Aerobic rice genotypes displayed greater adaptation to water-limited cultivation and tolerance to polyethyleneglycol-6000 induced stress

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Abstract Water scarcity and drought have seriously threatened traditional rice cultivation practices in several parts of the world including India. In the present investigation, experiments were conducted to see if the water-efficient aerobic rice genotypes developed at UAS, Bangalore (MAS25, MAS26 and MAS109) and IRRI, Philippines (MASARB25 and MASARB868), are endowed with drought tolerance or not. A set of these aerobic and five lowland high-yielding (HKR47 and PAU201, Taraori Basmati, Pusa1121 and Pusa1460) *indica* rice genotypes were evaluated for: (i) yield and yield components under submerged and aerobic conditions in field, (ii) root morphology and biomass under aerobic conditions in pots in the nethouse, (iii) PEG-6000 (0, -1, -2 and -3 bar) induced drought stress at vegetative stage using a hydroponic culture system and (iv) polymorphism for three SSR markers associated with drought resistance traits. Under submerged conditions, the yield of aerobic rice genotypes declined by 13.4–20.1 % whereas under aerobic conditions the yield of lowland *indica*/Basmati rice varieties declined by 23–27 %. Under water-limited conditions in pots, aerobic rice genotypes had 54–73.8 % greater root length and 18–60 % higher fresh root biomass compared

to lowland *indica* rice varieties. Notably, root length of MASARB25 was 35 % shorter than MAS25 whereas fresh and dry root biomass of MASARB25 was 10 % and 64 % greater than MAS25. The lowland *indica* were more sensitive to PEG-stress with a score of 5.9–7.6 for Basmati and 6.1–6.7 for non-aromatic *indica* rice varieties, than the aerobic rice genotypes (score 2.7–3.3). A set of three microsatellite DNA markers (RM212, RM302 and RM3825) located on chromosome 1 which has been shown to be associated with drought resistance was investigated in the present study. Two of these markers (RM212 and RM302) amplified a specific allele in all the aerobic rice genotypes which were absent in lowland *indica* rice genotypes.

Keywords Aerobic · Drought · *Indica* · Polyethyleneglycol 6000 · Root traits · SSR

Introduction

Increasing scarcity of water has threatened the traditional rice cultivation practices all over the world (Tuong and Bouman 2003). The situation is further aggravated by drought, global warming, methane emission, adverse climatic changes, over-pumping of ground water causing aquifer resources to decline and the high ‘cost’ of water (Tuong and Bouman 2003). Irrigation for agriculture consumes 2/3 of the world’s fresh water and rice alone consumes more than 50 per cent of the water used for irrigation in Asia. Overexploitation of groundwater has caused serious problems in many parts of India including Haryana and Punjab; groundwater tables have dropped on average by 0.5–1.0 m y^{-1} in states of India (Bouman and Tuong 2001). By 2025, it is expected that 2 million ha of Asia’s irrigated dry season rice and 13 million ha of its irrigated wet season rice will

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experience “physical water scarcity,” and most of the ~22 million ha of irrigated dry season rice in South and South-east Asia will suffer “economic water scarcity” (Tuong and Bouman 2003).

Decreasing water resources for rice cultivation has prompted research on the development of water efficient ‘aerobic rice’ varieties by combining the drought-resistant characteristics of upland varieties with the high-yielding traits of lowland varieties (Belder et al. 2005). Aerobic rice is a production system in which specially developed varieties are grown in well-drained, non-puddled and non-saturated soils. The soil is therefore “aerobic” or with oxygen throughout the growing season, as compared to traditional flooded fields, which are “anaerobic.” Rice is characterized by a shallow root system compared with other cereal crops, having limited water extraction below 60 cm (Fukai and Inthapan 1988). The form of the rice root system also varies with the cultivation method (Yoshida and Hasegawa 1982). In upland conditions with direct sowing, the root system generally develops deeper than in transplanted plantings in lowland conditions (Gowda et al. 2011). Compared with traditional lowland rice cultivation, water inputs in aerobic rice were less than 50 % (only 470–650 mm), water productivities were 64–88 % higher, gross returns were lower by 28–44 % (345–633 \$ ha⁻¹) and labour use were 55 % lower. It is hard to say if aerobic rice will replace the traditional rice varieties, but there is no doubt that aerobic rice may find its place in water-short irrigated lowlands, fields on upper slopes or terraces in undulating, rainfed lowlands, favourable uplands etc.

