Indian J. Genet., **63**(3): 197-201 (2003)

Molecular marker analysis for root length in a diverse germplasm of rice (*Oryza sativa* L.)

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(Received: January 2003; Revised: August 2003; Accepted: August 2003)

Abstract

Rice (*Oryza sativa* L.) is among the most important crops in the world. The productivity of rice in rainfed regions is constrained by drought. Among the root traits contributing to drought resistance, root system parameters play an important role in the regulation of plant growth through water and nutrient uptake from deep soil layers. The present study was conducted to validate molecular markers known to be associated with maximum root length. Seventy-nine diverse lines and six checks were used. Variation was observed for several root morphological traits *viz.*, maximum root length, total root number, root volume, root dry weight, shoot dry weight, plant height, number of tillers, total dry weight and root to shoot weight ratio. Most of these traits showed significant correlation and appeared interrelated. RAPD and microsatellite primers, which have been established as markers for maximum root length, were used for screening rice genotypes. Single-marker analysis showed significant association between the markers and maximum root length. Thus, these markers can be used for marker-assisted selection in plant breeding programmes involving diverse parents.

Key words: Rice, core and donor lines, microsatellite, RAPD, root morphology

Introduction

Rice (*Oryza sativa* L.) is among the most important crops in the world. It is grown on 148 million hectares spanning a wide range of environments. About one forth of the world's rice area is under rainfed lowlands and another 13% is in rainfed uplands (1). Drought is a major constraint in increasing productivity of rice in the rainfed regions (2). Among the several factors contributing to enhance resistance to drought, roots are the main organ for plant water uptake (3). Breeding for a larger and deeper root system that could enable exploring a wider and deeper soil column contributes to enhance drought resistance in rice crop challenged by depleting moisture or continued dry spell during growth (4). Maximum root length and root dry weight are good indicators of drought resistance in upland rice. Plants having deeper root system would colonize a large soil volume and improve the water uptake from the deeper soil strata where water is expected to be available. This would help in maintaining good plant water potential, which has a positive effect on yield under stress (5).

Quantifying root traits is difficult, time-consuming and most often destructive. Thus, it is difficult to manipulate such traits through conventional breeding. Use of molecular markers provides a potentially effective selection technique for crop improvement and has advantages over selection based on phenotype alone (6). Several types of molecular markers, *viz.*, RFLP, RAPD, microsatellites and AFLP have been developed. PCR-based markers such as microsatellites and RAPDs have shown great promise in genetic diversity analysis, genome mapping, gene tagging and marker-assisted selection (MAS) because they are technically simple, time saving, highly informative, and require small amount of DNA. Using markers in MAS permits rapid identification of individuals that contain favourable genes. Earlier, we identified two DNA markers (OPBH14, a RAPD marker and RM201, a microsatellite marker) linked to maximum root length in DH population derived from a cross between IR 64 and Azucena (4). In the present study, we screened these two markers across 85 diverse rice genotypes to assess the reliability.

Materials and methods

Plant materials: Seventy-nine Core (C) and Donor (D) lines along with six checks *viz.*, Azucena, Co 39, IR 20, IR 36, Jaya and Moroberekan were used in this study. Seeds of these lines were obtained from International Plant Molecular Breeding Programme, IRRI, Philippines. Core lines (C) represent varieties popularly grown in different parts of the world, while donor lines (D) are genotypes having one or few traits of economic importance such as disease resistance, drought

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tolerance, etc. These 79 C and D lines have been collected from different parts of the world.

Evaluation of root traits: Root parameters were evaluated in the Marker-Assisted Selection Laboratory greenhouse at the University of Agricultural Sciences, Bangalore, India during summer 2001. The experiment was laid out with three replications in randomized complete block design. Seeds were sown in poly venyl chloride pipes measuring 1 meter in length and 18 cm in diameter, which were filled with a mixture of sandy-clay loam and FYM in 4:1 proportion. After germination, one seedling was allowed to grow in each pipe. Plants were watered daily throughout the experiment. Root sampling was done 70 days after sowing (DAS). Pipes were removed carefully and completely submerged in water overnight to loosen the soil. Later, the roots were cleaned thoroughly with water. Observations consisted of maximum root length (MRL) in cm, total root number (TRN), root volume (RV) in cc, root dry weight (RDW) in g, shoot dry weight (SDW) in g, plant height (PHT) in cm and number of tillers per plant (NOT). Further, total dry weight (RDW+SDW = TDW) in g and root to shoot weight ratio (R/S) were computed.

