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# A Preliminary Study on the Identification of Molecular Marker Associated with Drought Response in Rice (*Oryza sativa* L.)

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*ABSTRACT. CT9993 and IR62266, two parental rice accessions differing in drought response were initially screened for DNA polymorphism using 95 RAPD primers. Out of the 48 polymorphic primers, 18 primers were used to further screen 24 rice accessions which included the parents, 9 drought resistant and 9 drought susceptible doubled-haploid lines derived from the parents, 3 landraces and a susceptible cultivar, IR20. The primer, OPAK2 produced a 700bp fragment, which was present very distinctly in CT9993, Norungan and 3 resistant DH lines. This band was absent in all the susceptible DH lines, IR62266 and IR20. Dendrogram constructed using the banding pattern generated by OPAK2 grouped the accessions into three distinct clusters. The cluster analysis indicated the diversity among the accessions and helped to identify rice genotypes with wide genetic distance for further use in breeding programs to generate a mapping population and to map drought resistant genes in rice. The RAPD marker $OPAK2_{700}$ which cosegregate with the drought resistance genotypes could be used to identify the drought resistance genotypes in the segregating population. The rice accessions were also evaluated for variation in germination and seedling vigour under reduced water potential and showed significant variation for root growth and early seedling vigour.*

## INTRODUCTION

About 39% of the world's rice area (59 million ha) is under rainfed ecosystems, where yields are seriously affected by drought. Eastern India alone leads all other countries by a wide margin in rainfed lowland rice with 15 million ha, where farmers identified drought stress as the foremost constraint to higher yields (Widawsky and O'Toole, 1995). Rice yield losses due to drought are estimated at US$ 3.51 billion annually. Incorporation of drought resistance thus has great implications in boosting rainfed rice production. Several putative traits conferring drought resistance in rice have been identified (Nguyen *et al.*, 1997). However, selection for these traits in conventional breeding for drought resistance is difficult due to labour-intensive nature of measuring most putative traits.

Molecular markers assist breeders to indirectly select for traits that are otherwise difficult to score. Genetic linkage maps composed of various DNA markers have been constructed and used for mapping agronomically useful genes in rice. Molecular markers (mainly restriction fragment length polymorphisms - RFLPs) linked to various drought resistance component traits have been identified in rice (Zhang *et al.*, 2001; Zheng *et al.*, 2000). Though RFLP markers are robust, the procedure involves hazardous radioactive chemicals and is tedious. Identification of polymerase chain reaction (PCR) based non-radioactive markers will improve the efficacy of marker-assisted selection (MAS) for

drought resistance improvement. Random amplified polymorphic DNAs (RAPDs), a class of PCR based markers, also detect DNA polymorphism similar to RFLPs. In addition, RAPDs require less quantity of template DNA, suitable for automation, non-radioactive and facilitate rapid genotyping of large germplasm. RAPDs have been used in mapping of insect and nematode resistance loci (Eastwood *et al.*, 1994; Nair *et al.*, 1995). RAPDs have been employed to screen rice cultivars for salt sensitivity/resistance (Erickson *et al.*, 1995). However, information relating to RAPD markers linked to drought resistance is scanty. Hence, a preliminary study was conducted to identify RAPD marker(s) associated with response to water stress in rice.

## MATERIALS AND METHODS

A doubled-haploid (DH) line population was developed between drought tolerant CT 9993-5-10-1-M and drought sensitive IR62266-42-6-2 (abbreviated as CT9993 and IR62266, respectively) at the International Rice Research Institute (IRRI), Philippines and Centro International de Agricultura Tropical (CIAT), Columbia through anther culture. CT9993 is a *japonica* from uplands and has deep thick roots with higher penetration ability of simulated soil hard pan but has low osmotic adjustment (OA) capacity (Babu *et al.*, 2001). IR62266 is an *indica* from lowlands and has shallow thin roots with low penetration ability but has high OA. A subset of this population was subjected to phenotyping for root traits and OA (Zhang *et al.*, 2001) and field drought tolerance (Babu *et al.*, 2000). Based on relative drought tolerance, 9 resistant and 9 susceptible DH lines were chosen along with the parents. Three land races, namely, Norungan, Kallurundaikar and Nootripathu from rainfed uplands of Tamil Nadu State, India and the drought sensitive check, IR20 were also included. The relative ranking of the 24 accessions for root traits, OA and drought tolerance index is given in Table 1.

