# Estimation of genetic diversity in rice (*Oryza sativa* L.) genotypes using SSR markers under salinity stress

**Fatemeh Gholizadeh[1]* and Saeed Navabpour[2]**

*1. Young Researchers and Elite Club, Mashhad Branch, Islamic Azad University, Mashhad, Iran*
*2. Department of Plant Breeding and Biotechnology, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran*

## Abstract

In order to study the genetic diversity in rice (*Oryza sativa* L.), 29 genotypes consisting land races, pure and improved lines were evaluated using simple sequence repeat (SSR) markers. A total of 30 SSR primers were used to amplify some part of rice genome in germplasms, the PIC values ranged from 0.07 (RM 340) to 0.71 (RM 7426) with an average of 0.45. The results showed a total number of 106 amplified bands. Among them, the primer RM7426 showed the highest number alleles while the lowest was observed for RM340 primer. Average number of observed alleles in total genotypes was 3.53. The lowest PIC value was observed in RM445, RM466, RM3345, and RM7424 primers and the highest PIC value was observed in RM7426, RM1337, RM47, and RM5430 primers. PCA components explained 84.40% of variation. The clustering patterns of the genotypes were assigned into three clusters based on their response to salinity and morpho-physiological characteristics. Cluster analysis grouped the genotypes in salt tolerant, intermediate tolerant and sensitive classes. The results showed that information from SSR data can complement information obtained from quantitative methods.

*Keywords:* rice (*Oryza sativa*); salinity stress; SSR markers; cluster analysis

**Gholizadeh, F.** and **S. Navabpour** 2014. 'Estimation of genetic diversity in rice (*Oryza sativa* L.) genotypes using SSR markers under salinity stress. *Iranian Journal of Plant Physiology* 4 (3), 1039-1046.

## Introduction

Rice belongs to the genus Oryza in the grass family (Gramineae). There are 22 species in the genus, of which only two are cultivated: O. sativa, which was domesticated in the humid tropics of South and Southeast to East Asia and which was domesticated in the Niger basin in Africa (Khush, 1997). Rice is a salt-sensitive crop and increasing its salt tolerance has enormous implications. The strategy to overcome salinity stress is genetic improvement of salinity tolerance in the available varieties (Epstein et al., 1980). Plant growth is adversely affected by salinity, a major environmental stress that limits agricultural production. In the most commonly cultivated rice cultivars, young seedling were very sensitive to salinity whereas rice was more salt tolerant at germination than other stages (Zeng and Shanon., 2000). Genetic diversity in plants has been traditionally assessed using morphological or physiological traits. The

*Corresponding author
*E-mail address*: fatima.gholizadeh64@yahoo.com
Received: January, 2014
Accepted: March, 2014

assessment of phenotype may not be a reliable measure of genetic differences as gene expressions are influenced by environment. Further, this is aggravated in screening for salt tolerance as any change in the environment alters salt tolerance among the genotypes (Yeo et al., 1990). On the other hand, identified genetic variations based on DNA polymorphism are abundant and independent of environmental factors. DNA markers that differentiate genotypes are more reliable and convenient than physiological or morphological characteristics in the identification and characterization of genetic variation (Zeng et al., 2004). Among various PCR based markers, SSR markers are more popular in rice because they are highly informative, mostly monolocus, co-dominant, easily analyzed and cost effective (Chambers and Avoy, 2000). About 2240 microsatellite markers are now available through the published high-density linkage map (McCouch et al., 2002). The genetic variation, as identified by morphological characters and molecular markers, may be useful in breeding for abiotic stress. Therefore, the present investigation was undertaken with the objective of estimating genetic diversity in a set of salt tolerant genotypes using SSR markers and morpho-physiological characteristics.