DNA isolation and RAPD-microsatellite loci amplification: Genomic DNA of 85 accessions were isolated from frozen leaf tissue by miniprep method (7). The final DNA concentration was adjusted to 50 ng/µl. A RAPD primer OPBH14 (Operon Technologies Inc., USA) and primer for microsatellite marker RM201 (Research Genetics Inc., USA) were used to amplify target DNA loci. The PCR reaction for RAPD was carried out in a volume of 20 µl containing 50 ng genomic DNA, 20 ng primer, 10mM Tris-HCl (pH 8.4), 50mM KCl, 1.8mM $MgCl_2$, 100mM each of dNTPs and 1 unit of *Taq* DNA polymerase (Bangalore Genei, India). For microsatellite amplification, 10 ng of each primer was used. The PCR amplification was performed on a M J Research Thermal Cycler (PTC-100) with 35 cycles of 1 min 94°C, 1 min at 38°C and 2 min at 72°C followed by final extension at 72°C for 5 min. For microsatellite amplification, annealing temperature was raised to 56°C. After PCR amplification, 8 ml of 3x loading buffer (10mM NAOH, 95% formamide, 0.25% bromophenol blue and 0.25% xylene cyanol) was added to amplified products. OPBH14 amplified products were loaded on 1.4% agarose gels in 1× TAE buffer and resolved at 5 V/cm for 2 1/2 h. The gels were stained with ethidium bromide and visualized under UV light. RM201 amplified products were loaded on 5% non-denaturing polyacrylamide gels (1 mm thick and 38 cm long) in 0.5% TBE buffer and resolved at 8 V/cm for 8 h. for detection of DNA fragments, the gels were silver stained (8) and photographed for documentation.

Data analysis: Analysis of variance and simple correlation were performed for root and shoot traits. The correlation coefficients were computed (9). The marker data obtained from screening of OPBH14 and RM201 on 85 genotypes were used. The bands were scored as 1 for the individuals having IR 64 type band (shallow-rooted genotype) and 3 for the Azucena-type band (deep-rooted genotype). Single-marker analysis was carried to find out the association of markers with root length. R^2 values were determined out to find the amount of variability explained by these markers.

Results and discussion

Variation in root morphology: In this study, root traits were evaluated in a total of 85 accessions (Table 1) from different countries. So far, there have been no detailed studies on root traits on these lines. Significant variation for all the traits confirmed the presence of high genetic diversity among the genotypes studied. D lines were found to be superior in MRL (D-82), RDW (D-32), TRN (D-79), PHT (D-86), SDW (D-19), and TDW (D-18). These results revealed that there are many donor lines with favorable root characteristics, MRL and RDW, which contribute to drought resistance, and thus they could be useful in breeding for drought resistance in rice.

Correlation among root traits: Simple correlation are summarized in Table 2. MRL, RV, TRN, and RDW were found to be positively and significantly associated at 0.01 level of probability. Therefore indirect selection of these traits is possible in this population. The results are in accordance with earlier findings (10, 11, 12 and 13). MRL and TDW were significantly and positively correlated with all the characters. Selection for these two traits greatly increased the selection efficiency for other traits as well as resulting in improved drought resistance. Since the root traits were found to be positively correlated with PHT and NOT, selection based on the shoot traits is very important, especially because of the difficulty in monitoring root development as compared to the shoot.