### RAPD analysis

Genomic DNA was isolated from frozen fresh leaf tissue following CTAB extraction procedure (Gawel and Jarret, 1991). The final DNA concentration was adjusted to 25 ng/µl. Ninety five random primers namely, OPK 1-20, OPAK 1-20, OPR 1-20, OPS 1-20, OPF 7, 11, 14, 18, 20, OPAH 4, 9, 10, 13, 17, OPU 2, 12, 13, 14 and 15 (Operon Technologies Inc., USA) were initially used to amplify RAPD loci of the parents, CT 9993 and IR62266. The primers had a GC content of 60-70%. PCR reactions were performed in a volume of 15 µl containing 50 ng of genomic DNA, 10 mM Tris buffer (pH-8.0), 2 mM $MgCl_2$, 50 mM each of dATP, dCTP, dGTP and dTTP, 6 ng of primer and 0.6 units of *Taq* DNA polymerase (Bangalore Genei Pvt. Ltd., India). Reaction was performed in a thermocycler (PTC-100™, MJ Research Inc., USA) with 35 cycles of 1 min at 94°C, 1 min at 37°C and 1 min at 72°C with initial denaturation for 5 min at 94°C and final extension at 72°C for 7 min. After PCR amplification, 3 µl of 10× loading buffer was added to the amplified products and were run on 1.5% agarose for 4 h. Gels were stained with ethidium bromide and the banding patterns were documented using a gel documentation system (Alphaimager 1200, Alpha Innotech Corpn., USA). DNAs of resistant and susceptible lines were not bulked since several physiological traits contribute in drought tolerance under field conditions.

**Table 1.** **Relative ranking of the 24 rice accessions for root penetration index (RPI), root thickness (RT), osmotic adjustment (OA) and drought tolerance index (DTI).**

| Accession | | Designation | Ranking |
|---|---|---|---|
| CT9993 | | CT 9993-5-10-1-M | High RPI, high RT, low OA and high DTI |
| IR62266 | | IR 62266-42-6-2 | Low RPI, low RT, high OA and low DTI |
| Resistant DH line | 1 | IR 68586 CA 6 | High RPI, high RT, high OA and high DTI |
| | 2 | IR 68586 F2 CA 148 | High RPI, high RT, low OA and high DTI |
| | 3 | IR 68586 F2 CA 69 | Low RPI, medium RT, medium OA and high DTI |
| | 4 | IR 68586 F2 CA 3 | High RPI, medium RT, medium OA and high DTI |
| | 5 | IR 68586 F2 CA 25 | High RPI, medium RT, high OA and high DTI |
| | 6 | IR 68586 F2 CA 90 | High RPI, high RT, high OA and high DTI |
| | 7 | IR 68586 F2 CA 4 | High RPI, high RT, medium OA and high DTI |
| | 8 | IR 68586 F2 CA 27 | Medium RPI, medium RT, low OA and high DTI |
| | 9 | IR 68586 F2 CA 88 | High RPI, high RT, medium OA and high DTI |
| Susceptible DH line | 1 | IR 68586 CA 27 | Medium RPI, medium RT, low OA and low DTI |
| | 2 | IR 68586 F2 CA 14 | Medium RPI, medium RT, high OA and low DTI |
| | 3 | IR 68586 CA 14 | Medium RPI, medium RT, high OA and low DTI |
| | 4 | IR 68586 CA 30 | Medium RPI, high RT, low OA and low DTI |
| | 5 | IR 68586 F2 CA 35 | Low RPI, medium RT, low OA and low DTI |
| | 6 | IR 68586 F2 CA 7 | Low RPI, medium RT, low OA and low DTI |
| | 7 | IR 68586 F2 CA 37 | Low RPI, medium RT, medium OA and low DTI |
| | 8 | IR 68586 F2 CA 128 | Medium RPI, RT and OA and low DTI |
| | 9 | IR 68586 CA 19 | Medium RPI, medium RT, low OA and low DTI |
| Norungan | | Norungan | High RPI, high RT and high DTI |
| Kallurundaikar | | Kallurundaikar | High RPI, high RT and high DTI |
| Nootripathu | | Nootripathu | High RPI, high RT and high DTI |
| IR20 | | IR20 | Low RPI, low RT, medium OA and low DTI |

*DTI based on leaf relative water content, leaf rolling and drying scores, relative biomass and relative yield under water stress in the field.

