amount of inhibitor protein than control rice seeds, which indirectly indicates the presence of the α-amylase inhibitor gene. Biochemical and bioassay studies proved the expression pattern in seeds of six transgenic lines, which revealed the α-amylase activity. Thus there was an indication of the expression of the α-amylase inhibitor gene in rice seeds. This approach would also minimize the risk of insects developing resistance to the inhibitor.

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Validation of markers linked to maximum root length in rice (*Oryza sativa* L.)

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Root characters/traits are difficult to record and require destructive sampling of the plants. DNA-based molecular markers represent a non-destructive method for gathering information regarding the root characteristics. Two root length specific markers, BH14 and RM201 were utilized to determine the maximum root length of 81 diverse genotypes. Polymerase chain reaction

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analysis revealed all deep-rooted cultivars amplifying 1.57 kb and 140 bp band and shallow-rooted cultivars 1.40 kb and 158 bp band for BH14 and RM201, respectively. Single marker analysis statistically linked the markers to maximum root length, BH14 (R^2 22.24%, P = 0.0001) and RM201 (R^2 14.62%, P = 0.0004).

Keywords: Maximum root length, marker-assisted selection, molecular markers, polymerase chain reaction, single-marker analysis.

DROUGHT is a major challenge to food production worldwide. Compared to maize and sorghum, rice has a shallow root system that makes it susceptible to water stress[1]. Several physiological and morphological traits have been reported to improve the performance of crops affected by drought. Root system morphology is one of the important components of drought resistance[2]. The root system plays an important role in the regulation of water uptake and extraction from deep soil layers. Large genetic variation in root morphology has been reported in germplasm adapted to different agro-ecological conditions[3]. The difference between shallow-rooted and deep-rooted varieties lies in root penetration of the soil layers deeper than 30 cm from the surface[4]. Root characteristics are cumbersome to evaluate when compared to shoot or above-ground characters and sometimes results in their damage while phenotyping, leading to erroneous values. DNA-based molecular markers circumvent this problem by amplifying specific-sized band for deep-rooted and shallow-rooted cultivars. Two polymerase chain reaction (PCR)-based DNA markers were

Table 1. Means of the root length along with marker data in diverse accessions of rice

Genotype	BH14	RM201	MRL (cm)	Genotype	BH14	RM201	MRL (cm)
IR64	3	3	10.50	ARC 1135	3	3	28.00
IR 53901-11-1-2-3R	3	3	22.00	Milyang 54	3	3	26.50
Suweon 318	3	3	30.00	Suweon 9654	3	3	29.00
V20B	3	3	34.00	S307	3	3	21.00
IET 12606	3	3	30.00	CAO 9246-36	1	1	33.50
IR 35454-18-1	3	3	27.50	Suweon 290	3	3	29.00
Madhu	3	3	24.00	IR 62829B	3	3	27.00
IR 50360-12-1	3	3	25.00	Sarasa B	3	3	26.50
IR 64608B	3	3	52.50	IR 35366-28-3	3	3	28.75
Azucena	1	1	40.50	IR 40750 R	3	3	19.50
IET 13155	3	3	25.00	IR 9761	3	3	28.00
IET13554	3	3	23.50	IET 13553	3	3	47.50
Moroberekan	1	1	39.50	IR 3429-50	3	3	20.00
Norin 20	3	3	35.50	PR 108	3	3	25.50
PR 103	3	3	25.00	IR 50360-12	3	3	29.00
IR 32841-46-IR	3	3	13.50	NGL 3935	3	3	27.00
IET 131553	3	3	26.50	IR 74	3	3	35.00
IR 52251-3-1	3	3	15.00	MM 125 A	3	3	22.50
Taichung 62	3	3	23.50	IET 13552	3	3	38.00
IR 49461-12-3-3	1	3	47.00	Mustual	3	3	26.00
IET 5995	3	3	35.50	Budda	1	3	31.50
IR 30864	3	3	17.50	Pusa 205	3	3	18.00
Pusa 33B	3	3	19.00	Irylon-1	1	3	37.50
Adt-34	3	3	27.00	Jaya	3	3	27.50
IR 21819-20	3	3	22.50	IR 58025 B	3	3	26.50
CTH-1	3	3	30.50	IR 660707 B	3	3	32.00
Sukanandi	3	3	19.50	IR 12645	3	3	35.00
IET 13550	3	3	25.00	Arkavathi	3	3	24.50
CO 32	3	3	23.50	IR 18356-93	3	3	47.00
Milyang 46	3	3	24.50	MO 9-Mukam	3	3	17.00
Pusajaldhidhan	3	3	23.00	Korea 1	3	3	29.50
IR 31358-90	3	3	23.00	IR 23283-109	3	3	24.00
Mingola	3	3	28.00	IR 40572 R	3	3	26.50
IR 52	1	3	33.00	Kanakam	3	3	23.50
Getu	1	1	57.00	Erramallalu	3	3	31.00
IR 13603-30	3	3	42.50	ESSH-2	3	3	17.50
M210	3	3	23.50	IRRI-Jap-12	1	1	33.00
MP 114	3	3	15.50	IR 5657-33	3	3	38.50
IET 5656	3	3	18.00	IR-20	3	3	22.50
Vikas	3	3	15.00	IR 29723 R	3	3	28.00
				IR 28	3	3	19.50

