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DIVERSITY ANALYSIS OF RICE VARIETIES DIFFERING IN SALT TOLERANCE

M.H.M. Ammar[1], Samah Monir[1], R.K. Singh[3], T. Mohapatra[2] and N.K. Singh[2]

1 Rice Research and Training Center, Sakha 33717, Egypt

2 National Research Center on Plant Biotechnology, Indian Agricultural Research Institute, New Delhi – 110012, India.

3 Central Soil Salinity Research Institute, Karnal – 132001, India.

ABSTRACT

Two hundred seventy five Sequence Tagged Microsatellite (STMS) markers covering the whole rice genome were used to screen polymorphism among eight rice genotypes with different levels and mechanisms of salt tolerance. The set included five salt tolerant varieties; Pokkali, CSR10, CSR11, CSR27and IR4630-22-2-5-1-3; one moderately tolerant variety; CSR30 and two international sensitive checks, IR28 and MI-48. The moderately tolerant, aromatic rice variety CSR30 was the most divergent genotype from the other genotypes (less than 44% similarity) and formed a separate group. The second group consisted of CSR27 and IR4630-22-2-5-1-3 which is also in correspondence with their pedigree since both of them are derived from a common salt tolerant rice genotype, Nona Bokra. The third group included two salt sensitive genotypes MI-48 and IR 28. The fourth group composed of Pokkali, CSR10 and CSR11. The CSR10 and CSR11 were very similar at the molecular level, showing 96.8% similarity, and confirming that the molecular assessment is in correspondence with their pedigree. A unique and double banding pattern was observed in Pokkali suggesting a duplication of these segments.

Key words: ***Rice, Genetic similarity, Diversity, Salt tolerance, Polymorphism, STMS***

INTRODUCTION

Rice (*Oryza Sativa* L.) is the stable food for more than half of the world's population, most of them in the developing countries. Besides its economic significance, rice is rich in genetic diversity in the form of thousands of land races, varieties and wild progenitor species. However, a series of biotic and a biotic stresses limit rice productivity worldwide. Soil salinity is considered as one of the major and most widespread abiotic stress limiting rice production in many rice-growing areas (Mishra *et al* 1988), not only in arid and semi arid regions, but also in sub-humid and humid zones (Gregorio 1997). Salinity affects rice growth in varying degrees at all stages starting from germination through maturation. Stress-tolerant high-yielding varieties along with efficient crop management practices will help reduce food gap in many of the rice growing areas and insure food security. Although salt tolerance is a difficult trait to manipulate due to its quantitative nature and low selection efficiency, recent advances in molecular marker technology, make it possible to assess polymorphism, map, tag, and finally clone the genes controlling salt tolerance. Sequence

tagged microsatellite marker (STMS) system is considered an ideal marker system for rice, because of its co-dominant nature, high level of polymorphism, their abundance and distribution throughout the rice genome at an average distance of 0.2 cM (McCouch *et al* 1988 and 2002). Since genetic variation is a pre-requisite for any successful breeding program, the aim of this study was to assess the genetic diversity among selected rice varieties known for their salt tolerance.

MATERIALS AND METHODS

A total of eight rice genotypes were selected, including six tolerant or moderately tolerant varieties, namely, Pokkali, CSR10, CSR11, CSR27, CSR30, IR4630-22-2-5-1-3, and two sensitive varieties, namely IR 28 and MI-48. The pedigree and some basic features of these genotypes are summarized in Table (1). Seeds of the rice genotypes were obtained from the Central Soil Salinity Research Institute (CSSRI), Karnal, Haryana. DNA isolation and purification was carried out using CTAB method (Murray and Thompson1980). The DNA was quantified using gel assay method and then PCR was performed. A total of 275 pairs of STMS primers were used for the screening purpose. The PCR was performed in 96-well microtitter plates in 10µl PCR volume containing 50 ng of template DNA, 5 pmole of each of forward and reverse primers, 0.1mM dNTP's, 1x PCR buffer (10mM Tris,pH 8.0, 50mM KCl and 50mM ammonium sulphate), 1.8mM $MgCl_2$, and 0.2 units of Taq DNA polymerase. Initial denaturation at 94°C for 5 minutes was followed by 35 cycles of amplification with template denaturation at 94°C for 1 minute, primer annealing at 55.7°C for 1 min and primer extension at 72°C for 2min. After the end of the 35th cycle, a final extension at 72°C for 7 min was given followed by storage at 4.0 °C. The PCR products were separated using either 3% Metaphor agarose (BMA, USA) stained with gel star dye (BMA, USA) or a Native PAGE (10% acrylamide, 0.08% crosslinking in 0.5x TBE, stained with Et Br solution (1 mg/l). The STMS banding pattern was then scored and used to prepare the matrix. Employing the computer package NTSYS .pc (Ralf 1998), Jaccord's similarity coefficients were calculated and used to establish genetic relationship among the genotypes based on unweighted pair group method of arithmetic averages (UPGMA) and sequential agglomerative hierarchical nested (SAHN) clustering.