Table 1
Details of genotypes

| S. NO | Genotypes | Origin |
|---|---|---|
| 1 | IR74099-3R-2-2 | IRRI, Philippines |
| 2 | IR74099-3R-2-3 | IRRI, Philippines |
| 3 | IR74095-AC-30 | IRRI, Philippines |
| 4 | IR74099-3R-5-3 | IRRI, Philippines |
| 5 | IR74095-AC32 | IRRI, Philippines |
| 6 | IR54447-3B-10-2 | IRRI, Philippines |
| 7 | IR74095-AC37 | IRRI, Philippines |
| 8 | IR74095-AC38 | IRRI, Philippines |
| 9 | IR74095-AC40 | IRRI, Philippines |
| 10 | IR65195-3B-19-1-1 | IRRI, Philippines |
| 11 | IR65-185-3B-8-3-2 | IRRI, Philippines |
| 12 | IR65192-3B-1-1-3 | IRRI, Philippines |
| 13 | IR59418-7B-19-2 | IRRI, Philippines |
| 14 | IR59418-7B-20-1 | IRRI, Philippines |
| 15 | IR59418-7B-27-3 | IRRI, Philippines |
| 16 | IR63311-B-6-2-1-3 | IRRI, Philippines |
| 17 | IR67075-2B-2-2 | IRRI, Philippines |
| 18 | IR67075-2B-5-2 | IRRI, Philippines |
| 19 | IR67075-2B-15-1 | IRRI, Philippines |
| 20 | IR65192-3B-14-1-1 | IRRI, Philippines |
| 21 | IR65192-4B-3-2 | IRRI, Philippines |
| 22 | IR28 | IRRI, Philippines |
| 23 | IR65192-4B-6-1 | IRRI, Philippines |
| 24 | Gharib | Landrace, Iran |
| 25 | Khazar | IR2071-625/TANU74, Iran |
| 26 | Shahpasand | Landrace, Iran |
| 27 | IR65192-4B-4-2 | IRRI, Philippines |
| 28 | IR50 | IRRI, Philippines |
| 29 | Sepidroud | Sadry/IR8/IR28. Iran |

IRRI: International Rice Research Institute

## Materials and Methods

### Plant material

This study was carried out at the Department of Plant Breeding and Biotechnology, Faculty of Crop Science, Gorgan University of Agricultural Science and Natural Resources, Iran. Twenty-nine rice genotypes (Table 1) consisting land races, pure and improved lines were chosen in the study. Accessions were obtained from the Rice Research Institute of IRAN and International Rice Research Institute.

### Morpho-physiological characteristics

The experiment was conducted in the experimental area of the Department of Plant Breeding and Biotechnology, Gorgan University of Agriculture Science and Natural Resources. All the twenty-nine genotypes were sowed at the split plot array based on randomized complete block design with three replicates at the controlled condition. Each genotype was planted in two rows per replication. Salinity tolerance was evaluated using the method of Gregorio et al. (1993). Rice genotypes were investigated under 8 dS.m-1 salinity level. This experiment was conducted in phytotron glasshouse maintained at 29/21 day/night temperature and minimum relative humidity of 70% during the day. Pre-germinated seeds of each cultivar were sown in holes made on Styrofoam sheets with a nylon net bottom. Three seeds were sown per hole, with 10 holes per test entry. The sheets were then floated on distilled water for 3d after which nutrient solution (Yoshida et al., 1976) were used for 14 days. Salinity treatment was introduced corresponding to electrical conductivities (EC) of 8 ds.m-1 21 days after planting. The culture solution was renewed weekly and the pH was adjusted daily to 5.5 by adding either NaOH or HCl. Fourteen days after salinization, morpho-

physiological characteristic including root and shoot dry weights, chlorophyll content, biomass, Na+/K+ ratio, and leaf area were measured and cultivars were evaluated using the method of Grigorio et al. (1997).

## DNA extraction and SSR marker analysis

Total genomic DNA was extracted from young leaves using CTAB method (Saghi Maroof et al., 1994). Thirty SSR primers were selected in this study. Thirty SSR marker Covering all the 12 chromosomes of rice, were selected from the Genome Databases, Rice Genes Microsatellite Markers. These primer sequences were synthesized by MWG Biotec Inc Germany. PCR reactions were carried out in PTC (A Programmable Thermal Cycler). The volume of the reaction mixture was 20 µL which consisted of 4 µL DNA sample and 16 µL Master mix. The temperature cycles were programmed as 94 °C for 4 min, 94°C for 45 s, 55 °C for 45 s, 72 °C for 1 min for 35 cycles and additional temperature of 72 °C for 5 min for extension and 4 °C for cooling.