Besides water scarcity, rice crop in northern parts of India is subjected to frequent spells of drought resulting from falling monsoon, poor quality of water and inadequate supply of water through canals at critical stages (Bouman 2002). It is thus important to ensure that aerobic rice genotypes are also endowed with drought tolerance. The first drought tolerant aerobic rice officially released in India was MAS946-1 in 2006–07, developed through marker assisted selection, by University of Agricultural Sciences, Bangalore, which promised 6.5–7.0 tons of grain yield and 7.0 tons of fodder yield and consumes 40 per cent of water as against submerged conditions (Hemamalini et al. 2000). Other varieties such as PMK3 and MAS26 have also been developed for aerobic rice cultivation (Jeyaprakash et al. 2005; Veeresh et al. 2011). In this paper, we report the evaluation of five water-efficient aerobic (MAS25, MAS26, MAS109, MASARB25 and MASARB868) and five lowland high-yielding (HKR47 and PAU201, Taraori Basmati, Pusa1121 and Pusa1460) *indica* rice genotypes for yield and yield components under submerged and aerobic conditions in field, root morphology and biomass under aerobic conditions in pots in a nethouse, PEG-6000 (0, –1, –2 and –3 bar) induced drought stress at vegetative stage using a

hydroponic culture system and polymorphism for three SSR markers associated with drought resistance traits.

Material and methods

Plant material

The experimental material was comprised of Basmati (Taraori Basmati, Pusa1121 and Pusa Basmati 1460), *indica* (PAU201 and HKR47) and aerobic rice genotypes (MAS25, MAS26, MAS109, MAS-ARB25 and MAS-ARB868). MAS25, MAS26 and MAS109 are the aerobic rice varieties developed by University of Agricultural Sciences, Bangalore. MAS-ARB25 (IET-20649) and MAS-ARB868 (IET-20653) are the drought tolerant rice genotypes developed from IR64/Azucena/xx IR64 crosses at IRRI, Manila, Philippines.

Field evaluation of rice genotypes under submerged and aerobic conditions

The crop was raised during the kharif season of 2008–09 at CCS HAU, Rice Research Station, Kaul (Kaithal). Three lines of 2.5 m length of each variety were grown in Augmented Design using the traditional flooded as well as direct-seeded aerobic cultivation practices. Traditional flooded and aerobic cultivation practices were carried out as described earlier (Bouman et al. 2002). For each genotype, six plants were randomly selected and observations were recorded on the following parameters: plant height (cm), effective number of tillers per plant, panicle length (cm), number of panicles per plant, number of grains per panicle, 1000-grain weight and yield per plant (g).

Evaluation of root-shoot morphology under aerobic conditions in pots

Fifteen seeds of each genotype were soaked in water and incubated in seed germinator maintained at 28°C for 48 hrs prior to sowing. The germinated seeds were transferred to the floating culture trays containing 1000 ml Yoshida solution in green house. After recording observation on root length and root volume, fifteen-days-old seedlings were transferred to the pots (25 cm diameter × 25 cm height) [one plant/pot] filled with soil. After every fifteen day, the pots were irrigated with full-strength nutrient solution for the first 45 days, and with half-strength nutrient solution thereafter (Yoshida and Hasegawa 1982). The pots were irrigated with water (1 l) after every two days for first ten days and then with 750 ml water after every fifth day till harvesting. After 120 days, six plants were randomly taken out from the pots and washed

Table 1 Summary of mean±S.E., critical difference and coefficient of variation for some agronomic and quality traits in ten rice genotypes grown under submerged and water limited conditions