RAPD-Microsatellite DNA polymorphisms: The marker data obtained from screening of the two markers, OPBH14 and RM201, on 85 genotypes are given in Table 1. Out of 85 varieties screened with OPBH14, 61 individuals showed IR 64 type band and their mean was 37.10 cm, whereas the rest (24 accessions) showed Azucena type band (1.57 kb) and their mean was 44.20 cm. Similarly, out of 85 genotypes screened with microsatellite marker RM201, 60 individuals showed IR 64 type band (158bp) and their mean was 37.30 cm, whereas 25 genotypes showed Azucena type bands (147 bp) with a mean of 43.40 cm. The mean of individuals showing Azucena type band was significantly

Table 1. Mean values of rice genotypes along with markers data in diverse accessions of rice

Accession name			Mean values								DNA markers data	
No	Line No.	Name	PHT	NOT	MRL	RV	TRN	RDW	SDW	TDW	R/S	OPBH14	RM201
1	C-02	BR 24	71.7	13.3	34.0	14.0	43.3	2.5	11.8	14.2	0.2	1	1
2	C-03	93072	80.3	5.3	32.0	7.3	39.0	1.0	5.4	6.4	0.2	1	1
3	C-05	C 418	61.0	10.7	45.3	23.0	95.0	4.0	10.1	14.1	0.4	3	3
4	C-07	Cheng-Hui 448	74.3	10.0	55.0	20.0	56.3	2.8	14.7	17.5	0.2	1	1
5	C-08	Feng-Ai-Zan	69.7	7.0	41.0	10.7	57.7	1.1	14.0	15.1	0.1	1	1
6	C-09	Gang 16	77.7	10.7	37.7	13.0	41.7	1.6	13.3	14.9	0.1	1	3
7	C-13	R 644	77.0	8.3	27.7	10.0	32.3	0.5	6.9	7.4	0.1	1	1
8	C-14	NA	55.3	11.7	29.0	14.3	38.3	0.7	6.5	7.2	0.1	1	1
9	C-15	Shen-Nong 89366	74.0	7.3	40.0	16.7	62.7	2.0	18.7	20.7	0.1	1	3
10	C-16	Y 134	70.3	11.0	41.0	15.0	55.0	0.7	9.7	10.5	0.1	1	3
11	C-17	Yunhui 290	81.0	7.0	31.0	13.3	65.3	2.4	10.4	12.8	0.2	3	3
12	C-19	Yu-Xiang-Zan	71.0	14.7	36.3	17.3	64.7	3.2	16.8	20.0	0.2	1	1
13	C-20	Zao-Xian 14	69.7	12.7	31.7	20.7	53.3	2.5	13.4	15.8	0.2	1	1
14	C-21	Zhong 413	77.7	6.7	33.0	15.3	38.3	1.9	12.0	13.9	0.2	1	1
15	C-22	Zhong-You-Zao 81	51.3	11.3	30.0	12.3	52.7	1.4	7.3	8.7	0.2	1	1
16	C-24	Bg 90-2	86.0	15.3	46.3	28.3	66.7	5.3	13.8	19.2	0.4	3	3
17	C-25	Basmati 370	66.7	8.3	43.0	7.3	28.3	0.8	10.6	11.4	0.1	3	3
18	C-26	Co 43	72.3	10.0	39.3	18.3	50.7	2.0	12.7	14.7	0.2	1	1
19	C-29	Rasi	71.0	7.3	30.5	14.7	25.0	0.7	7.6	8.2	0.1	1	1
20	C-30	TKM 9	66.7	18.3	39.3	15.7	60.7	1.8	19.5	21.2	0.1	1	1