## Cluster analysis

The banding patterns were scored for RAPD primer OPAK2 in each rice accession starting from the small size fragment to large sized ones. Presence and absence of each band was scored as 1 and 0, respectively. The scores were used to create a data matrix to analyse genetic relationship using the NTSYS-pc program version 2.02 (Exeter software, New York, USA) as described by Rohlf (1990). A dendrogram was constructed based on Jaccard's similarity coefficient, using the marker data for all the rice accessions following Unweighed Pair Group Method (UPGMA) (Jaccard, 1908).

## Evaluation of drought response

Rice seeds (25/replication) were germinated using 0 and -0.25 MPa polyethylene glycol-6000 solution. Germination and root length were determined 14 days after sowing.

Seedling vigour index was calculated by multiplying germination percentage and root length (Abdul-baki and Anderson, 1973).

## RESULTS AND DISCUSSION

All the 95 primers amplified scorable PCR products. A total of 634 PCR products were generated in both the parents. Among the 95 primers, 48 were found to amplify polymorphic bands between the parents. The extent of polymorphism generated by individual primers ranged from 12-80%, with majority primers producing 16-25% polymorphism (Table 2). While OPAK11 generated less polymorphism (12%), OPAK10 and 13 produced greater polymorphism (80% each). Out of the 48 polymorphic primers,

**Table 2. Polymorphic primers and the extent of polymorphism among the parental accessions, CT9993 and IR62266.**

| S. No. | Primer | Total number of bands | Polymorphic bands | Polymorphism (%) |
|---|---|---|---|---|
| 1 | OPK 02 | 5 | 1 | 20 |
| 2 | OPK 08 | 3 | 1 | 33 |
| 3 | OPK 09 | 4 | 2 | 50 |
| 4 | OPK 14 | 6 | 2 | 33 |
| 5 | OPK 15 | 4 | 1 | 25 |
| 6 | OPK 19 | 6 | 1 | 16 |
| 7 | OPAK 01 | 4 | 1 | 25 |
| 8 | OPAK 02 | 12 | 7 | 58 |
| 9 | OPAK 05 | 4 | 1 | 25 |
| 10 | OPAK 06 | 7 | 2 | 28 |
| 11 | OPAK 09 | 5 | 2 | 40 |
| 12 | OPAK 10 | 5 | 4 | 80 |
| 13 | OPAK 11 | 8 | 1 | 12 |
| 14 | OPAK 13 | 5 | 4 | 80 |
| 15 | OPAK 14 | 13 | 5 | 38 |
| 16 | OPAK 16 | 8 | 2 | 25 |
| 17 | OPAK 19 | 11 | 6 | 54 |
| 18 | OPAK 20 | 6 | 3 | 50 |
| 19 | OPS 01 | 5 | 2 | 40 |
| 20 | OPS 02 | 4 | 1 | 25 |
| 21 | OPS 04 | 5 | 1 | 20 |
| 22 | OPS 07 | 5 | 2 | 40 |
| 23 | OPS 08 | 4 | 1 | 25 |
| 24 | OPS 09 | 5 | 2 | 40 |
| 25 | OPS 12 | 5 | 1 | 20 |
| 26 | OPS 13 | 5 | 2 | 40 |
| 27 | OPS 16 | 4 | 2 | 50 |

*Continued .....*

Table 2. Cont'd .....

