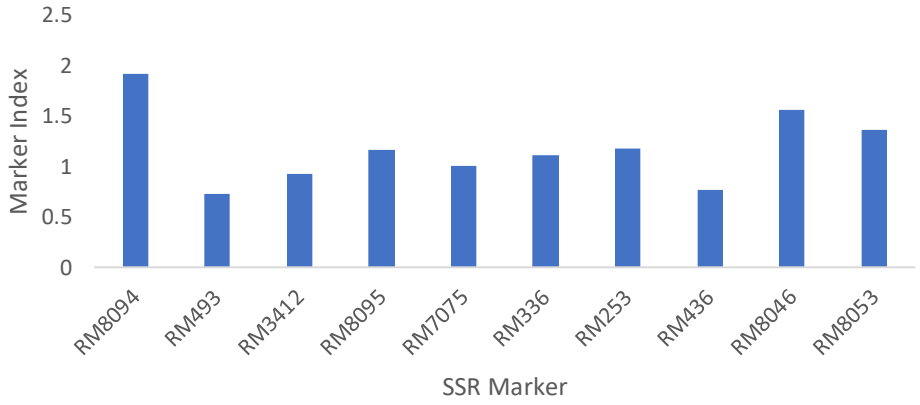

Figure 4: Marker Index for 10 SSR markers for 33 rice landraces used for screening salinity tolerance (Source: Ali *et al*., 2014).

The SSR markers RM 8094 was found to be the most superior for this analysis based on PIC coupled with MI value followed by RM336 and RM8046. The higher PIC with higher MI value indicates that all these primers were capable of distinguishing among genotypes and highly informative.

3.1.4 Gene diversity and Discriminatory Power (D) value

Gene diversity and D value are another two-important measures for determining the ability of any SSR marker in genetic differentiation study. In both cases, the higher value indicates, the higher capacity of a particular marker. The genetic diversity of 19 loci for the 24 genotypes of rice (including modern varieties and coastal salt tolerant landraces in Bangladesh) was estimated by Shakil *et al*. (2015). The gene diversity ranged from 0.4340 to 0.8819 with an average of 0.6924, indicating a moderate level of diversity existing within the genotypes surveyed.

Table 2: Gene diversity and Discriminatory Power (D) value for 19 SSR marker found among 24 rice genotypes

Marker	Gene Diversity	D value
RM5	0.8229	0.8587
RM493	0.8819	0.9203
RM279	0.8403	0.8768
RM227	0.6424	0.6703
RM131	0.4792	0.5000
RM26	0.7326	0.7645
RM31	0.6354	0.6630
RM217	0.7500	0.7826
RM125	0.6979	0.7283
RM18	0.4340	0.4529
RM72	0.7604	0.7935
RM257	0.6910	0.7210
RM219	0.8056	0.8406
RM171	0.6840	0.7138
RM222	0.5104	0.5326
RM21	0.8576	0.8949
RM224	0.7700	0.8080
RM117	0.4965	0.5181
RM17	0.6632	0.6920
Mean	**0.6924**	**0.7227**

(Source: Shakil *et al*., 2015)

The highest genetic diversity (0.8819) was recorded in locus RM493, and the lowest genetic diversity (0.4340) was detected in locus RM18 (Table 2). The discriminatory power value (D value) is an estimation of the efficiency of a primer for varietal identification; *i.e.,* the probability that two randomly chosen varieties have different patterns. The D values calculated for every marker, where RM493 had the highest value (0.9203) and was the best marker among them (Table 2). The gene diversity and D values revealed that RM493 and RM21 were considered as the best markers for the identification of these 24 rice genotypes, followed by RM5, RM72, RM219, RM224, and RM279. The discriminating power of the primer does not

only depend on the number of alleles it generates but also on the frequencies of the different banding patterns.

3.1.5 Genetic dissimilarity index

A dissimilarity matrix of shared SSR alleles was used to determine the level of relatedness among the rice genotypes. Pair-wise genetic dissimilarity estimates ranged from 0.00 to 1.00 as shown in Table 3. In dissimilarity matrix, *Oryza rufipogon* showed maximum dissimilarity with rest of the genotypes. In particular, *O. rufipogon* and CSR6 showed the highest dissimilarity (lowest similarity), whereas as CSR4 and Canning showed the lowest dissimilarity (highest similarity). It was found that SR26B and Nonabokhra, SR26B and CSR6, Canning and CSR6, and CSR6 and CSR4 were much closer genotypes with lower dissimilarity index that is 0.11, 0.17, 0.20, and 0.20, respectively (Ganie *et al*., 2014). This information found with the help of SSR markers which are very useful while designing a breeding program for salinity tolerance. Because the crossing between two genetically distant parents will result in more number of useful segregants in F_2 (second filial) generation.