Table 1. Slat tolerant indica rice genotypes allay with sensitive varieties and their important characteristics

S.No.	Name	Parentage	Tolerance mechanism	Level of salt tolerance
1	Pokkali	Local type, Kerala	Na^+ accumulation	Tolerant
2	CSR10	M40-431-24-114/ Jaya	Na^+ exclusion K^+ accumulation	Tolerant
3	CSR11	M40-431-24-114/ Basmati 370	Na^+ exclusion K^+ accumulation	Tolerant
4	CSR27	Nona Bokra / IR5657-33-2	Na^+ accumulation Tissue tolerance	Tolerant
5	CSR30	BR4-10 /Pakistan Basmati	K^+ accumulation	Moderate
6	IR4630-22-2-5-1-3	Nona Bokra derived IRRI line	Na^+ accumulation K^+ accumulation	Tolerant
7	MI-48	Pelita1-1//H4/H501	Low K^+ Uptake	Sensitive
8	IR28	IRRI line	-	Sensitive

RESULTS AND DISCUSSIONS

A total of 275 STMS primer pairs covering the whole rice genome were used to screen a set of 8 rice varieties with different levels and mechanisms of salt tolerance (Fig.1). Seventy percent of all the tested primers showed polymorphic pattern among these genotypes. The number of polymorphic alleles ranged from two with RM190 to five with RM333. The aromatic and moderately tolerant rice variety CSR30, a derivative from Pakistani Basmati, was the most diverse of all other tested genotypes (Table 2). On the other hand, CSR10 and CSR11 were the closest pair of genotypes (96.7% similarity) since they share a common parent (M40-431-24-114). The most divergent pair of genotypes was Pokkali and CSR30, with a similarity coefficient of 41.6%. A dendrogram was constructed based on the Jaccord's similarity coefficient, in which the eight genotypes were grouped into four main classes (Fig. 2). Variety CSR30 was alone in a separate group as the most divergent genotype, followed by the two salt tolerant varieties, IR4630-22-5-2-2 and CSR27 in the second group. Both of these genotypes are derived from the salt tolerant genotype Nona Bokra and this explains their presence in the same group. The two salt sensitive varieties IR28 and MI-48 were clustered in the third group and finally the last group included

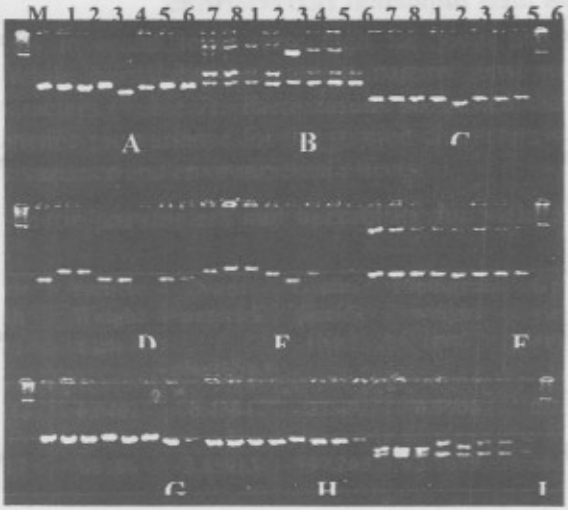

Figure 1. Polymorphism survey of the eight genotypes using STMS markers, the primers shown are A) RM242, B) RM47, C) RM201, D) RM249, E) RM217, F) RM213, G) RM231, H) RM60, and I) RM210, M is 100bp ladder. Rice genotypes (from left to right) are: 1) Pokkali, 2) CSR10, 3) CSR11, 4) CSR27, 5) CSR30, 6) IR4630-22-2-5-1-3, 7) MI-48 and 8) IR28