Cultivation conditions	Variety	Height (cm)	Tiller no.	Panicles/plant	Panicle length (cm)	Grains/panicle	1000 grain weight (g)	Yield/plant (g)
Submerged	Taraori Basmati	133.0±1.69	16.0±1.91	14.3±1.69	24.8±0.76	76.8±2.77	29.2±0.49	22.5±0.79
	Pusa 1121	110.8±1.49	19.0±1.89	17.3±1.71	26.3±0.84	64.7±2.93	28.9±1.17	23.0±1.88
	Pusa Basmati 1460	100.8±1.74	13.0±1.05	12.3±0.99	29.0±1.18	79.0±4.09	30.2±0.52	23.4±2.24
	HKR47	98.8±2.23	15.0±1.05	16.2±1.01	22.2±0.48	69.7±5.24	28.3±0.50	24.6±1.68
	PAU201	89.0±2.92	10.0±1.07	17.8±1.01	23.8±0.65	68.8±3.32	28.1±0.40	24.2±0.74
	MAS25	101.2±1.85	13.0±0.99	12.2±0.95	24.5±0.43	60.9±3.16	26.4±4.54	17.4±2.46
	MAS26	104.0±1.29	14.0±1.32	12.8±1.25	22.3±0.56	70.7±1.84	25.0±0.35	17.9±1.12
	MAS109	91.7±1.75	18.0±3.34	13.3±1.28	20.7±0.76	60.2±2.18	24.6±0.54	16.7±0.99
	MAS-ARB25	96.7±1.02	15.0±1.12	13.5±1.12	23.8±0.31	68.3±3.17	24.6±0.92	18.8±1.21
	MAS-ARB868	101.5±1.34	12.0±0.40	10.7±0.49	21.8±0.91	69.8±2.27	23.2±0.85	16.4±0.86
Aerobic	Taraori Basmati	111.5±0.85	14.0±0.83	12.3±1.05	23.7±0.88	48.7±2.62	24.9±0.49	16.9±1.71
	Pusa1121	101.7±2.23	15.0±0.76	14.3±1.02	25.8±1.33	60.2±2.96	28.3±0.65	16.7±1.01
	PBI460	108.8±1.14	12.0±0.92	11.0±0.73	22.8±1.05	69.5±3.80	28.7±0.59	18.1±0.62
	HKR47	85.0±2.42	13.0±1.47	12.3±1.48	20.8±0.70	66.3±3.29	27.1±0.73	18.1±2.16
	PAU201	74.5±1.18	14.0±0.73	12.8±0.79	22.0±0.79	59.7±2.39	26.0±0.22	17.8±1.17
	MAS25	83.5±1.20	15.0±0.76	15.0±0.68	23.3±0.49	70.2±2.83	30.3±0.71	20.4±2.13
	MAS26	80.5±1.95	16.0±0.58	14.0±0.52	22.7±1.28	74.8±2.43	29.2±0.36	21.4±0.72
	MAS109	65.7±1.05	17.0±1.26	14.8±1.35	19.2±1.25	66.3±2.17	28.5±1.01	20.9±2.62
	MAS-ARB25	76.7±0.99	12.0±1.02	14.7±1.20	24.3±0.56	71.6±2.19	30.7±0.77	21.8±3.20
	MAS-ARB868	83.3±1.59	14.0±0.70	12.0±0.78	20.2±0.31	73.2±2.85	29.0±0.79	19.9±0.79
Total	CD	4.735	3.011	3.163	2.330	8.576	1.868	4.638
	SE(d)	2.381	1.897	1.590	1.172	4.313	0.939	2.848
	SE(m)	1.684	1.341	1.125	0.829	3.050	0.664	1.926
Varieties	CV	4.354	22.579	22.579	8.701	10.843	5.707	18.392
		113.80 ^a	5.02 ^a	5.84 ^a	12.12 ^a	11.65 ^a	23.13 ^a	13.61 ^a
Cultivation condition		437.25 ^a	1.28	0.19	9.85 ^a	0.18	3.71	10.02 ^a
Variety x Condition		15.96 ^a	2.70 ^a	2.55 ^b	3.64 ^a	9.00 ^a	0.0082 ^a	31.66 ^a

^a significance at 1 % level, ^b significance at 5 % level

Table 2 Variation for root length, fresh and dry root weight in lowland *indica* and aerobic rice varieties under water limited conditions in pots