Table 3: Dissimilarity matrix of ten rice genotypes based on 1-0 matrix of SSR fingerprints

Varieties	CSR4	CSR6	Canning	IR36	Kalonuniya	Nonabokhra	Pokkali	Porteresia	SR26B	*O. rufipogon*
CSR4	0.00									
CSR6	0.20	0.00								
Canning	0.00	0.20	0.00							
IR36	0.23	0.34	0.23	0.00						
Kalonuniya	0.27	0.22	0.30	0.25	0.00					
Nonabokhra	0.30	0.95	0.27	0.38	0.31	0.00				
Pokkali	0.22	0.27	0.22	0.46	0.42	0.23	0.00			
Porteresia	0.52	0.54	0.52	0.38	0.44	0.50	0.55	0.00		
SR26B	0.31	0.17	0.31	0.36	0.30	0.11	0.22	0.46	0.00	
O. rufipogon	0.95	1.00	0.52	0.87	0.87	0.96	0.98	0.87	0.95	0.00

(Source: Ganie *et al*., 2014)

3.1.6 Clustering of genotypes based on SSR marker data

Unweighted neighbor-joining tree presented in Figure 5 revealed the genetic relatedness among 12 genotypes of rice collected from Brunei Darussalam using 15 SSR markers. The genotypes were assessed for their tolerance against salinity (Ishak *et al*., 2015). Genotypes that are derivatives of genetically similar types clustered together in the figure. The genotypes were clustered into three major groups. Group 1 consisted of Sp1 while Group 2 consisted of Kuaci and the other ten cultivars are in Group 3. Group 3 was further sub-divided into five groups with Salleh diverted from the other nine cultivars. It is because of similar breeding material were used for the development of these genotypes or in other words they have the same ancestry. Data shown in Figures 5 showed that Kuaci and Sp1 were most genetically different from the other ten rice cultivars.

It can happen because of different types of material have been used for the breeding of these varieties. As these 15 SSR markers was successfully discriminate the group of 12 salt tolerant rice genotypes, they can be considered as beneficial markers for genotypic differentiation.

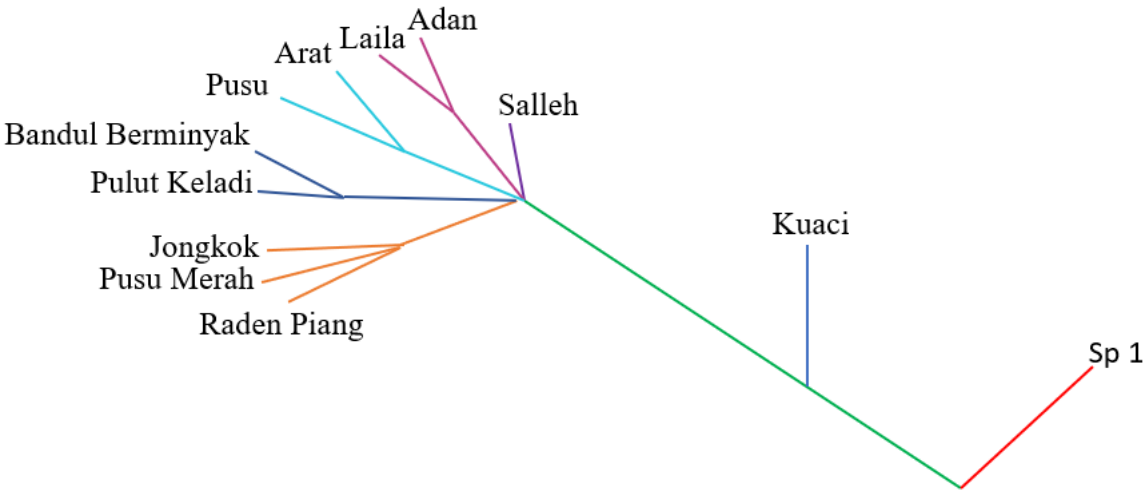

Figure 5: Tree based on neighborhood joining method showing the genetic relationship among 12 rice cultivars investigated using 15 SSR markers (Ishak *et al*., 2015).

A matrix of similarity was created from all pairs of 33 rice genotypes. The genetic relationships among rice genotypes are presented in a dendrogram based on informative SSR loci (Figure 6). All genotypes grouped into two major branches in the dendrogram with less than 10% similarity based on Jaccard similarity index. One branch unambiguously represents the subspecies, *japonica* rice. Another branch represents either the subspecies, *indica*, or the hybrids between *japonica* rice and *indica* rice. The only exception was a cultivar of *japonica*

rice, Daeyabyeo, which grouped with the genotypes of *indica* rice. Below the main japonica branch in the dendrogram, most genotypes grouped into three clusters, A1, A2, and A3, at 57, 47, and 48% similarity, respectively. Below the main *indica* branch in the dendrogram, most genotypes grouped into two clusters, B1 and B2, at about 30% similarity. Among the genotypes of *japonica* rice, most genotypes derived from Egypt grouped into Cluster A1 while those derived from Philippines and California grouped into Cluster A2 and A3, respectively. Among the genotypes of *indica* rice, Clusters B1 and B2 consist primarily of the genotypes derived from Egypt and Philippines, respectively. The genotypes of 'GZ5291-7-1-2', 'Agami,' 'L205', and 'Pokkali' were not included in these clusters (Zeng *et al*., 2004).

The breeding lines which was developed from the salt tolerant variety like Pokkali and Nonabokhra were ranked at the top for its salt tolerance in previous evaluation trials (Zeng *et al*., 2002). Moreover, IR63731-1-1-4-3-2 and Nona Bokra grouped into the same cluster. This indicates that the genetic backgrounds between the two genotypes are identical.