Table 2.Genetic similarity index between pairs of cultivars

	Pokkali	CSR10	CSR11	CS R27	CSR30	IR4630	MI-48	IR28
Pokkali	**1.000**							
CSR10	**0.649**	**1.000**						
CSR11	**0.653**	**0.968**	**1.000**					
CS R27	**0.570**	**0.596**	**0.594**	**1.000**				
CSR30	**0.416**	**0.431**	**0.435**	**0.437**	**1.000**			
IR4630	**0.567**	**0.630**	**0.621**	**0.641**	**0.433**	**1.000**		
MI-48	**0.620**	**0.624**	**0.627**	**0.575**	**0.433**	**0.626**	**1.000**	
IR28	**0.605**	**0.651**	**0.643**	**0.590**	**0.395**	**0.604**	**0.635**	**1.000**

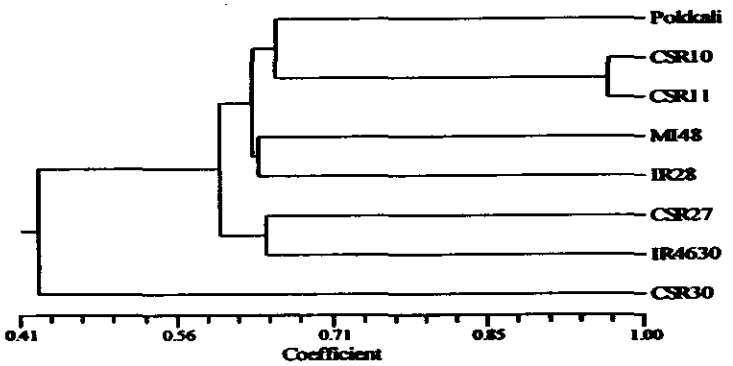

Figure 2. Dendrogram of genetic relationship among the eight rice varieties using Jaccord's coefficient.

three genotypes, the two salt tolerant and the most similar genotypes CSR10 and CSR11 along with the international salt tolerant check Pokkali. This is also quite explainable as Pokkali rice variety is a local type found in Kerela region, while a common parent of both CSR10 and CSR11, namely M40-431-24-114, is also a local type found in West Bengal region. This may suggest that Pokkali and M40-431-24-114 are actually related genotypes since they were found in the costal regions, and both are salt tolerant genotypes, or may have a common parentage, but got diverged from each other due to geographical isolation.

These results clearly demonstrate the existence of significant amount of molecular polymorphism even among the closely related *indica* genotypes and hence the possibility of salt tolerant variety development using such genetic pool. It also emphasizes the importance of molecular markers in germplasm diversity assessment and the strong correspondence between the molecular fingerprints and the pedigree of a particular genotype. It is also clear that the overall diversity index is basically correlated with the pedigree rather than with the salt tolerance *per se* performance. The lack of common band(s) distinguishing salt tolerant genotypes from the sensitive ones is basically because of the complexity of the trait and the different mechanisms operating in each salt tolerant genotype. This emphasizes the urgent need of mapping QTLs for salinity tolerance to detect a linked DNA marker(s). Doing so will greatly help in improving selection efficiency for salt tolerance and enables map based cloning of these genes for trait manipulation.

An interesting observation was that, the long existing, widely adopted salt-tolerant rice variety Pokkali showed unique double bands with four microsatellite primers (RM128, RM160, RM280 and RM145) while other varieties showed only single bands as shown in Figure (3). These are

reproducible patterns and their presence may have some significance in the wide adaptability of this variety. These STMS markers are located on chromosome 1 (RM128), 2 (RM145), 4 (RM280) and 9 (RM160) where QTLs related to salt tolerance have been previously identified (Gregorio 1997 and Flowers *et al* 2000). Gregorio, (1997) identified one QTL on chromosome 1 on the long arm, close to *saltol* gene, and one QTL on chromosome 4, using Pokkali /IR29 recombinant inbred line (RIL) population. Flowers et al (2000) used another RIL population derived from Nona Bokra/Pokkali//IR4630-22-2-5-2-3/IR10167-129-3-4, and found QTLs on chromosome 1 , 6 and chromosome 9. This suggests a correlation between those extra bands and regions on the chromosomes related to stress tolerance and /or the wide adaptability of this genotype. The extra and unique bands in Pokkali could either be due to duplicate loci for these markers or in a rare case scenario could represent residual heterozygosity that needs to be confirmed and then relationship with the QTLs for salt tolerance needs to be explained in genetic fine mapping studies.