21	C-31	Cisanggarung	76.0	12.3	44.0	18.3	60.3	6.4	19.9	26.3	0.3	1	1
22	C-33	Amol 3 (Sona)	67.3	8.0	23.7	14.3	27.7	1.6	6.9	8.5	0.2	1	3
23	C-35	IR 64	65.7	10.3	33.3	16.0	43.0	3.8	12.7	16.5	0.3	1	1
24	C-36	Nipponbare	59.0	10.7	30.3	13.0	54.3	1.1	8.7	9.8	0.1	3	3
25	C-37	Gayabyeo	71.3	17.3	46.0	28.3	93.0	6.1	19.2	25.3	0.3	3	3
26	C-38	Iksan 438	64.3	10.3	32.0	23.7	50.7	0.8	9.8	10.6	0.1	3	3
27	C-39	Ilmibyeo	60.0	10.3	44.0	15.7	65.0	0.9	11.5	12.4	0.1	3	3
28	C-40	Milyang 23	53.0	11.0	33.3	13.3	38.3	0.7	7.5	8.2	0.1	1	1
29	C-44	Manawathukha	54.7	12.3	41.3	11.3	64.3	0.8	8.8	9.6	0.1	1	1
30	C-45	Shwe Thwe Yin Hyy	70.7	14.7	34.0	21.7	53.7	1.3	5.6	6.9	0.2	1	1
31	C-46	Bg 300	70.0	14.0	40.7	17.3	49.3	2.1	15.4	17.6	0.1	1	1
32	C-47	Bg 94-1	66.7	15.0	37.7	27.7	61.7	1.9	16.4	18.3	0.1	1	1
33	C-48	CR 203	77.7	14.3	33.0	12.3	51.7	1.2	13.3	14.6	0.1	1	1
34	C-49	OM 997	74.7	20.7	37.7	33.3	65.3	3.5	19.1	22.6	0.2	1	1
35	C-50	PSB RC 28	60.0	12.7	39.3	11.7	35.3	1.7	8.5	10.2	0.2	1	1
36	C-51	PSB RC 66	80.3	16.7	46.0	35.0	58.0	7.1	20.4	27.5	0.3	1	1
37	C-52	IR 64	73.3	13.3	50.5	13.3	47.3	1.2	19.1	20.3	0.1	1	1
38	C-53	TEQING	64.7	12.7	37.0	16.0	48.3	0.8	11.8	12.6	0.1	1	1
39	C-55	IR 66897B	60.0	14.0	41.0	16.0	81.3	2.9	20.9	23.9	0.1	1	1
40	C-58	Dhan 4	69.3	14.7	50.7	24.3	78.3	3.5	20.1	23.6	0.2	3	3
41	C-59	Pusa (Basmati 1)	61.3	14.3	38.7	18.7	45.3	2.2	11.2	13.5	0.2	1	1
42	D-03	Babaomi	96.7	12.3	27.7	9.3	62.3	1.4	19.3	20.7	0.1	1	3
43	D-04	Diantun 502	72.3	13.3	33.7	13.0	46.3	1.6	12.7	14.3	0.1	3	1
44	D-08	Jiangxi-Si-Miao	85.7	11.7	47.0	15.0	45.3	2.4	12.6	15.0	0.2	3	1
45	D-13	Peng-Shan-Tie-Gan-Zan	74.0	12.0	22.3	13.3	56.7	1.1	1.7	2.8	0.7	1	1
46	D-18	Yu-Qui-Gu	88.3	11.7	27.7	23.0	50.0	1.7	25.4	27.1	0.1	1	1
47	D-19	Zhong 123	117.0	13.0	46.3	21.7	67.7	5.3	34.7	39.9	0.2	3	1
48	D-23	Khao Daeng	94.0	15.7	32.7	18.3	41.7	4.3	19.5	23.9	0.2	1	1
49	D-24	Madhukar	94.0	16.7	42.3	22.7	51.7	3.3	20.2	23.5	0.2	3	3
50	D-25	Milagrosa, Zawa Banday	86.0	9.7	40.7	11.7	41.3	2.3	12.3	14.7	0.2	3	3
51	D-29	Bhavani	67.3	14.7	34.7	14.3	50.0	1.1	9.1	10.1	0.1	1	1
52	D-30	IR 50	59.3	17.7	27.3	13.0	52.7	1.0	12.9	13.9	0.1	1	1
53	D-31	Jhona 349	85.3	11.3	58.0	35.3	55.3	6.5	16.1	22.5	0.4	3	3

(*Table 1 contd.*)

Table 1. Contd.