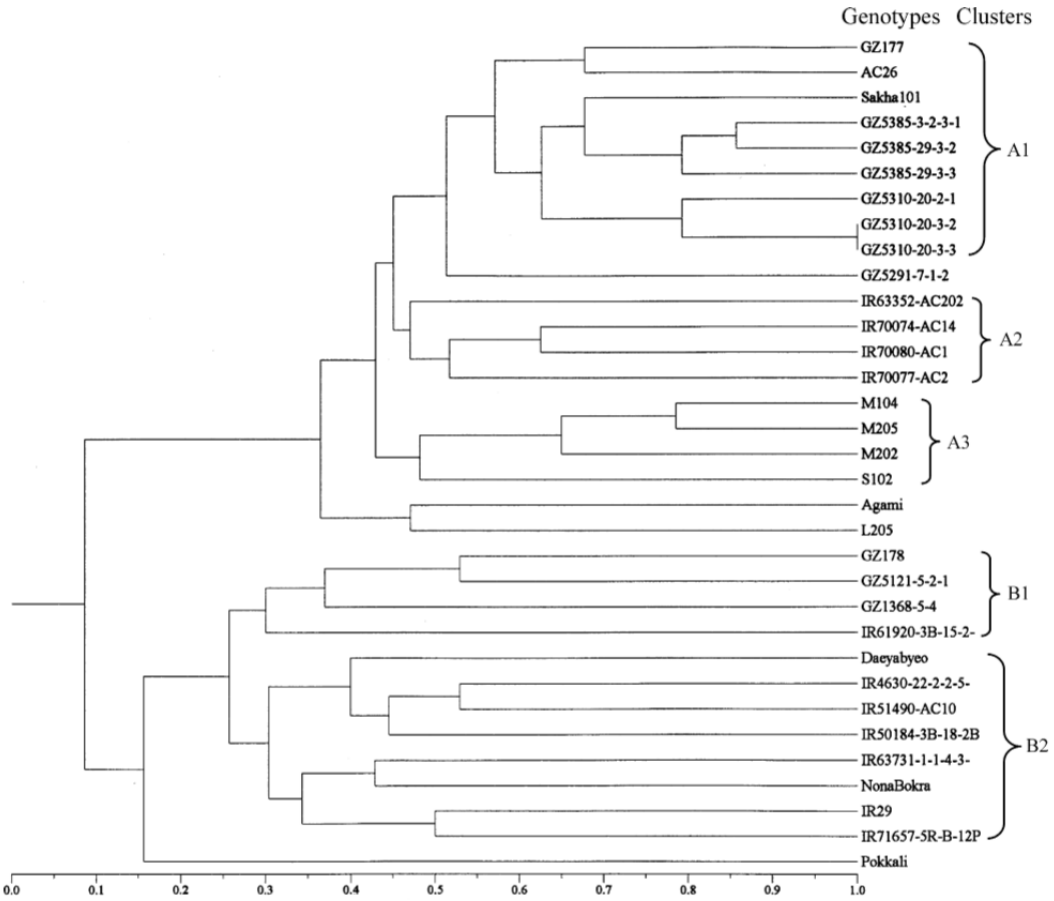

Figure 6: Clusters of 33 rice genotypes based on Jaccard's similarity index calculated from the data matrix of 25 microsatellite loci (Source: Zeng *et al*., 2004).

3.1.7 Population structure

Models with putative numbers of subpopulations (K) from 1 to 10 with admixture and correlated allele frequencies were considered for developing population structure of 40 rice genotypes. Seven independent runs with burn-in of 10,000 and run the length of 100,000 iterations for each K were implemented. Evanno's ΔK were used to determine the K-value (Evanno *et al*., 2005). The optimum value of K was then used to determine inferred ancestries. An individual was assigned to a specific population if it had more than 0.8 membership in that population, whereas individuals with membership probabilities less than 0.8 were assigned to an admixed group. 40 genotypes of rice that are salt tolerant, cold tolerant and high yielding variety were used for determining population structure with 12 SSR markers (Vanniarajan *et al*., 2012). The K value showed highest pick at 3 (Figure 7). Thus, the total population is divided into three subpopulations.

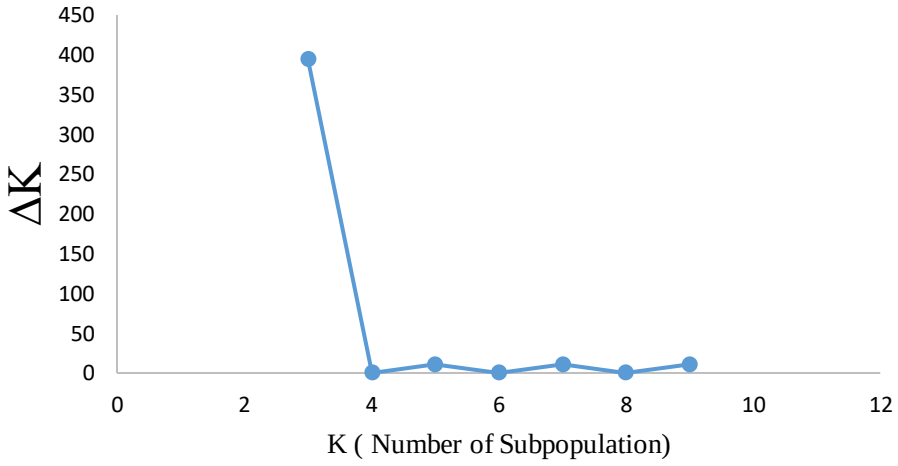

Figure 7: Analysis of population structure showing values of K for determining optimum number of subpopulations for total population (Source: modified from Vanniarajan *et al*., 2012)