Amplification products were separated by denaturing polyacrylamide gel electrophoresis (PAGE) on mini vertical units using either 6% polyacrylamide gels with Ix TBE buffer at constant voltage of 70 V for a period of 45 min to 1 h. After electrophoresis, gels were stained according to silver-staining method (Cho et al., 1996) and (Creste et al., 2001). The gel was visualized in photographs taken using a scanner instrument. Clearly resolved, unambiguous bands were scored visually for their presence or absence with each primer. The scores were obtained in the form of matrix with '1' and '0', which indicate the presence and absence of bands in each variety respectively.

## Data analysis

Statistical analysis of morpho-physiological data was performed using one-way ANOVA and the difference between the mean values was compared at $p \leq 0.01$ using Duncan Multiple Range Test. Statistical analyses for the SSR marker data were conducted using the software NTSYS-pc version (2.0 Rohlf., 1997).

The morpho-physiological characteristics were standardized prior to cluster analysis. Cluster analysis was then conducted on the taxonomic distance matrix with the un weighted Pair Group Method based on Arithmetic Average (UPGMA) and a dendrogram was generated based on the genetic distance matrix. For analyses based on SSR markers data from all the markers were used to estimate the similarity on the basis of the number of shared bands. Similarity was calculated with SIMQUAL function of NTSYS that computes a variety of similarity and dissimilarity coefficients for qualitative data. The similarity matrix values based on Jaccard coefficient of similarity were calculated. The similarity matrix thus generated was used to generate dendrogram based on UPGMA method. In order to estimate the congruence among dendrograms, cophenetic matrices for which marker and index type were computes and compares using the Mantel test.

Principal component analysis was performed in order to highlight the resolving power of the ordination. Polymorphic information content (PIC) that provides an estimate of the discriminatory power of a locus or loci, by taking into account not only the number of alleles that are expressed, but also relative frequencies of those alleles, was estimated using the formula suggested by Botstein et al. (1980):

$$PIC = 1 - \sum_{j=1}^{n} P_{ij}^2$$

where $P_{ij}$ is the frequency of the j*th* allele for the i*th* marker, and summed over n alleles.

## Results

## Genetic diversity based on SSR markers data

Thirty SSR markers were used for amplifying DNA segments from genomic DNA of twenty-nine genotypes. A total of 106 alleles were scored from the thirty SSR markers. The number of alleles per primer ranged from 2 to 6. On average, 3.53 alleles per locus were observed. The Polymorphism Information Content (PIC) ranged from 0.07 to 0.71 with an average of 0.45 (Table 2). The lowest PIC value were observed in

RM445, RM466, RM3345, RM7424 primers and the highest PIC value was observed in M7426, RM1337, RM47 and RM5430 primers. These results were consistent with those reported by Cho et al. (2000). For seven markers (RM416, RM478, RM5430, RM5473, RM7426, RM184, and RM1337), the tolerant and moderately tolerant genotypes had alleles that were not found in the sensitive genotypes. The size of the amplified segment ranged from 85-110 bp for RM7424 to 185-215 bp for RM445 (Table 2).

The microsatellite markers were able to distinguish between different rice genotypes. The high degree of polymorphism of microsatellite markers allows rapid and efficient identification of rice genotypes. The microsatellite markers classified the rice genotypes into II clusters. Each cluster distinguishes the genotypes clearly from the other. Cluster I had all the tolerant and moderately tolerant genotypes and Cluster II had all the sensitive genotypes (Fig. I). The tolerant genotypes of IR74099-3R-2-2, IR67075-2B-2-2, Gharib and Shahpasand were included into clusters I. The sensitive genotypes of IR50, IR28, IR74095-AC40, IR65195-3B-19-1-1, Khazar, and Spidroud were included into clusters II (Fig. I). The Twenty-nine accessions used in this study clustered in the same order using the UPGMA cluster analysis based on the Jaccard coefficient (Fig. I). A high cophenetic correlation (r = 0.94) between the original similarity matrix and those given by the clustering process was observed. The two major tolerant and moderately tolerant groups, and sensitive were resolved in the dendrogram. Most of the genotypes fall into tolerant and moderately tolerant group. There was overlapping of tolerant genotypes within the cluster. PCA components explained 84.40% of variation (Fig. II). Similar results were reported earlier by Zeng et al. (2004).