| S. No. | Primer | Total number of bands | Polymorphic bands | Polymorphism (%) |
|---|---|---|---|---|
| 28 | OPS 17 | 5 | 1 | 20 |
| 29 | OPS 19 | 6 | 1 | 17 |
| 30 | OPS 20 | 5 | 2 | 40 |
| 31 | OPR 01 | 5 | 2 | 40 |
| 32 | OPR 02 | 6 | 1 | 17 |
| 33 | OPR 03 | 4 | 1 | 25 |
| 34 | OPR 06 | 7 | 2 | 35 |
| 35 | OPR 07 | 12 | 3 | 25 |
| 36 | OPR 08 | 5 | 2 | 40 |
| 37 | OPR 10 | 3 | 1 | 33 |
| 38 | OPR 11 | 6 | 5 | 83 |
| 39 | OPR 12 | 9 | 3 | 38 |
| 40 | OPR 13 | 4 | 1 | 25 |
| 41 | OPR 20 | 5 | 1 | 20 |
| 42 | OPF 07 | 4 | 1 | 25 |
| 43 | OPF 18 | 5 | 3 | 60 |
| 44 | OPAH 10 | 2 | 1 | 50 |
| 45 | OPAH 13 | 4 | 3 | 75 |
| 46 | OPAH 17 | 7 | 3 | 43 |
| 47 | OPU 13 | 2 | 1 | 50 |
| 48 | OPU 15 | 2 | 1 | 50 |

18 primers (OPAK 2, 10, 11, 13, 14, 16 and 19, OPR 6, 7, 11 and 14, OPS 5 and 16, OPF 18, OPAH 10 and 13 and OPU 13 and 15) consistently produced distinct polymorphic bands between the parents. These primers were further used in screening of resistant and susceptible DH lines, land races and IR20 to detect the RAPD fragment(s) co-segregating with drought response. The primer, OPAK2 produced 4 polymorphic bands, which are unique to the drought resistant parental accession, CT9993 (Plate 1). Out of these 4 bands, one particular fragment of 700bp length ($OPAK2_{700}$) was also amplified in 3 resistant DH lines namely, IR 68586 CA 148, IR 68586 CA 3, IR 68586 CA 4 and in the land race, Norungan. Interestingly, this $OPAK2_{700}$ fragment was absent in all the susceptible DH lines, IR62266, the susceptible parent and IR20, the drought sensitive check. $OPAK2_{700}$ fragment may be associated with root trait(s), since all the accessions in which this fragment was absent were ranked under poor root system (Table 1). However, this needs to be verified through bulk segregant analysis using lines selected based on a single root trait (*e.g.*, thickness). Further, it could not be generalised that this fragment may be associated with drought tolerance, since this fragment is absent in several resistant accessions as well. It is common knowledge that not only root traits but several other physiological attributes such as phenology, OA, water use efficiency, cuticle resistance *etc.*, also confer drought resistance. While these attributes have a positive impact on plant performance under drought, they may be negatively correlated among themselves. For instance, though both contribute to drought resistance, deep and thick root system and OA are negatively

correlated in rice (Babu *et al.*, 2001). Hence bulk segregant analysis using resistant and susceptible accessions selected for a specific trait will be a useful approach towards MAS for drought resistance improvement (Quarrie *et al.*, 1999).