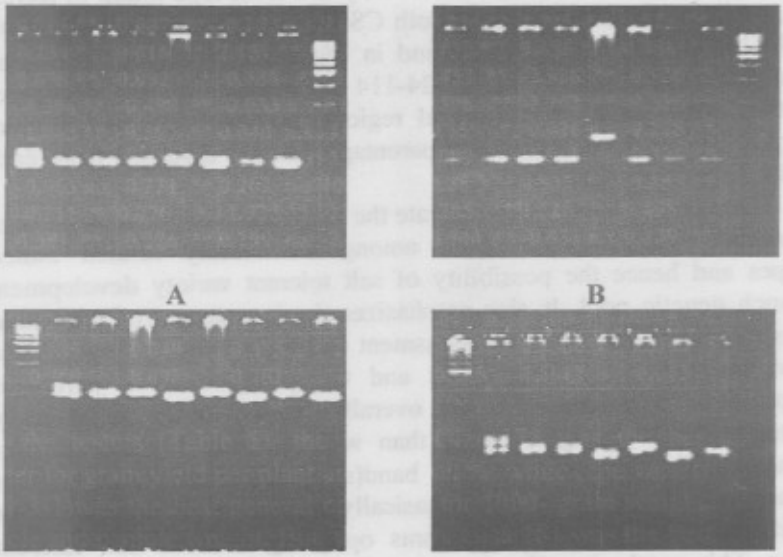

Figure 3. Pokkali (P1), showing additional unique band for some of the tested STMS markers; A) RM128; B)RM160 ;C) RM280 and D) RM145. Rice genotypes (from left to right) are: 1) Pokkali, 2) CSR10, 3) CSR11, 4) CSR27, 5) CSR30, 6) R4630-22-2-5-1-3, 7) MI-48 and 8) IR28

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تقدير درجة الاختلافات الوراثية لأصناف أرز مختلفة في تحملها للملوحة

مجاهد حلمي عمار[1]، سماح منير[1]، أر. ك. سنج[2]، ت. موهاباترا[3]، إن. ك.سنج[2]

[1] مركز البحوث و التدريب في الأرز -سخا – مصر

[2] المركز القومي لبيوتكنولوجيا النبات – المعهد الهندي للبحوث الزراعية – نيودلهي – الهند

[3] المعهد المركزي لبحوث ملوحة الأراضي – كرنال- هريانا- الهند

أجريت هذه الدراسة لدراسة درجة الاختلاف الوراثي لثمانية أصناف أرز باستخدام 275 مطم جزيئي SSR (التوابع القصيرة) . الأصناف الثمانية لختلفت فيما بينها في كل من ميكانيكيات و مستوي تحمل الملوحة وشملت هذه الأصناف خمسة أصناف متحملة للملوحة وهي Pokkali , IR4630-22-25-1-3, CSR10, CSR11, CSR27 و صنف متوسط التحمل ذو خلاية عطرية CSR30 و اثنين من الأصناف الحساسة الدولية للملوحة و هي MI-48 , IR28 . و أظهرت النتائج أن الأصناف قد توزعت في 4 مجموعات حيث ضمت المجموعة الأولي صنفا واحدا وهو CSR30 حيث كان اكثر الأصناف اختلافا مسجلا أقل من 44% في درجة التشابه . المجموعة الثانية شملت كلا من IR4630-22-25-1-3, CSR27 مما يتفق مع سجلات النسب حيث

أن كلا منهما منفرد من الصنف Nucellaire المتحمل للملوحة . و كذلك شملت المجموعة الثالثة كـلا مـن MI-48, IR28 و هما سلالتان شديدي الحساسية للملوحة . أما المجموعـة الرابعـة و الأخيـرة فقـد شـملت CSR10, CSR11,Pokkali . وقد اظهر الصنفان CSR10, CSR11 درجة التشابه بينهما 96.8% ممـا يؤكد التوافق بين سجلات النسب و التباين الجزيئي حيث انهما يشتركان في نفـس الأم (M40-431-24-114) . وقد وجدت ظاهرة المواقع المتوسطة في الصنف Pokkali و تم اقتراح تفسير لها.