Accession name			Mean values								DNA markers data	
No	Line No.	Name	PHT	NOT	MRL	RV	TRN	RDW	SDW	TDW	R/S	OPBH14	RM201
54	D-32	Karnal Local	90.0	15.7	39.7	50.0	83.3	14.5	19.0	33.5	0.8	3	3
55	D-35	NA	103.7	11.3	38.7	19.0	27.3	0.9	10.5	11.4	0.1	1	1
56	D-36	TB 154E-TB-2	71.3	12.0	33.7	17.7	59.3	1.6	10.7	12.3	0.1	3	1
57	D-37	Binam	87.3	9.3	45.7	14.0	50.0	1.6	15.1	16.7	0.1	3	3
58	D-38	Domsiah	88.0	7.3	30.7	15.0	49.0	2.3	7.0	9.3	0.3	3	3
59	D-39	Taron Molaii	90.7	15.7	47.0	15.0	60.7	1.7	19.9	21.6	0.1	3	3
60	D-42	MR 167	72.0	11.0	45.3	23.7	50.0	1.3	10.5	11.8	0.1	1	1
61	D-44	Innmayebaw	78.0	5.3	41.0	15.7	47.3	0.7	8.4	9.1	0.1	1	1
62	D-48	Basmati 385	79.0	14.7	36.7	16.3	44.0	1.3	16.2	17.5	0.1	3	3
63	D-49	IR 6	76.3	10.3	28.5	13.7	44.3	0.8	13.1	13.9	0.1	1	1
64	D-56	OM 1706	83.0	15.3	37.0	16.7	60.0	3.1	19.6	22.8	0.2	1	1
65	D-58	X 21	73.7	14.7	33.3	24.3	66.7	0.9	5.8	6.7	0.2	1	1
66	D-59	X 22	45.0	10.7	33.0	9.3	45.0	0.4	3.4	3.7	0.1	1	1
67	D-60	X 23	77.0	17.7	40.7	22.7	81.7	1.9	6.6	8.5	0.3	1	1
68	D-61	C 71	64.3	11.7	31.0	14.7	60.3	0.8	6.8	7.6	0.1	1	1
69	D-66	Pahk maw peuhn meuang	101.0	15.0	46.3	27.3	60.0	6.8	21.1	27.9	0.3	1	1
70	D-67	Haoannong	71.7	12.7	36.7	21.7	73.0	4.4	15.4	19.8	0.3	1	1
71	D-73	Pokhreli	105.3	7.7	40.3	17.7	68.3	0.8	12.5	13.3	0.1	1	3
72	D-75	Khole marshi	95.0	7.3	38.0	13.3	38.3	0.5	11.4	12.0	0.0	1	1
73	D-76	Jumli marshi	110.0	8.0	41.0	16.0	37.7	0.9	16.8	17.8	0.1	1	1
74	D-77	Govind	61.3	12.0	34.7	11.7	52.7	0.5	8.4	8.9	0.1	1	1
75	D-78	UPR 191-66	85.0	14.7	47.3	18.3	59.0	2.2	14.0	16.1	0.2	1	1
76	D-79	ASD 18	60.0	14.0	27.0	17.3	101.3	1.9	11.3	13.2	0.2	3	1
77	D-82	Phalguna	103.7	16.7	59.3	41.7	55.0	9.3	29.0	38.4	0.3	1	1
78	D-85	Budda	70.0	13.3	35.3	18.7	58.3	2.0	10.9	12.9	0.2	1	1
79	D-86	Doddabyranellu	122.3	15.7	50.0	36.7	84.7	5.0	19.0	24.0	0.3	1	1
80	IR 20		63.3	14.3	27.7	10.0	42.0	0.6	9.5	10.1	0.1	1	1
81	IR 36		74.3	14.3	48.0	26.7	70.0	3.3	15.9	19.2	0.2	1	1
82	Jaya		65.0	10.0	33.0	13.3	72.3	4.9	8.0	12.8	0.6	1	1
83	CO-39		91.0	14.0	33.0	17.3	59.3	2.3	23.7	26.0	0.1	1	1
84	Moroberekan		107.0	5.7	85.7	80.0	86.7	14.1	17.1	31.2	0.8	3	3
85	Azucena		95.0	14.7	79.0	43.0	64.0	3.3	19.7	23.1	0.2	3	3