BH14, score = 1, 1.57 KB, Deep-rooted; RM201, score = 1, 140 BP, Deep-rooted; BH14, score = 3, 1.40 KB, Shallow-rooted; RM201, score = 3, 158 BP, Shallow-rooted.

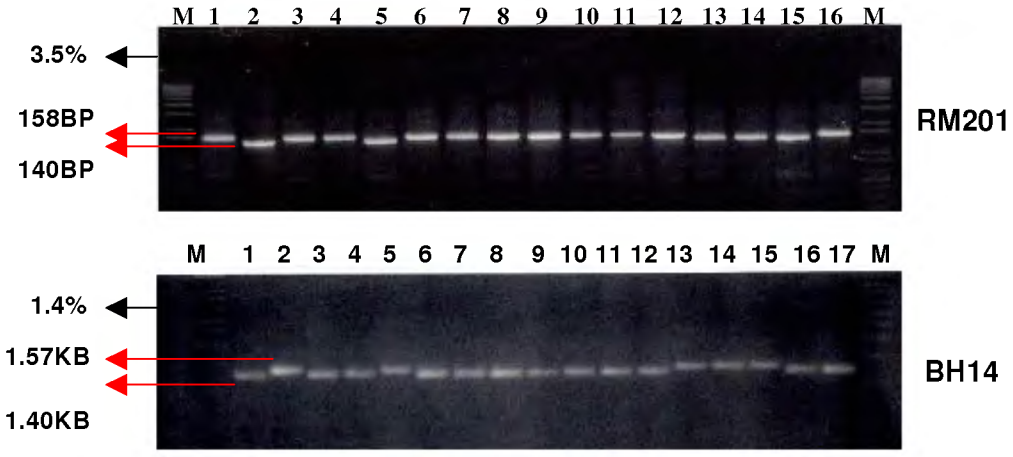

Figure 1. Marker profile of diverse cultivars of rice for RM201 and BH14 markers. M, Ladder; 1, IR64; 2, Azucena; 3, Jaya; 4, IR20; 5, Moroberekan; 6, CO 32; 7, Norin 20; 8, Pusa 33B; 9, Taichung 62; 10, Vikas; 11, Mingola; 12, MM125A; 13, CAO 9246-36; 14, IRRI-Jap-12; 15, Getu; 16, IR28; 17, Arkavathi (BH14 only).

utilized to check 81 diverse genotypes for their maximum root length (MRL). A sequence characterized amplified region (SCAR) marker BH14 (ref. 5) and a rice microsatellite marker RM201 (ref. 6) were employed in this study. The two root length specific markers identified deep-rooted and shallow-rooted diverse rice varieties. They showed statistically significant association with MRL.

The experiment was conducted in Hebbal, University of Agricultural Sciences, Bangalore in a randomized complete block design with two replications. The 81 diverse genotypes were sown in polyvinyl chloride pipes of 100 cm length and 18 cm diameter. The pipes were compacted from the top with sandy loam soil and well decomposed farmyard manure[7] in the ratio of 4 : 1 and 3 seeds per genotype were directly sown into the pipes. Ten days after germination, the seedlings were thinned leaving only one seedling in each pipe. The plants were watered twice weekly. After 65 days from the date of sowing, the shoots were cut at the base and the pipes were submerged in water overnight to loosen the soil. The following day, soil was eased out of the pipes and the roots were thoroughly cleaned with a jet of water. The intact root system was stored in polythene bags for recording observations. The maximum root length was measured from the collar region to the tip of the longest root (Table 1).