Table 2
Allelic variation and PIC values for SSR markers identified in 29 rice genotypes

| SSR LOCUS | Allele No. | PIC Values | AT (C) | AL (bp) |
|---|---|---|---|---|
| RM466 | 3 | 0.120 | 55 | 180-210 |
| RM488 | 3 | 0.489 | 55 | 140-175 |
| RM262 | 3 | 0.422 | 55 | 165-180 |
| RM236 | 3 | 0.566 | 55 | 150-195 |
| RM416 | 5 | 0.622 | 55 | 100-120 |
| RM5626 | 3 | 0.349 | 55 | 170-200 |
| RM478 | 5 | 0.716 | 55 | 168-200 |
| RM562 | 4 | 0.401 | 66 | 170-210 |
| RM5430 | 4 | 0.649 | 61 | 98-130 |
| RM7426 | 6 | 0.644 | 55 | 115-150 |
| RM6283 | 3 | 0.437 | 55 | 150-180 |
| RM7389 | 3 | 0.581 | 55 | 100-115 |
| RM11 | 5 | 0.568 | 55 | 130-180 |
| RM445 | 4 | 0.070 | 55 | 185-215 |
| RM152 | 3 | 0.580 | 55 | 130-150 |
| RM3342 | 3 | 0.476 | 55 | 105-145 |
| RM7424 | 3 | 0.247 | 50 | 88-110 |
| RM5702 | 3 | 0.340 | 55 | 110-140 |
| RM184 | 4 | 0.604 | 55 | 170-200 |
| RM3152 | 3 | 0.466 | 55 | 180-200 |
| RM144 | 3 | 0.390 | 55 | 150-200 |
| RM1341 | 5 | 0.550 | 55 | 170-190 |
| RM276 | 3 | 0.456 | 55 | 120-190 |
| RM1337 | 5 | 0.670 | 55 | 170-210 |
| RM5642 | 3 | 0.318 | 55 | 130-150 |
| RM5473 | 3 | 0.604 | 55 | 100-125 |
| RM3345 | 3 | 0.202 | 55 | 100-117 |
| RM5140 | 3 | 0.357 | 55 | 178-190 |
| RM3827 | 3 | 0.344 | 55 | 130-150 |
| RM340 | 2 | 0.328 | 55 | 138-150 |

AL: Allele Length in bp; AT : Armealing Temperahire; PIC: Polymorphic Information Content

Table 3
Variance analysis of traits evaluated in seedling stage of twenty-nine cultivars at two salinity levels

| Sources of Variation | d.f | root dry weight | shoot dry weight | chlorophyll content | Leaf area | biomass | $Na^+$ / $K^+$ ratio |
|---|---|---|---|---|---|---|---|
| SL | 1 | 0.126** | 5.88** | 113.93** | 734.99** | 7.738** | 43.14** |
| Error sl | 2 | 0.00005 | 0.00032 | 0.092 | 0.042 | 0.0006 | 0.008 |
| C | 28 | 0.0009** | 0.0444** | 9.061** | 10.95** | 0.054** | 0.06** |
| SL×C | 28 | 0.0002** | 0.042** | 5.08** | 2.08** | 0.005** | 0.05** |
| Error c×sl | 112 | 0.00001 | 0..0002 | 0.08 | 0.022 | 0.0003 | 0.0004 |
| CV (%) | | 5.99 | 2.99 | 0.87 | 1.94 | 2.84 | 5.66 |

**: Significant at 1%. SL: Saliinity Levels (8 $ds.m^{-1}$), C: Cultivars

## Genetic diversity and cluster analysis based on morpho-physiological characteristics

In an attempt to evaluate the similarity among different rice genotypes in relation to growth and physiological responses to salinity, a cluster analysis was performed using eight physiological characters which gave high correlation with the level of salinity tolerance. Morpho-physiological characteristics of each genotype were measured in each replication. Analysis of variance revealed significant genotypic differences (Table 3). Taxonomic distance based on plant morpho-physiological characteristics was estimated after standardization. The matrix of average taxonomic distance was estimated using Euclidian distance coefficient. The cluster analysis was conducted on average taxonomic distance with UPGMA method. Measurement of association using UPGMA revealed that twenty-nine genotypes were grouped into three clusters (Fig. III and Table 4). Among them, cluster II was found to have large number of genotypes. When the genotypes in each cluster were compared with the morpho-physiological data, it was found that genotypes in cluster I were susceptible, genotypes in cluster II were moderately tolerant, and genotypes in the cluster III were tolerant.