NA = Not available; 1 - IR 64 type band; 3 - Azucena type band; Moro = Moroberekan

Table 2. Simple correlation among root and shoot traits in rice

Characters	PHT	NOT	MRL	RV	TRN	RDW	SDW	TDW
NOT	0.005							
MRL	0.427**	0.080						
RV	0.439**	0.240*	0.709**					
TRN	0.090	0.367**	0.336**	0.492**				
RDW	0.404**	0.208*	0.550**	0.817**	0.479**			
SDW	0.571**	0.401**	0.454**	0.413**	0.306**	0.489**		
TDW	0.582**	0.398**	0.543**	0.606**	0.414**	0.724**	0.952**	
R/S	0.228*	0.070	0.313**	0.627**	0.455**	0.778**	0.052	0.312**

*,**Significant at P = 0.05 and P = 0.01 level, respectively

different from the mean of individuals showing IR 64 type band ($p < 0.01$) in both of markers. Correlation analysis revealed significant and positive correlation between MRL and the markers ($r = 0.38$, $p < 0.01$ and $r = 0.28$, $p < 0.05$ for OPBH14 and RM201, respectively). Single marker analysis revealed highly significant association between each of the markers OPBH14, RM201 and root length ($p < 0.01$ and $p < 0.05$, respectively). OPBH14 could explain 13.78% of variability and RM201 could explain 8.29% of variability

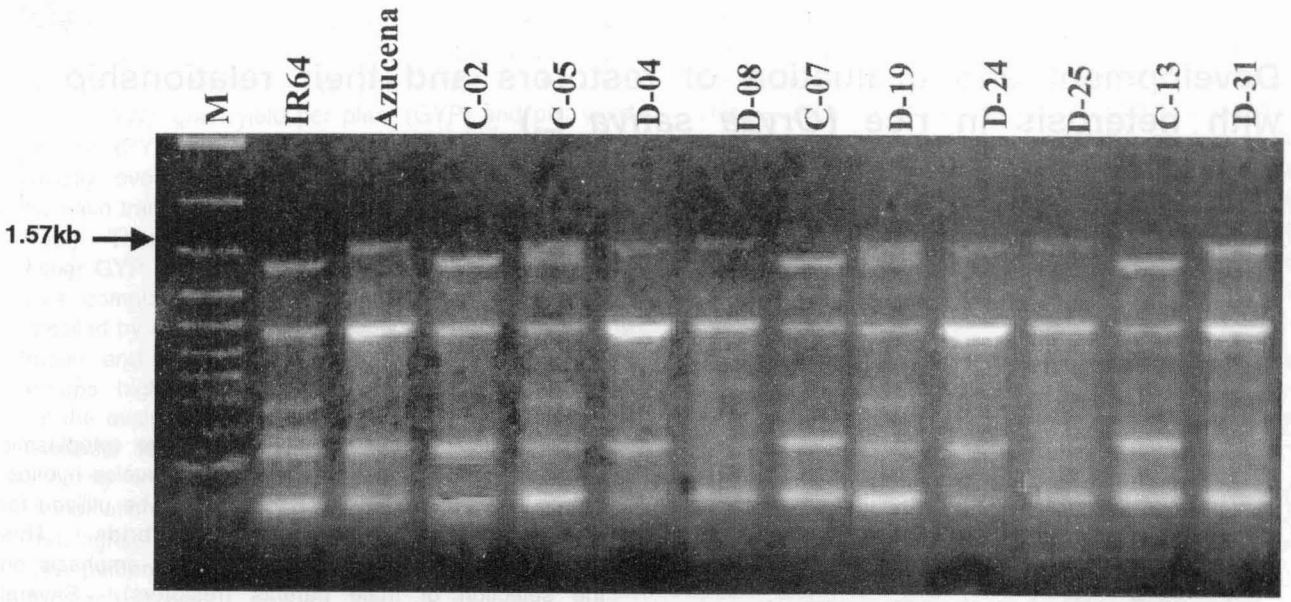

Fig. 1. Detection of root length specific bands generated by RAPD primer OPBH14 in rice accessions (M-2kb DNA ladder)