Variety	Root length (cm)	Shoot length (cm)	Fresh root weight (g)	Fresh shoot weight (g)	Total plant weight (g)	Dry root weight (g)	Dry shoot weight (g)	Total dry weight (g)
Taraori Basmati	35.0±1.51	120.3±2.84	3.5±0.47	28.7±2.49	34.7±2.90	1.06±0.08	8.8±0.32	13.9±0.94
Pusa 1121	28.8±1.25	107.8±2.39	3.2±0.54	20.4±1.34	23.0±0.87	0.95±0.03	8.9±0.46	11.9±0.54
Pusa Basmati 1460	33.5±2.92	110.2±2.70	4.1±0.48	29.9±1.24	32.3±0.27	1.09±0.09	8.8±0.39	12.5±0.70
HKR47	31.2±0.87	101.3±2.39	3.5±0.48	21.9±0.52	23.5±0.80	0.94±0.04	9.5±0.11	13.1±0.98
PAU201	41.2±1.68	106.5±2.88	4.3±0.30	21.3±0.69	22.2±0.34	1.48±0.05	10.2±0.60	14.8±0.39
MAS25	54.2±3.42	97.8±4.12	5.1±0.65	24.0±1.51	31.1±0.34	1.32±0.12	7.9±0.60	14.1±0.40
MAS26	48.8±1.35	89.0±2.46	4.6±0.49	21.5±0.45	25.5±0.69	1.04±0.05	8.5±0.40	12.7±0.48
MAS109	36.8±2.97	89.3±2.96	3.7±0.37	22.2±1.25	22.2±0.42	0.99±0.04	8.4±0.47	12.4±0.40
MAS-ARB25	35.5±3.23	98.8±1.60	5.6±1.18	23.5±1.29	29.5±2.00	2.17±0.21	8.9±0.19	14.6±0.64
MAS-ARB868	33.8±0.98	97.0±2.00	3.7±0.27	22.0±0.44	22.3±0.48	1.23±0.15	7.6±0.74	14.8±0.34
CD	6.351	7.72	1.003	3.620	4.638	0.286	1.322	1.771
SE(d)	3.153	3.832	0.816	1.797	2.303	0.142	0.656	0.879
SE(m)	2.229	2.710	0.577	1.270	1.628	0.101	0.464	0.621
CV	14.226	6.52	13.145	13.221	15.631	19.756	13.011	11.288
Analysis of variance								
Basmati	7.18 ^a	4.12 ^b	3.47 ^b	7.98 ^a	17.51 ^a	1.16	0.02	2.02
<i>Indica</i>	62.69 ^a	3.12	1.96	12.09 ^a	2.28	154.77 ^a	1.22	2.57
Aerobic	12.23 ^a	1.79	1.60	4.93 ^a	4.98 ^a	13.61 ^a	1.03	5.90 ^a
Aerobic, <i>indica</i> , Basmati	7.20 ^a	3.99 ^b	1.14 ^b	10.20 ^a	1.66	3.34 ^b	8.80 ^a	2.26

^a significance at 1 % level, ^b significance at 5 % level

properly with tap water and observations were recorded for root length (cm), fresh and dry root weight (g), fresh and dry shoot weight (g) and total plant weight (g). Shoots were cut at the crown level and roots were washed in running water to remove the soil. The complete removal of soil was done on a 3 mm sieve. Care was taken to remove the root system completely. The roots were thoroughly cleaned and straightened by repeated dipping and rinsing in buckets of clean water. Then the plants, roots and shoots were dried at 70°C for 72 h before weighing for measuring dry biomass (Painawadee et al. 2009).

Effect of PEG-6000 induced water stress conditions

A hydroponic culture system was designed to determine the response of these rice varieties to PEG-6000 induced water stress in net-house. Experiment was conducted in four sets. The seeds of these varieties were allowed to germinate directly onto nylon mesh supported on floating thermocol sheets (specially-designed sheets having holes of 1cm diameter with fine nylon mesh attached on the lower surface) in Yoshida solution. One set served as a control and in the other three sets, ten days old seedlings were given stress (PEG-6000) to achieve water deficit of −1, −2 and −3 bars respectively. The experiment was carried out under natural climatic conditions in a net-house during rice growing season. Solutions were renewed every week and water level was adjusted on alternate days. After five weeks of PEG-6000 treatment, data was recorded for seedling growth (root and shoot length), seedling biomass (fresh and dry root and shoot weight) and relative water content (RWC) in the presence and absence of PEG-6000. The relative water content was calculated by recording fresh, dry and turgid weight of seedlings after stress. Turgid weight was recorded after soaking the leaves in water overnight. Relative water content was estimated using the following formula: $RWC = \frac{\text{fresh weight} - \text{dry weight}}{\text{turgid weight} - \text{dry weight}} \times 100$. Each variety was scored according to IRRI standard evaluation system (1988) with some modifications for seedling growth, seedling biomass and relative water content on a 1–9 scale, where lower score state for tolerance and higher score for sensitivity. The scores were averaged and used to assess the PEG-6000 tolerance levels of rice varieties.

