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Estimation of genetic diversity among parents and F3 mapping population developed between a salt tolerant and salt susceptible rice variety

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ABSTRACT

Hybridization was made between a high yielding, salt susceptible rice variety IR 28 and a salt tolerant rice cultivar Pokkali to generate a mapping population aiming to tag the salt tolerant gene(s) in rice. Phenotyping of 129 F_3 segregants was done which showed varying degree of tolerance towards excess salt (12 dSm^{-1} NaCl) provided artificially. Phenotyping of the segregants and parents was done based upon tolerance (0-9 scale) towards salt stress. Three groups (highly tolerant, 0-1, moderately tolerant, 2-4 and susceptible 5-9) were formed based upon tolerance norms. Genetic analysis using RAPD grouped the segregants cultivar into two major classes with two sub-classes each. RAPD was found to be potential to tag the gene(s) as indicated by character analysis using UPGMA

Key words: *rice, salt tolerance, hybridization, phenotyping, genotyping*

Soil salinity is a major abiotic stress limiting rice production in coastal, arid and semi-arid regions globally. Cultivation of salt tolerant rice varieties is the most appropriate route to harness higher production from salt affected soils. In India, vast land measuring about 8.6 million ha is reported to be under salt stress with excess salinity (Anon, 2004). In the union territory of Andaman and Nicobar Islands, about 3000 ha of soil is found to be affected with salinity which possesses immense potential to produce additional quantity of 9000 tons of rice if properly exploited (Chowdhuri and Mandal, 2001). Therefore, development of varieties having both salt tolerance as well as high yielding characters is desirable. It is well known that environment significantly influences the growth and expression of the character like tolerance and susceptibility of any genotype. In this crossroad, molecular markers may play pivotal role to identify the true tolerant genotypes and it is possible to tag salt tolerant gene(s) by using a segregating mapping population. It is well known that RAPD primers (Williams *et al*., 1990; Welsh and Mc Clelland, 1990) are highly compatible to develop a molecular map and tagging of gene(s). Use of RAPD in genetic mapping and population genetics studies have widely documented both in animal and plant kingdom (Braden and Havey, 1995; Damasco *et al*., 1996). At individual level, RAPD marker can also be applied for parentage analysis, while at population level, RAPD deemed to detect hybrid populations, species or subspecies (Hadrys *et al*., 1992).

Keeping all these in purview and to tag the salt tolerance gene(s) in rice, an attempt was made to develop a mapping population and to assess genetic diversity of parents and F_3 mapping populations through RAPD analysis. Altogether 129 F_3 individuals along with their parents (IR28 and Pokkali) were phenotyped under artificially simulated salt stress (12 dSm^{-1} NaCl) and profiled at DNA level.

MATERIALS AND METHODS

Segregating F_3 population was developed from IR28 (*indica*) x Pokkali (*indica*) to combine high yielding ability of IR28 with salt-tolerance from Pokkali. F_1 seeds from hybridized spikelets were collected. Those were planted to grow F_1 plants. F_2 seeds were obtained by selfing and were grown in the experimental farm. Single seed from each F_2 plant was taken and those seeds were bulked to grow F_3 generation following

single seed decendent (SSD) method. Tillers from individual F_3 plants at active tillering stage were split into two, half was grown in cemented pots under normal soil condition and other half was grown in long narrow cemented pots artificially stressed with NaCl (12 dSm^{-1}) for phenotyping. Artificially simulated salt stressed segregants were phenotyped in 0-9 scale following standard screening protocol as advocated by International Rice Research Institute, Philippines (SES, IRTP, IRRI, 1988). DNA isolation was done from tender leaves of split segregants grown under normal soil following CTAB method (Murray and Thompson, 1980). Twenty arbitrary decamer primers from Operon Technologies, USA (viz. OPD 01, 02, 04, 05, 06, 08, 10, 13, 15, 19; OPZ 02, 03, 06, 07, 11, 13, 14, 15, 17, 19) were employed for PCR analysis. Amplified products were profiled by agarose gel electrophoresis (1.2%) and documented with gel documentation system. PCR amplified bands were scored based upon the presence (1) or absence (0) of clear, visible and reproducible bands. Two alleles (0/1) were assigned for a polymorphic locus and one allele (0/0 or 1/1) for a monomorphic locus. Within each lane, bands were scored as present if their intensity was found to be at least 10% of that of monomorphic reference band within that lane. Similarity index of bands in common between two accessions was estimated using the formula of Nei and Li (Nei and Li, 1979). Using dice coefficient, a similarity matrix involving 129 F_3 accessions and both of their parents was generated with the software NTSYS-pc version 2.02e. Dendrograms was constructed using an unweighted pair group method with arithmetical averages (UPGMA) to check the genetic similarity between F_3 individuals along with the parents.