Table 3. Mean root length of plants showing different banding patterns

Marker	Banding pattern	
	1 (IR 64)	3 (Azucena)
RM201	37.3	43.4
OPBH14	37.1	44.2
Overall mean	39.1	

in MRL, which is on par with the results obtained earlier (4). Hence, OPBH14 is more closely associated with MRL. It can be concluded that OPBH14 and RM201 can be used to select for MRL and related traits in breeding programs involving diverse parents.

Acknowledgement

Funds received by H. E. Shashidhar from The Rockefeller Foundation, New York, USA (2000 FS028) is thankfully acknowledged. The Indian Council for Cultural Relations (India) and Damascus University (Syria) merit scholarship to Mr. Adnan Kanbar is gratefully acknowledged.

References

1. **Khush G. S.** 1997. Origin, dispersal, cultivation and variation of rice. Plant Mol. Biol., **35**: 25-34.
2. **Nguyen H. T., Babu R. C. and Blum A.** 1997. Breeding for drought resistance in rice: physiology and molecular genetics consideration. Crop Sci., **7**: 1426-1434.
3. **O'Toole J. C.** 1982. Adaptation of rice to drought-prone environment. *In*: Drought resistance in crops with emphasis on rice. IRRI, Philippines: 195-213.
4. **Shashidhar H. E., Sharma N., Venuprasad R., Toorchi M., Chandrashekar M., Adnan Kanbar and Hittalmani S.** 2001. PCR-based markers for quantitative traits associated with drought resistance and validation across populations and germplasm. 8th National Rice Biotechnology Network Meeting, 21-25 October, 2001, Aurangabad, India: 47-50.
5. **Mumbani B. and Lal R.** 1983. Response of upland rice varieties to drought stress. II. Screening rice varieties by means of variable moisture regimes along a top sequence. Plant and Soil, **73**: 73-94.
6. **Tanksley S. D., Young A. H., Paterson A. H. and Bonierbale M. W.** 1989. RFLP mapping in plant breeding: new tool for an old science. Theor. Appl. Genet., **75**: 257-264.
7. **Zheng K., Huang N., Bennett J. and Khush G. S.** 1995. Rapid micro-level extraction of DNA in rice, IRRI discussion paper, series No. **12**: pp. 24.
8. **Bassam B. J., Caetano-Anolles G. and Gresshoff P. M.** 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal. Biochem., **80**: 81-84.
9. **Weber C. R. and Moorthy B.** 1952. Heritable and non-heritable relationship and variability of oil content and agronomic characters in the F_2 generation of soybean crosses. Agron. J., **44**: 202-209.
10. **Ekanayake I. J., O'Toole J. C., Garrity D. P. and Masajo T. N.** 1985. Inheritance of root characters and their relations to drought resistance in rice. Crop Sci., **25**: 927-933.
11. **Yadav R. S., Courtois B. and Huang N.** 1995. Mapping genes controlling root morphology in a doubled haploid population of *indica* × *japonica* cross of rice. Theor. Appl. Genet., **94**: 619-32.
12. **Hemamalini G. S.** 1997. Molecular mapping of low-moisture stress induced response in rice (*Oryza sativa* L.) roots at peak vegetative stage. Unpubl. M.Sc. thesis. University of Agricultural Sciences, Bangalore.
13. **Gireesha T. M., Shashidhar H. E. and Hittalmani S.** 2000. Genetics of root morphology and related traits in an *indica-indica* based mapping population of rice (*Oryza sativa* L.). Res. on Crops, **1**: 208-215.