Microsatellite marker analysis

For microsatellite marker analysis, genomic DNA was extracted from young leaf samples of parental rice genotypes using the modified CTAB method (Saghai-Marooof et al. 1984). A small set of three previously developed rice microsatellite markers (RM212, RM302 and RM3825) associated with drought resistant traits were used for the identification and discrimination of polymorphism

Fig. 1 Correlation between root length and yield under aerobic conditions

(Kanagaraj et al. 2010). PCR amplification, denaturing polyacrylamide gel electrophoresis and silver staining were carried out as described earlier (Jain et al. 2004).

Statistical analysis

Mean values were taken from the measurement of six replicates and standard error of the means was calculated. Difference between means were determined by one way ANOVA and Duncan's Multiple Range test ($p < 0.05$). Analyses were done using Statistical Package for Social Sciences (SPSS) for window version 13.0.

Results and Discussion

Field evaluation of rice genotypes under submerged and water-limited conditions

The experiment was carried out to study the field performance of five aerobic and five low-land *indica* rice

Fig. 2 Correlation between root biomass and yield under aerobic condition

Table 3 Effect of PEG-6000 induced water stress on seedling growth, biomass and relative water content in lowland *indica* and aerobic rice genotypes

	Variety	PEG-6000 Stress	Root length (cm)	Shoot length (cm)	Fresh root weight (g)	Fresh shoot weight (g)	Dry root Weight (g)	Dry shoot weight (g)	RWC (%)
Aromatic <i>indica</i> (Basmati)	Taraori Basmati	Control	31.33 ^a	48.50 ^a	0.244 ^a	0.675 ^a	0.071 ^b	0.317 ^d	93.09 ^a
		-1 bar	26.83 ^b	44.67 ^b	0.232 ^b	0.664 ^b	0.084 ^{ab}	0.335 ^c	63.94 ^b
		-2 bar	23.86 ^c	42.83 ^{bc}	0.216 ^c	0.654 ^{bc}	0.091 ^{ab}	0.353 ^b	56.96 ^c
	Pusa 1121	-3 bar	20.48 ^d	40.17 ^c	0.205 ^d	0.646 ^c	0.105 ^a	0.379 ^a	46.15 ^d
		Control	29.66 ^a	46.33 ^a	0.249 ^a	0.660 ^a	0.073 ^c	0.307 ^d	93.12 ^a
		-1 bar	26.17 ^b	43.17 ^b	0.239 ^{ab}	0.653 ^{ab}	0.085 ^b	0.337 ^c	61.23 ^b
	Pusa Basmati 1460	-2 bar	24.39 ^c	41.50 ^{bc}	0.227 ^{bc}	0.644 ^c	0.089 ^c	0.360 ^b	54.02 ^c
		-3 bar	21.83 ^d	40.17 ^c	0.213 ^c	0.632 ^d	0.101 ^a	0.377 ^a	44.35 ^d
		Control	30.67 ^a	46.17 ^a	0.266 ^a	0.659 ^a	0.071 ^a	0.318 ^a	93.23 ^a
Non-aromatic <i>indica</i>	HKR47	-1 bar	28.33 ^b	44.50 ^{ab}	0.259 ^{ab}	0.652 ^{ab}	0.083 ^b	0.338 ^b	63.19 ^b
		-2 bar	26.33 ^b	42.50 ^{bc}	0.245 ^{bc}	0.628 ^{bc}	0.092 ^c	0.359 ^c	58.11 ^c
		-3 bar	22.17 ^c	40.83 ^c	0.232 ^c	0.619 ^c	0.101 ^d	0.373 ^d	46.96 ^d
	PAU201	Control	29.67 ^a	48.00 ^a	0.267 ^a	0.678 ^a	0.070 ^a	0.320 ^d	93.24 ^a
		-1 bar	25.33 ^b	46.17 ^b	0.258 ^{ab}	0.667 ^a	0.087 ^b	0.337 ^c	67.27 ^b
		-2 bar	24.17 ^{bc}	43.17 ^c	0.247 ^b	0.659 ^{ab}	0.094 ^c	0.363 ^b	56.95 ^c
	MAS25	-3 bar	21.83 ^c	40.50 ^d	0.238 ^c	0.646 ^c	0.101 ^d	0.382 ^a	50.82 ^d
		Control	31.67 ^a	48.70 ^a	0.276 ^a	0.670 ^a	0.074 ^c	0.319 ^c	93.17 ^a
		-1 bar	29.83 ^a	43.50 ^b	0.262 ^{ab}	0.661 ^b	0.087 ^b	0.328 ^c	68.67 ^b
Aerobic	MAS26	-2 bar	26.00 ^c	42.67 ^{bc}	0.251 ^{bc}	0.642 ^{bc}	0.091 ^{ab}	0.354 ^b	59.33 ^c
		-3 bar	22.83 ^d	39.33 ^c	0.232 ^c	0.633 ^c	0.103 ^a	0.381 ^a	47.79 ^d
		Control	38.50 ^a	41.67 ^a	0.292 ^a	0.677 ^a	0.078 ^a	0.341 ^d	95.07 ^a
	MAS109	-1 bar	35.33 ^b	41.50 ^a	0.287 ^{ab}	0.673 ^a	0.092 ^b	0.357 ^c	79.79 ^b
		-2 bar	33.17 ^{bc}	39.00 ^b	0.272 ^{bc}	0.659 ^b	0.111 ^c	0.370 ^b	73.56 ^c
		-3 bar	29.50 ^c	36.50 ^c	0.260 ^c	0.649 ^b	0.128 ^d	0.389 ^a	66.23 ^d
	MASARB25	Control	33.83 ^a	42.67 ^a	0.264 ^a	0.658 ^a	0.077 ^c	0.332 ^c	94.84 ^a
		-1 bar	31.00 ^b	40.50 ^b	0.258 ^a	0.645 ^{ab}	0.092 ^{bc}	0.359 ^b	78.79 ^b
		-2 bar	29.67 ^{bc}	38.50 ^c	0.252 ^{ab}	0.639 ^b	0.099 ^b	0.377 ^{ab}	71.88 ^c
		-3 bar	28.17 ^c	37.17 ^c	0.245 ^b	0.620 ^c	0.120 ^a	0.383 ^a	65.44 ^d
		Control	35.67 ^a	42.67 ^a	0.268 ^a	0.665 ^a	0.076 ^c	0.342 ^a	94.15 ^a
		-1 bar	31.67 ^b	40.83 ^b	0.259 ^{ab}	0.649 ^b	0.098 ^b	0.351 ^b	75.27 ^b
		-2 bar	30.50 ^b	38.33 ^c	0.248 ^b	0.634 ^{bc}	0.110 ^{ab}	0.368 ^{bc}	71.68 ^c
		-3 bar	27.67 ^c	37.17 ^c	0.234 ^c	0.625 ^c	0.119 ^a	0.373 ^c	65.03 ^d
		Control	32.00 ^a	43.17 ^a	0.302 ^a	0.660 ^a	0.074 ^c	0.338 ^a	93.96 ^a
		-1 bar	30.00 ^{ab}	39.83 ^b	0.290 ^{ab}	0.645 ^b	0.096 ^b	0.359 ^b	81.06 ^b
		-2 bar	27.50 ^{bc}	38.67 ^{bc}	0.278 ^{bc}	0.630 ^{bc}	0.104 ^{ab}	0.375 ^c	74.14 ^c