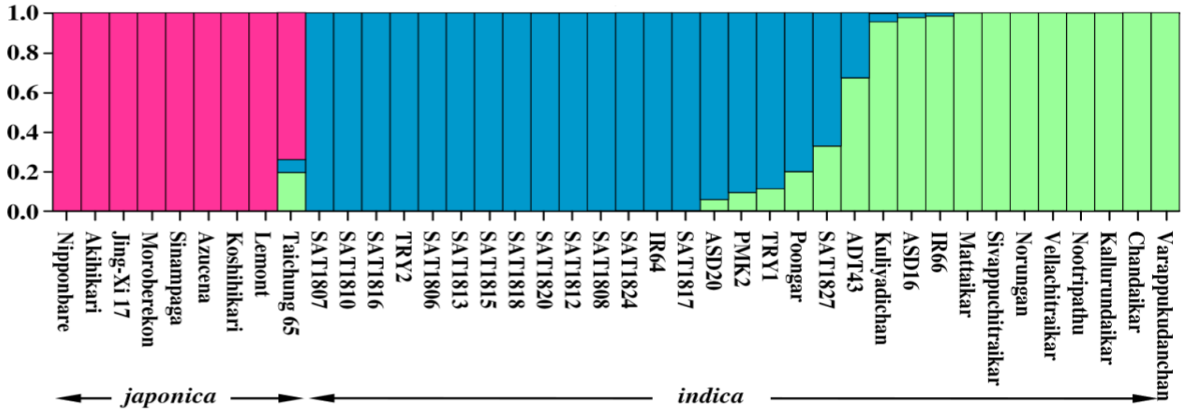

Figure 8: Bar plot showing distribution of genotypes within subpopulations (Source: Vanniarajan *et al*., 2012).

Figure 8 showed the bar plot diagram of the distribution of genotypes within subpopulations. Two subpopulations along with an admixture group were found. The salt tolerant varieties (which is indicated by SATVT) grouped into the same subpopulation as they have similar genetic constitution for the particular salt tolerant locus. The marker RM21 has the highest PIC (0.60) value among the markers can be considered as the suitable marker for grouping these 40 rice genotypes.

3.1.8 Analysis of Molecular Variance (AMOVA)

The analysis of molecular variance (AMOVA) based on SSR data also suggest significant genetic differences (p> 0.001) among the accessions and as per the analysis, 5 and 95 % of total genetic variation were caused by differences between and within the population, respectively (Figure 9) (Samal *et al*., 2016). The results indicate that accessions within the subpopulation are genetically diverse and can be used as potential source of parental lines for breeding program.

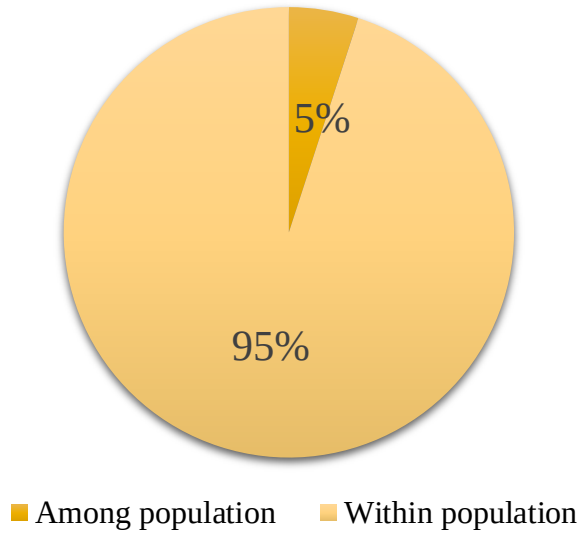

Figure 9: Analysis of Molecular Variance of salt tolerant rice genotypes based on 24 SSR loci (Source: Samal *et al*., 2016).

3.2 Reliability of SSR markers over other methods

The clustering pattern is different among these methods. Among methods of cluster analysis, the molecular analysis provides the maximum genetic differences among the test genotypes followed by morphological. Several reports suggested that molecular diversity provides

remarkably higher estimates of genetic diversity than morphological or physiological methods (Messmer *et al*., 1993; Beyene *et al*., 2005). Genotypes also swapped from one cluster to another cluster among different methods, and this pattern is somewhat irregular. Differences in clustering pattern and swapping of genotypes among different clusters in different methods of cluster analysis have been reported in some studies (Hanyong *et al*., 2004, Tar'an *et al*., 2005; Weiguo *et al*., 2007). These differences are not an indicator of the failure or limitation or weakness of the methods (Roldan-Ruiz *et al*., 2001). These results may be due to the diversity at the molecular level, which may not reflect in the diversity at the morphological or physiological level, as described by Karhu *et al*. (1996). To get similar diversity pattern among genotypes based on molecular and morphological diversity, the number of markers utilized in the molecular analysis should be increased to several thousand, and the morphological or physiological traits would contain all possible parameters. Another possible reason for this variation in clustering might be the environmental influence and genotype-environment interaction. Morphological and physiological characters are the ultimate expression of molecular constitution of a variety where some biochemical processes are involved. So, different types of clustering in different methods are not unusual (Hanyong *et al*., 2004). Some studies have also warned of the dangers of assuming that marker- QTL linkage will remain in different genetic backgrounds or different testing environments, especially for complex traits. Even when a single gene controls a particular trait, there is no guarantee that DNA markers identified in one population will be useful in different populations, especially when the population originates from distinctly related germplasm. Again, 3-D plot obtained from both morpho- physiochemical data and SSR data may differ in some cases as observed by Chattopadhyay *et al*. (2014).