RESULTS AND DISCUSSION

All the 129 F_3 segregants along with their parents were phenotyped in response to salt stress @ 12 dSm^{-1} under artificially simulated condition in 0-9 scale. As expected, the tolerant parent Pokkali displayed a score of 1 and the susceptible parent IR 28 showed a score of 8, indicating extreme tolerance and sensitivity, respectively towards salt stress. All segregants were found to have varying degree of tolerance towards excess salt. Based upon the reaction towards saline stress, the segregants were grouped into three major classes. The most tolerant group comprised of 22 lines and parent Pokkali, while the second group was consisted of 48 segregants that showed moderate salinity tolerance (scale 2-4). The third group was found to be highly susceptible, had remaining 59 segregants and the susceptible parent IR 28 (Table 1). Objective of this study was to use cheap and easy to handle RAPD marker to analyse F_3 segregants and thus assessing its reliability, efficiency and potential in tagging of salt tolerant gene(s). Molecular profiles as generated by some of the RAPD markers in segregating populations and the parents, which showed genetic relatedness among segregants and parents having various genomic constituents (Fig. 1). Multivariate (cluster) analysis of the genetic similarity data obtained using the arbitrary primers grouped the cultivars into two (I and II) major clusters (Fig. 2). Cluster I has 16 genotypes and it consists of two sub-clusters (Ia and Ib). Sub-cluster Ia is comprised of the high yielding susceptible parent IR28 along with another 12 segregants, and most of these segregants fall within the highly susceptible group. Another sub-cluster Ib consists of highly salt tolerant Pokkali along with two more tolerant segregants and showed about

Table 1. Distribution of F_3 segregants and parents based upon tolerance norms towards excess salt (12dSm^{-1} NaCl)

Salinity score (0-9 scale)*	Segregant	Numbers of segregants
0-1	Pokkali, 5, 9, 16, 17, 19, 26, 28, 38, 48, 50, 72, 77, 80, 81, 98, 100,117, 118, 123, 130, 131, 148	23
2-4	2, 3, 4, 6, 7, 8, 12, 13, 21, 24,27, 29, 30, 31,34, 39, 46, 54,61, 65, 67, 71,83, 84, 89, 91, 95, 96, 97, 99, 103, 106,111, 113,115, 116, 119, 120, 121, 122, 124, 132, 139,142, 145, 149,150,151	48
5-9	IR28, 1, 10, 11, 14, 15, 22, 23, 25, 32, 33, 36, 37, 41, 45, 47, 56, 62, 63, 64, 66, 68, 69,70, 73, 74, 76, 78, 82, 86, 87, 88, 90, 94, 101, 102, 104,105, 107, 109, 110, 112, 114, 125, 126, 127, 128, 129, 133, 134, 135, 136, 137, 138, 140, 141, 143,144, 146, 147.	60

* Salinity score (0-9 scale) following SES, IRTP, IRRI, 1988.
Scale: (0-1: tolerant, 2-4 moderate tolerant, 5-9 susceptible)