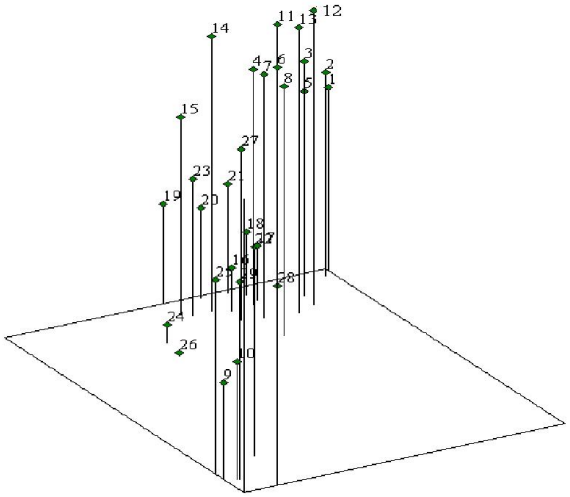

Figure II. Three dimensional plot of principal component analysis morpho-physiological data from the 29 rice genotypes; the numbers plotted represent individual cultivars corresponding to the ones listed in Table 1.

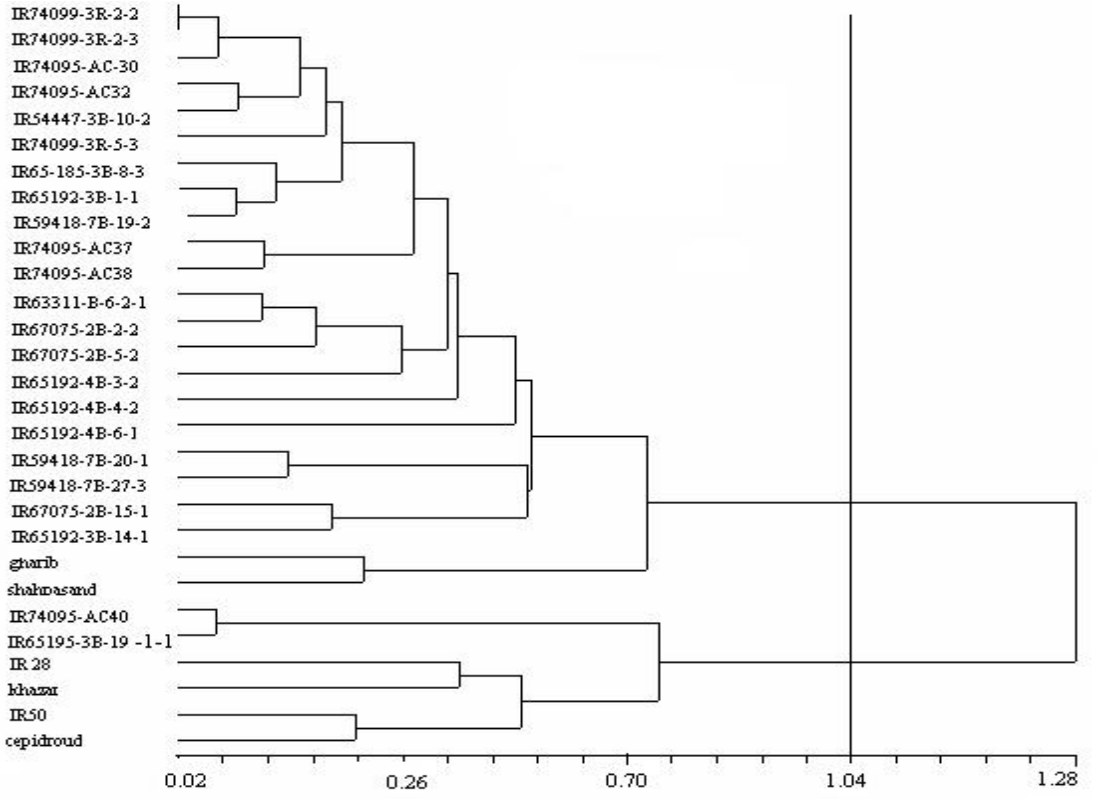


Figure I. Clustering of all 29 rice genotypes based on SSR marker data

Cluster analysis was carried out using appropriate procedures of the program SPSS 16.0. (2007).