Principal component analysis based on markers in the Saltol- QTL region of chromosome 1 led to the following positions on the 3-D plot: the moderately tolerant genotypes Rahspunjar and Nona Bokra were close to each other but distant from other genotypes including Pokkali, and SR 26B and Pokkali were also distant from each other (Figure 10). The positional symmetry of the salt-tolerant lines SR 26B, Pokkali, Rahspunjar, and Nona Bokra in both the 3-D plots shows that diversity in the Saltol QTL region varies more or less linearly with the phenotypic expression of salt tolerance. IR29 is commonly considered as salt susceptible variety. Morpho-physiological 3-D plot showed the position of IR29 near to salt tolerant SR 26B variety. It may happen due to interaction with the environment which helped IR29 to perform like a salt tolerant one. But, the actual scenario is quite evident in 3-D plot which

obtained from SSR data. IR29 was with other salt susceptible rice variety in this 3-D plot which indicates that IR29 is a salt susceptible variety. So, SSR data is more reliable in this case.

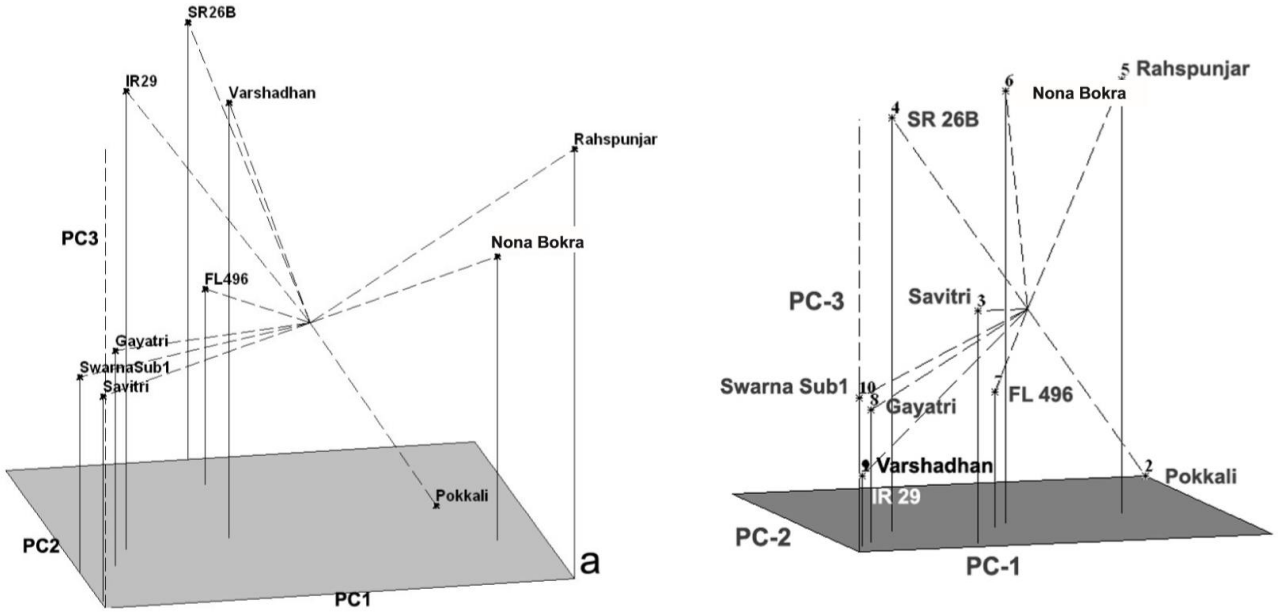

Figure 10: 3-D plot diagram showing the positional symmetry of morpho-physiological (a) and molecular (SSR based) salt tolerant and salt susceptible genotypes (Source: Chattopadhyay *et al*., 2014).

Again, dendrogram showing based on the physiological index and SSR marker data can provide a great comparison between the two methods. Tahjib-Ul-Arif *et al*. (2018) experimented to screen the salt-tolerant rice genotypes in the coastal region of Bangladesh. The dendrogram produced by using physiological indices showed three clusters at germination stage. Cluster I (moderately tolerant): Gajor Goria, Bina Sail, Vusharia, Sona Anjul, Tal Mugur, Til Kapor, Panbra, Patnai Balam, Dud Sail and Bolonga. Cluster II (susceptible): BINA dhan 7, BRRI dhan 29, Konkachur, Beto, Tillapur, Gota and Kolmilita. Cluster III (tolerant): BINA dhan 8, BRRI dhan 53, BINA dhan 10, BRRI dhan 40, FL 478, Nakraji, Komol Bhog and Sona Toly (Figure 11). UPGMA dendrogram based on Nei's (1973) genetic distance, summarizing data on differentiation among 25 rice genotypes according to SSR analyses (sub-cluster was cut at 50% of average Nei's genetic distance 0.3288). Cluster I (susceptible): Tal Mugur, BINA dhan 7, Patna Balam, Kolmilota, Dud Sail, Beto, Konkachur, Sona Anjul, Vusharia, BRRI dhan 29 and BRRI dhan 53. Cluster II, III, IV(tolerant): BRRI dhan 40, Bolonga, Til Kapor, BINA dhan 10, BINA dhan 8, Panbra, Sona Toly, FL 478, Bina Sail, Komol Bhog, Nakraji, Tilkapur, Gajor Goria and Gota (Figure 12).