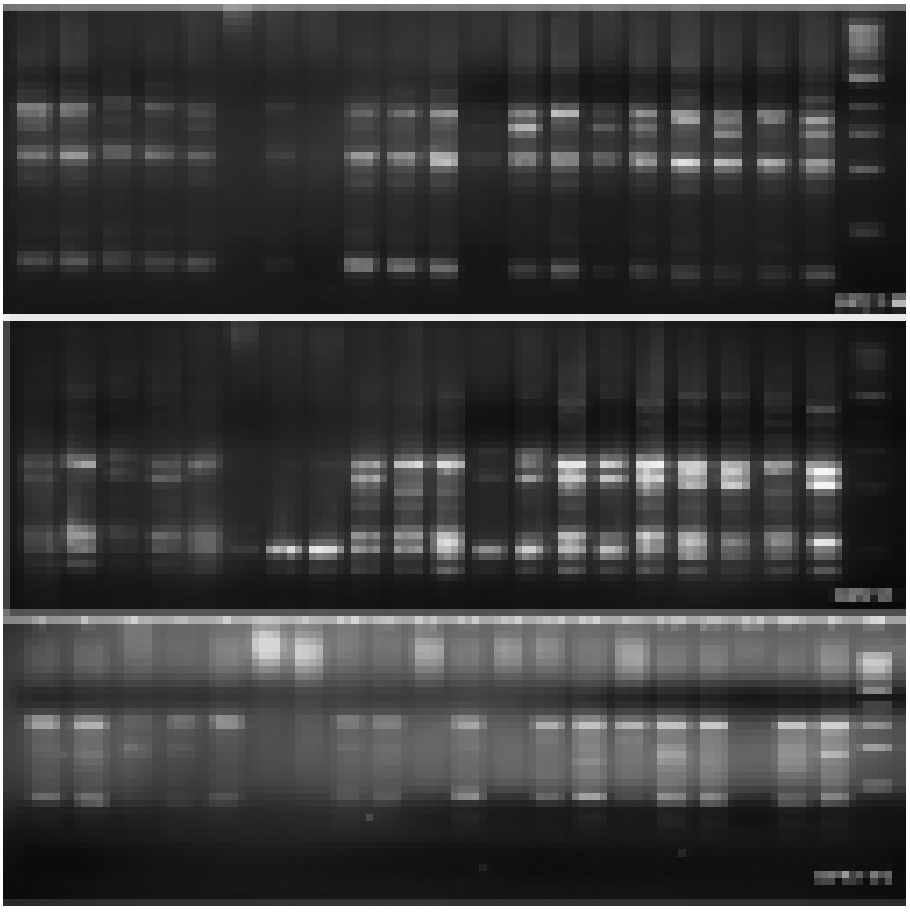

Fig. 1. RAPD-PCR profile of F_3 lines derived from IR28 x Pokkali, lanes 1-18 represent IRP 1, 2, 3, 4, 5, 6, 7, 10, 11, 12, 13, 14, 15, 16, 17, 19, 21, 22, respectively (F_3 segregants) IR: Parent IR 28, P: Pokkali, M: Molecular ruler 100bp ladder

76% similarity with Ia. Major cluster II is again consisted of two sub- clusters (IIa and IIb) with 78 and 37 segregants in each sub-cluster, respectively. Major sub-cluster IIa consists of 51 segregants which were found to be about cent percent genetically similar as depicted by RAPD primers. Distribution of the genotypes in IIa and IIb sub-clusters that showed about 70% genetic similarity, is not exactly based upon the scale of the salt tolerance which indicated that during genetic relationship estimation, other than the salt tolerance, few more genetic factors are expected to interplay within the genome. It was evident that, barring a few genotypes, RAPD primers used in the present study was able to identify most of the genotypes employed in the present study and thus found to be an effective and efficient tool in grouping and diversity estimation of the F_3 segregants. However, analysis of these segregants or further progenies with more RAPD primers is expected to lead to tagging of salt tolerant gene(s). RAPD profiles in individual lines were found to be different from each other and ample polymorphism was discernible in respect of the oligonucleotide primers used. RAPD pattern as shown by 18 segregants indicated distinct inclination towards either parent (only to one of the two parents). Further, the study prospects ample scope of RAPD to be used in molecular profiling. Bands common to tolerant check Pokkali are plausibly having more possibility to have links to salt tolerance character in this mapping population. This was observed in the mapping population based on phenotypic index for excess salt. i.e. the mapping population with high salt tolerance (score 1) were also grouped by RAPD markers in the same cluster. However, more random primers would be required for more appropriate grouping and tagging of salt tolerant gene(s). The present study involving a developing mapping population