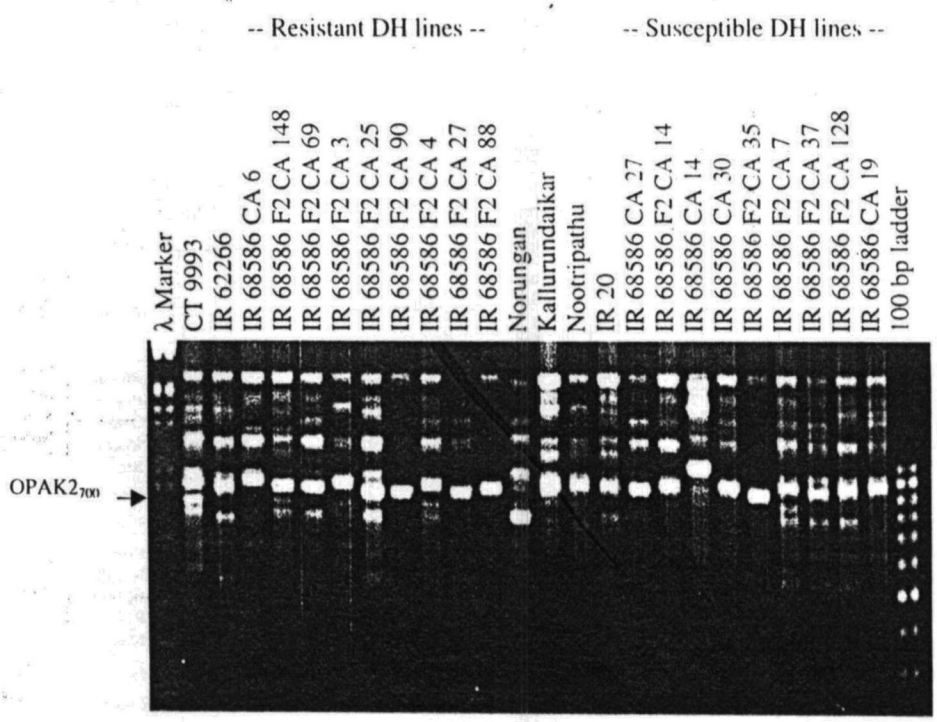


**Plate 1. Polymorphism detected with RAPD primer OPAK2.**

Cluster analysis was performed using the banding pattern generated by the RAPD primer OPAK2. The dendrogram (Plate 2) showed three main clusters at 0.50 similarity co-efficient. Cluster A consists of seven accessions, out of which six are drought resistant. However, the susceptible check IR20 also falls into this cluster but it has only 37% homology with the rest of the entries. The susceptible parent, IR62266 is grouped in B and the resistant line, IR68586-F2-CA-3 is grouped in cluster C. The dendrogram revealed that the drought resistant lines are diverse in origin, coming under different clusters. RAPD marker $OPAK02_{700}$ may serve as a putative marker which could be helpful in identifying the drought tolerant genotypes in the segregating population.

There was considerable variation among the accessions for germination and root growth at reduced water potential. At -0.25 MPa, germination and root growth were significantly reduced in all the accessions (Table 3). CT9993 produced longer roots. The land races had relatively higher germination and better root growth. IR20, IR62266 and the susceptible DH lines had low germination and less seedling vigour at -0.25 Mpa. The resistant DH lines had higher seedling vigour than the susceptible DH lines. These findings

were in accordance with the earlier results (Sadasivam *et al.*, 2000), where drought resistant rices had higher germination and seedling growth under PEG induced water stress.