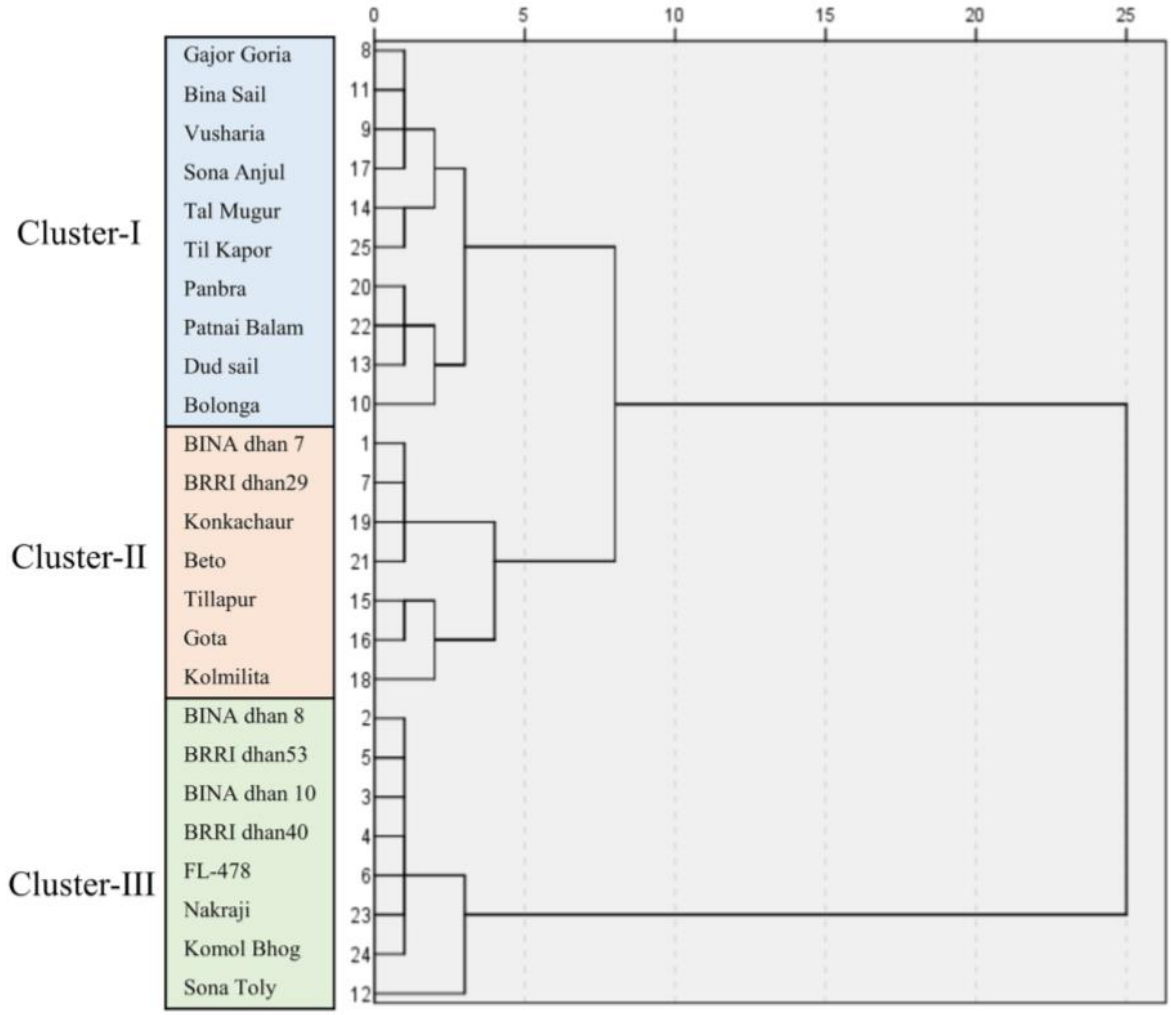

Figure 11: Dendrogram showing the clusters of 25 rice genotypes based on physiological indices (Source: Tahjib-Ul-Arif *et al*., 2018).

The genotypes Vusharia, Sona Anjul, Tal Mugur, Patnai Balam and Dud Sail performed like moderately tolerant to salt stress while considering the physiological indices. But they were clustered into the susceptible genotypes based on their genetic distances (Figure 11 and Figure 12). These four genotypes were clustered into the same group with the known susceptible rice variety like BINA dhan7, BRRI dhan29. So, they must be susceptible to salt stress genetically. Other genotypes produced the same result both in physiological indices and SSR marker-based analysis. This might happen due to the effect of the environment which influenced the genotypes to performed like tolerant ones. The results indicated that based on SSR marker data is much more reliable in case of identifying the rice genotypes based on their salt tolerance. These types of phenomenon do not indicate the physiological indices as a faulty method. It just indicated that in some special cases physiological indices may give some erratic results due environmental interactions. But molecular differentiation based on SSR marker data always

give the accurate results as it is based on the genetic constitution and there is no effect of environment.