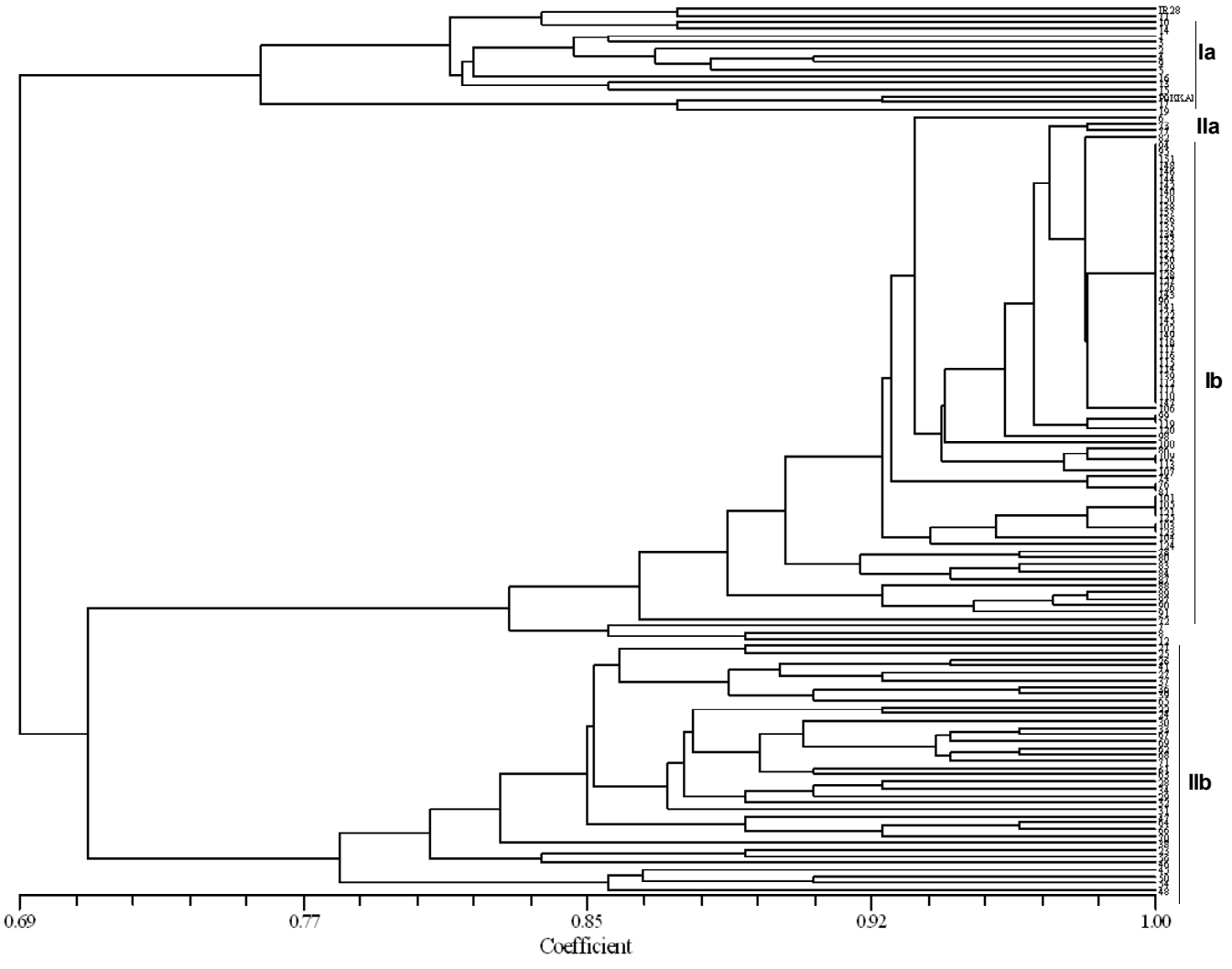

Fig. 2. Dendrogram showing genetic relatedness among parents and 129 F_3 segregating population in respect of RAPD primers. Numbers in the dendrogram represent different F_3 population

has displayed the extent of genetic diversity and identified suitable RAPD primers which be used in advance generation mapping population (F_6 to F_8) to tag salt tolerant gene(s) at the end of the entire study. Those markers in turn are expected to be reliably used in marker aided selection (MAS). This would help in rapid development of salt tolerant varieties more precisely with confidence in future. The present study also showed that RAPD is an easily applicable tool in unraveling the genetic diversity among the segregants and its virtue to identify ample polymorphism therein prospects its immense use in appropriate mapping of salt tolerant characters in rice.

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