## Discussion

In this study, most of genotypes were grouped into moderately tolerant cluster, while the other genotypes were clustered into tolerant or susceptible, and this was revealed based on morpho-physiological characteristics through cluster analysis (Table 4).

However, the genetic diversity in this subset of rice genotypes was relatively low compared to other reports. Although the average of the PIC values in this study (0.45) was higher than that determined by Akagi et al., (1997) in 59 japonica cultivars in Japan (0.37), it was lower than that determined by Panaud et al. (1996) in 24 rice cultivars. Breeders often have to deal with the tasks of genetic improvement in crops for tolerance to abiotic stress when the related mechanisms are not well characterized. One of the major approaches in molecular breeding is selection with the aid of molecular markers linked to the quantitative trait loci (QTLs) underlying the physiological or agronomical performance under stress when the candidate genes are not available. The QTLs controlling salt tolerance have been identified using molecular markers in tomato (Foolad et al., 1997) and rice (Koyama et al., 2001). In contrast to morphological trait, the molecular markers revealed polymorphism at the DNA level, suggesting a very powerful tool for characterization of genotype and estimation of genetic diversity.

Among them the microsatellite or simple sequence repeats (SSR) markers showed a high potential for identification and estimation of genetic diversity (Zhang et al., 1994). The SSR markers played an important role in studying the germplasm diversity in rice (Yu et al., 2005). The results indicated that SSR analysis could be a better method to study the genetic diversity in rice.