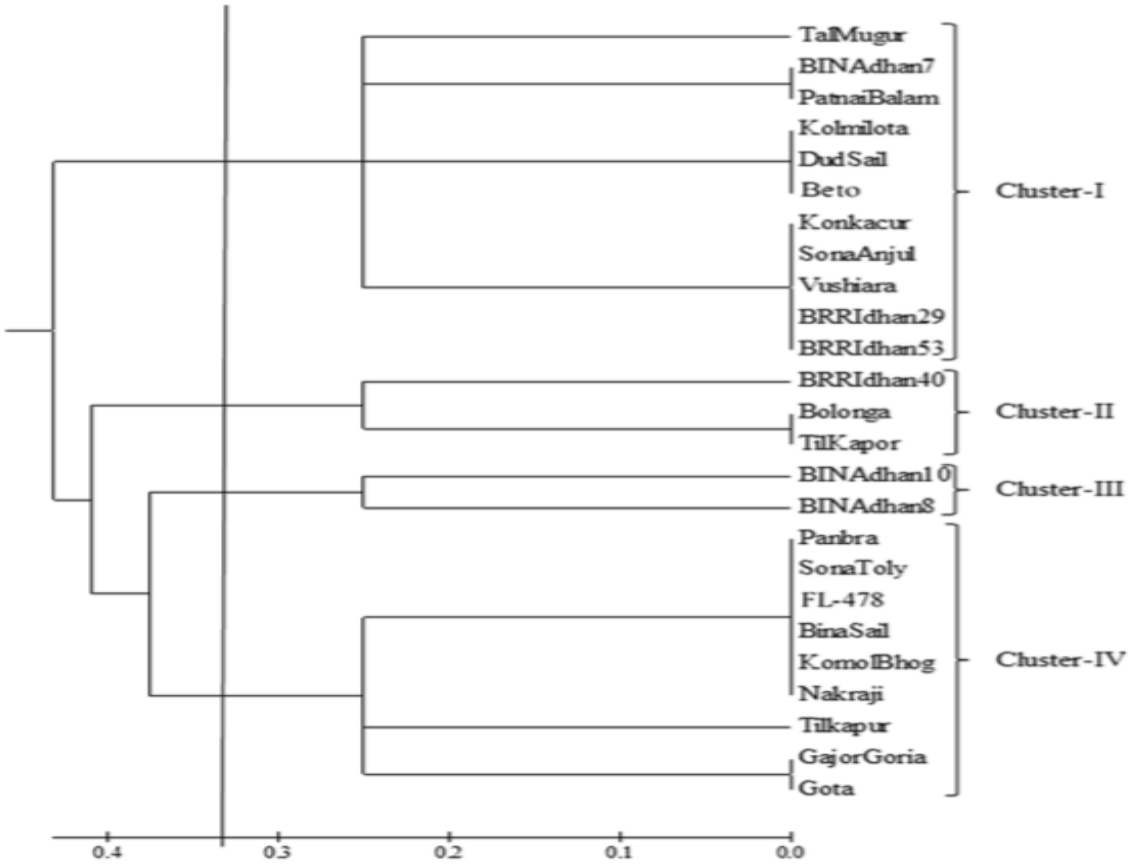

Figure 12: UPGMA dendrogram based on Nei's (1973) genetic distance, summarizing data on differentiation among 25 rice genotypes according to SSR analyses (Tahjib-Ul-Arif *et al*., 2018).

Moreover, the high efficiency of SSR markers comparing to other methods was also revealed by the experiment conducted by Vanniarajan *et al*. (2012). 40 rice genotypes of *japonica* and *indica* subspecies were used to differentiate the performance of agronomic characters and molecular characters by 12 SSR markers. The variety under the japonica subspecies are high yielding and cold tolerant but salt susceptible. Other varieties which under *indica* subspecies are high yielding and salt tolerant. Dendrogram produced from agronomic parameters showed that the salt tolerant *indica* varieties co-exist along with salt susceptible *japonica* variety (Figure: 13). Such incidence occurred because both *indica* and *japonica* varieties used in the experiment is high yielding. But the results obtained from SSR marker data isolated the salt tolerant varieties (which are indicated by SATVT) from the other varieties (Figure 14). It is possible because the used SSR markers were designed for the salt tolerant loci. The genotypes having the similar genotypic constitution were grouped together. As SSR marker reveal the actual genetic constitution of the rice genotypes they are much more reliable.