Genomic DNA was extracted from leaf tissues of 25-days-old seedlings of the 81 diverse varieties by modified cetyl trimethyl ammonium bromide method[8]. The DNA was quantified at 260 nm using a UV spectrophotometer. PCR was carried out using two DNA markers, viz. BH14 and RM201. The PCR reaction mixture contained 40 ng of template DNA, 0.2 µM of each primer pair (Sigma Aldrich), 1 mM dNTP's, 10X PCR buffer (1X is 10 mM Tris Hcl pH 8.8 at 25°C, 1.5 mM KCl and 0.1% triton X-100), 1 unit of taq polymerase (Bangalore Genei) and deionized water to get a total reaction volume of 20 µl. The PCR profile involved an initial denaturation of 94°C for 5 min followed by 35 cycles of 94°C for 1 min, 56°C for 1 min, 72°C for 1 min and a final extension for 5 min at 72°C. The amplified PCR products for BH14 and RM201 were resolved on 1.4% and 3.5% agarose gels in 1X TAE buffer. With BH14 marker, all the deep-rooted varieties amplified a 1.57 kb band and were assigned a score of 1 whereas the shallow-rooted varieties amplified a 1.40 kb band and were assigned a score of 3 (Figure 1). Interestingly with RM201 marker, all the deep-rooted varieties amplified a smaller sized band (140 bp) and were assigned a score of 1. The shallow-rooted varieties amplified a bigger sized band (158 bp) and were assigned a value of 3 (Figure 1).

Four genotypes (IR49461-12-3-3, IR52, Budda and Irylon 1) amplified the deep-rooted band with BH14 but amplified the shallow-rooted band with RM201 marker. This may be because the two MRL linked markers are independent of each other as BH14 is on chromosome ten[5] and RM201 is on chromosome nine[6]. Hence, the genes associated with the markers segregate independently. Fourteen genotypes amplified the shallow-rooted alleles with both BH14 and RM201 markers even though they were deep-rooted. This is due to the fact that root length, being a polygenic trait, is controlled by several genes. Hence, several markers tagged to root length controlling genes are required to distinguish the deep-rooted cultivars from shallow-rooted cultivars with 100% accuracy.

Single-marker analysis using SAS (ref. 9) was carried out on the two markers. They showed statistically significant association with maximum root length, with BH14 having a R^2 value of 22.24% and $P = 0.0001$. RM201 revealed a R^2 value of 14.62% with $P = 0.0004$. This proves that the markers are tightly linked to MRL, hence can be successfully employed to check the length of root system of the concerned genotypes.

A well-endowed root system is a vital mechanism of crop plants to grow under moisture deficit conditions. Root length is an indicator of the plant's ability to absorb water

from the deeper layers of soil and is influenced by better root penetrability. The same inference is reflected from the studies of several scientists[10–15]. Selection for root traits is arduous conventionally since it needs destructive sampling of the plants. As the expression of root characters is below the ground, the selection may not be much easier than that of the other characters which are above-ground level. Breeding varieties for improved roots is difficult because root traits are quantitative and have low heritability. Molecular markers can be used to identify linkage to quantitative trait loci (QTL) for rooting ability and these can be selected more easily in a breeding programme than the traits themselves[16]. Tagging of markers like BH14 and RM201 for traits conferring drought resistance, especially root-related traits helps in generating tools for marker-assisted selection, which in turn helps in accelerating crop improvement.

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Breeding systems and pollination modes of understorey shrubs in a medium elevation wet evergreen forest, southern Western Ghats, India

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This study on the reproductive biology and pollination modes of 22 species of understorey shrubs in 11 families was conducted in a medium elevation wet evergreen forest in the southern Western Ghats of India from 1994 to 1997. We evaluated whether this assemblage was predominantly outcrossing as in other tropical forests, and whether mating systems are related to pollination mode. We assessed whether species were hermaphrodites, dioecious or monecious. We assessed the breeding systems of each species with hand self-pollinations. About 55% of the species produced small white and inconspicuous flowers. The majority of the flowers opened at dawn and was visited by diurnal pollinators. The proportion of dioecious and monoecious species was lower than for other tropical forests. Among the hermaphrodites, the majority had mixed mating systems. Therefore the overall levels of obligate outcrossers (37%) were low compared with other tropical forests. We recognized 7 pollination modes: social bees, solitary bees, diverse insects, flies, sunbird, sphingid moth and *Xylocopa* sp. Among these the social bees, flies and diverse insects visited more species than the other groups. Species pollinated by flies and diverse insects tended to be significantly more outcrossing than those pollinated by bees and other solitary pollinators.

Keywords: Breeding systems, India, pollination mode, shrubs, Western Ghats.

INFORMATION on the sexual and breeding systems of tropical plants is important for understanding speciation processes in tropical forests and for the conservation of tropical biodiversity[1–5]. Data on the sexual and breeding

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