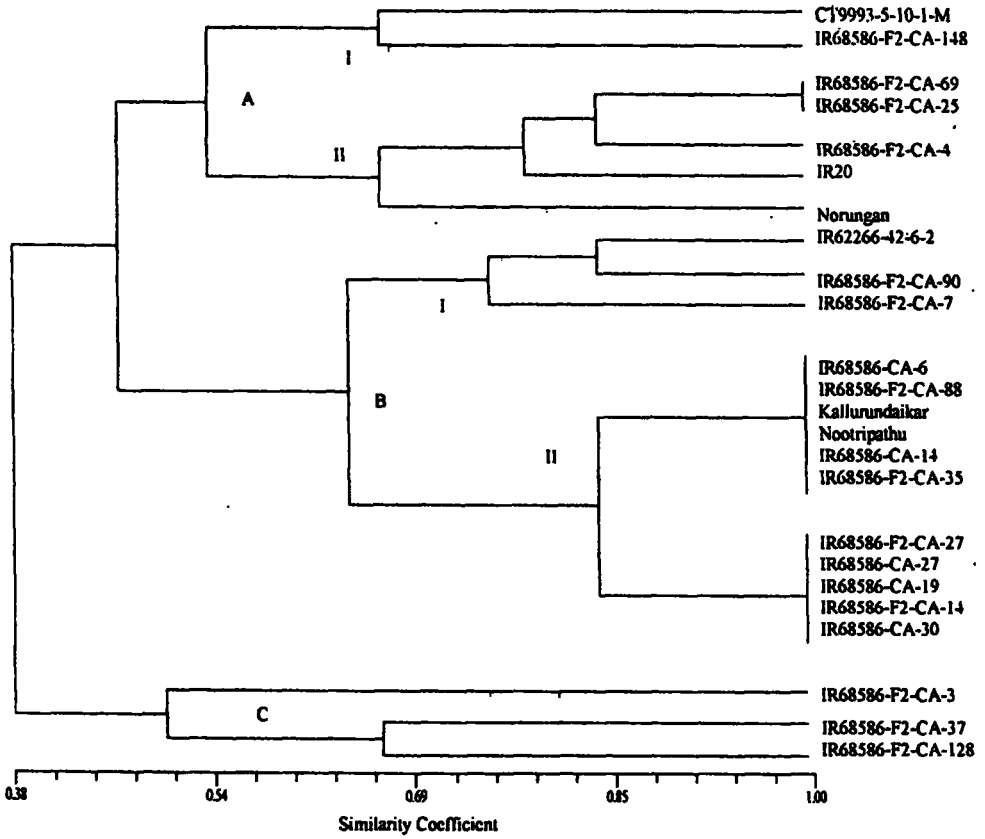


**Plate 2. A dendrogram generated by OPAK2.**

Though the genomic regions associated with drought resistance component traits are distributed throughout the genome, certain regions are known to contribute greater effects than others. OPAK2 generated a 700bp fragment which was absent in all the drought susceptible DH lines, IR62266 and IR20. This particular fragment was well amplified in CT9993, 3 resistant lines and Norungan. Further bulk segregant analysis using resistant and susceptible bulks, selected based on specific root trait, such as thickness or depth will reveal its value as a marker associated with drought tolerance in rice. Such a study will help in developing sequence characterised amplicon region (SCAR) marker for use in MAS. Experiments on these lines are currently in progress at this laboratory.

**Table 3. Variation among the rice accessions for germination and radicle growth under simulated water stress using -0.25 MPa polyethylene glycol-6000.**

| Accession | | Germination (%) | Root length (mm) | Seedling vigour index |
|---|---|---|---|---|
| CT 9993 | | 80 (100) | 18.4 (90) | 1472 |
| IR 62226 | | 70 (100) | 6.3 (45) | 441 |
| Resistant DH line | 1 | 80 (100) | 16.1 (123) | 1288 |
| | 2 | 70 (100) | 17.3 (96) | 1211 |
| | 3 | 80 (97) | 18.2 (87) | 1456 |
| | 4 | 70 (97) | 15.1 (99) | 157 |
| | 5 | 80 (100) | 19.5 (93) | 1560 |
| | 6 | 80 (100) | 20.3 (89) | 1624 |
| | 7 | 80 (100) | 19.8 (101) | 1584 |
| | 8 | 80 (97) | 18.3 (111) | 1464 |
| | 9 | 80 (100) | 20.8 (99) | 1664 |
| Mean | | 77.7 (98.9) | 18.4 (99.7) | 1429.6 |
| Susceptible DH line | 1 | 70 (100) | 5.0 (90) | 350 |
| | 2 | 70 (97) | 5.3 (48) | 371 |
| | 3 | 70 (97) | 5.4 (62) | 378 |
| | 4 | 60 (97) | 6.4 (65) | 384 |
| | 5 | 70 (97) | 7.3 (70) | 511 |
| | 6 | 70 (100) | 5.3 (65) | 371 |
| | 7 | 70 (97) | 8.6 (52) | 602 |
| | 8 | 70 (97) | 6.7 (70) | 469 |
| | 9 | 70 (100) | 8.5 (55) | 595 |
| Mean | | 68.9 (96.6) | 6.5 (64.1) | 477.9 |
| Norungan | | 80 (100) | 18.3 (83) | 1464 |
| Kallurundaikar | | 80 (97) | 20.37 (96) | 1630 |
| Nootripathu | | 80 (97) | 19.7 (98) | 1576 |
| IR20 | | 70 (93) | 5.4 (40) | 378 |
| Overall Mean | | 74.2 (97.8) | 13.04 (80) | 967.6 |
| CD (5% P) | | 11.2** | 0.29** | |

## CONCLUSIONS

The finding on OPAK02700 seems very valuable as a putative molecular marker linked to drought response in rice.

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