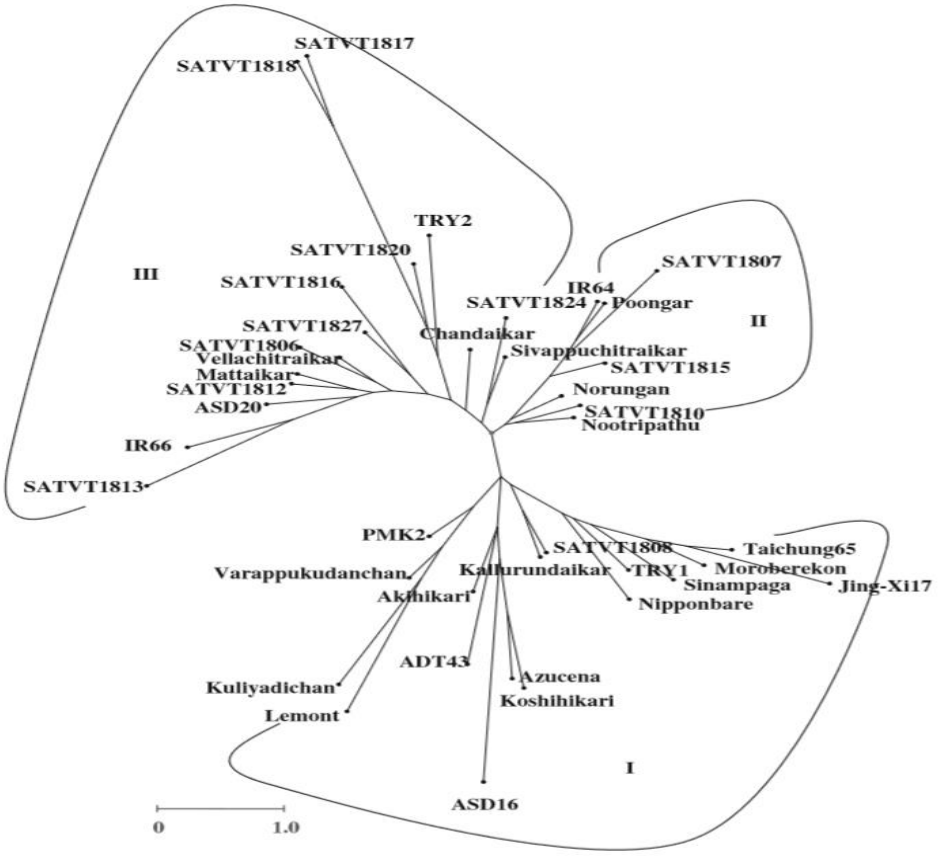

Figure 13: Dendrogram showing the clusters of 40 rice genotypes based on their agronomic traits (Source: Vanniarajan *et al*., 2012).

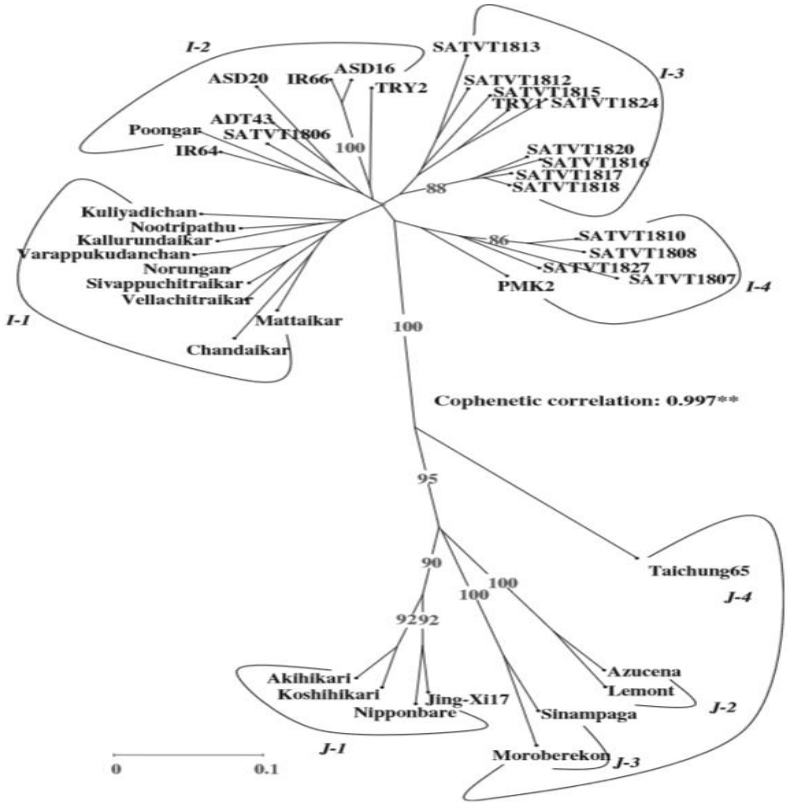

Figure 14: Dendrogram showing the cluster of 40 rice genotypes based on SSR marker data (Source: Vanniarajan *et al*., 2012).

All the previous results of different experiment revealed that the SSR markers are reliable than morphological and physiological characterization for grouping rice genotypes based on their salt tolerance. It does not mean that morphological and physiological characterization are not efficient. It only indicates that while comparing among the methods of differentiating salt tolerant rice genotypes SSR markers can provide the best results.

CHAPTER 4

CONCLUSIONS

From the above discussion following conclusions can be drawn:

- SSR markers are very useful for differentiating rice genotypes based on their salt tolerance. The marker RM8094 has the PIC value 0.82, Marker Index 1.92 and detect 14 alleles. The marker RM493 has the highest genetic diversity 0.8819 and D value 0.9203. RM10772 detected 12 alleles, RM336 has a PIC value of 0.84 and the D value for RM21 is 0.8949. All these results indicate that the marker RM8094, RM493, RM10772, RM336 and RM21 are the most suitable SSR markers for the discrimination among salt tolerant rice genotypes.
- SSR markers are reliable for grouping salt tolerant rice genotypes. Morphological and physiological characterization are also reliable but while comparing between SSR markers and other methods SSR markers are found to be more suitable method for salt tolerant rice differentiation.

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