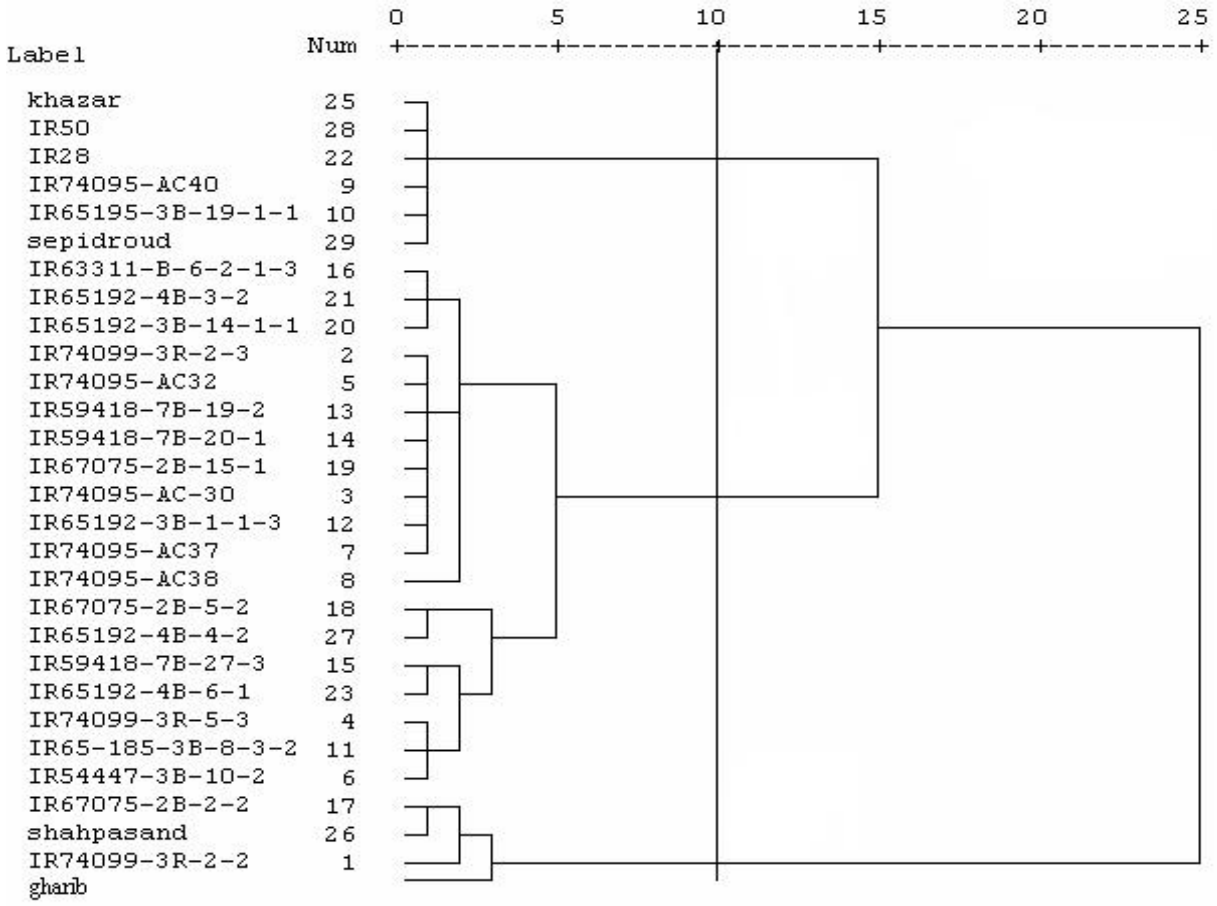


Figure III. Clustering of all 29 rice genotypes based on morpho-physiological data

Table 4
The grouping result for 29 genotypes rice based on morpho-physiological characters cluster analysis

| Group | Material |
|---|---|
| Susceptible | IR50 IR28, IR74095-AC40, IR65195-3B-19-1-1, Khazar, Spidroud |
| Moderately tolerant | IR74099-3R-2-3, IR74095-AC-30, IR74099-3R-5-3, IR74095-AC32, IR54447-3B- 10-2, IR74095-AC37, IR74095-AC38, IR65-185-3B-8-3-2, IR65192-3B-1-1-3, IR59418-7B-19-2, IR59418-7B-20-1, IR59418-7B-27-3, IR59418-7B-28-2, IR63311- B-6-2-1-3, IR67075-2B-5-2, IR67075-2B-15-1, IR65192-3B-14-1-1, IR65192-4B-3- 2, IR65192-4B-6-1, IR65192-4B-4-2 |
| Tolerant | IR74099-3R-2-2, IR67075-2B-2-2, Gharib, Shahpasand |

In sum, the results of this study provide some implications for engineering salt tolerance using microsatellite clusters. Evaluating and selecting salt tolerance among genotypes are not easy tasks because measurements of physiological and morphological phenotypes are highly affected by environmental factors. However, findings suggest that physiological phenotypes among different genotypes can be predicted based on their genetic similarity characterized by microsatellite markers. The best measure to analyze genetic diversity among genotypes would be with the use of all information, both from morpho-physiological characteristics and DNA-based markers. Molecular marker data and morpho-physiological data subjected to various numerical and taxonomical techniques measure the relationship among the genotypes (Kumar et al., 2003). The genotypes which were found to vary based on both morpho-physiological and molecular diversity can be used for further breeding program.

## Acknowledgements

We thank the International Rice Research Institute (IRRI), Rice Research Institute of Iran and Gorgan University of Agricultural Science & Natural Resource for their supports.

## References

**Akagi H, Y., Yokozeki** and **A. Inagaki.** 1997. 'Highly polymorphic microsatellites of rice consist of AT repeats, and a classification of closely related cultivars with these microsatellite loci'. *Theor. Appl. Genet. pp.* 94: 61–67.

**Botstein D, RL., White, M., Skolnick** and **RW. Davis.** 1980. 'Construction of a genetic linkage map in man using restriction fragment length polymorphisms'. *Am. J. Hum. Genet.* 32: 314–331.

**Creste S, A., TulmannNeto** and **A. Figueria.** 2001. 'Detection of single sequence repeat polymorphism in denaturing polyacrylamid sequencing gels by silver staining'. *Plant Mol. Biol. Reporter.* 19: 2999-3006.

**Chambers M** and **M. Avoy.** 2000. 'Comparision of microsatellite and amplified fragment length polymorphism markers for parentage analysis'. *Mol. Ecol.* 9: 1037–1048.

**Cho YG, M W., Blair, O. Panaud** and **SR. McCouch.** 1996. 'Cloning and mapping of variety-specific rice genomic DNA sequences: Amplified length polymorphisms (AFLP) from silver-stained polyacrylamide gels'. *Genome,* 39: 373–378.

**Cho YG, T., Ishii, S., Temnykh, X., Chen, L., Lipovich, SR., McCouch, WD., Park, N., Ayres** and **S. Cartinhour.** 2000. 'Diversity of microsatellites derived from genomic libraries and GenBank sequences in rice (*Oryza sativa* L.)'. *Theor. Appl. Genet.* 100: 713–722.

**Epstein E, JD., Norlyer, DW., Rush, RW., Kingsbury, DB., Kelly, GA., Cnningham** and **AF. Wrona.** 1980. 'Saline culture of crops: A Genetic approach'. *Science.* 210: 399–404.

**Foolad MR, T., Stoltz, C., Dervinis, RL., Rodriguez** and **RA. Jones.** 1997. 'Mapping QTLs conferring salt tolerance during germination in tomato by selective genotyping'. *Mol. Breeding.* 3: 269–277.

**Gregario GB** and **D. Sendhira.** 1993. 'Genetic analysis of salinity tolerance in rice'. *Theor. Appl. Genet.* 86: 333–338.

**Gregario GB, D. Sendhira** and **R. Mendoza.** 1997. 'Screening rice for salinity tolerance' . IRRI, Dis Peper. No. 22, Los Banos. Philipine.

**Koyama ML, A., Levesley, RMD., Koebner, TJ., Flowers** and **AR. Yeo.** 2001. 'Quantitative trait loci for component physiological traits determining salt tolerance in rice'. *Plant Physiol.* 125: 406–422.

**Khush GS.** 1997. 'Origin, dispersal, cultivation and variation of rice'. *Plant Mol. BioI.* 35: 25–34.

**Kumar P, K., Singh, Y., Vikal, LS., Randhawa** and **GS. Chahal.** 2003. 'Genetic diversity studies of elite cotton germplasm lines using RAPD markers and morphological characters'. *Indian J. Genet.* 63:(1), 5–10.

**McCouch SR, L., Teytelman , Y., Xu** and **BL. Katarzyna.** 2002. 'Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.)'. *DNA Research.* 9: 257–279.

**Panaud O, X., Chen** and **SR. McCouch.** 1996. 'Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (*Oryza sativa* L.)'. *Mol. Gen. Genet.* 252: 597–607.

**Rohlf FJ**. 1997. 'NTSYS-pc numerical taxonomy and multivariate analysis system'. *version 2.0. New York: Exeter Software.*

**Saghi Maroof MA, RM., Biyashev, GP., Yang, Q., Zhang** and **RW. Allard.** 1994.' Extraordinarily polymorphic microsatellites DNA in barely species diversity, choromosomal location, and population dynamics'. *Proceeding of the Academy of Sciences, USA. 91: 5466–5570.*

**SPSS Base**. 2007. 16.0 'Applications Guid'e. *SPSS Inc.*

**Yeo AR, ME., Yeo, SA., Flowers** and **TJ**. **Flowers.** 1990.'Screening of rice genotypes for physiological characters contributing to salinity resistance and their relationship to overall performance'. *Theor. Appl. Genet.* 79: 377–384.

**Yoshida S, DA., Forno, JH., Cock** and **KA. Gomez.** 1976. 'Labratory Manual for Physiological Studies of Rice'. *IRRI, Los Banos, Philippines.*

**Yu GQ, Y., Bao, CH., Shi, CQ., Dong** and **S. Ge.** 2005. 'Genetic diversity and population differentiation of Liaoning weedy rice detected by RAPD and SSR markers'. *Biochem. Genet.* 43(5-6): 261–270.

**Zeng L, TR., Kwon, X., Liu, C., Wilson, CM., Grieve** and **GB. Gregorio.** 2004. 'Genetic diversity analysed by microsatellite markers among rice (*Oryza sativa* L.) genotypes with different adaptation to saline soils'. *Plant Sci.* 166: 1275–1285.

**Zeng L.** and **MC. Shannon.** 2000.' Salinity effects on seedling growth and yield components of rice'. *Crop Sci.* 40:996–1003.

**Zhang Q, YL., Gao** and **SH. Yang.** 1994.' A diallel analysis of heterosis in elite hybrid based on RFLP and microsatellite'. *Theor Appl Genet.* 89: 185–192.