Table 3 (continued)

Variety	PEG-6000 Stress	Root length (cm)	Shoot length (cm)	Fresh root weight (g)	Fresh shoot weight (g)	Dry root Weight (g)	Dry shoot weight (g)	RWC (%)
MASARB868	-3 bar	26.33 ^c	37.00 ^c	0.269 ^c	0.622 ^c	0.126 ^a	0.384 ^d	69.79 ^d
	Control	33.17 ^a	44.17 ^a	0.302 ^a	0.657 ^a	0.080 ^c	0.346 ^c	93.88 ^a
	-1 bar	30.67 ^b	42.33 ^b	0.287 ^{ab}	0.641 ^{ab}	0.094 ^b	0.362 ^b	77.65 ^b
	-2 bar	28.17 ^c	40.33 ^{bc}	0.267 ^b	0.633 ^b	0.110 ^{ab}	0.373 ^{bc}	70.11 ^c
	-3 bar	27.17 ^d	38.67 ^c	0.251 ^c	0.629 ^b	0.122 ^a	0.384 ^c	65.68 ^d
Analysis of variance								
Variety								
PEG-6000 Stress								
Variety x PEG-6000 Stress								
		34.81 ^a	25.99 ^a	35.22 ^a	10.07 ^a	11.46 ^a	21.07 ^a	208.32 ^a
		132.13 ^a	116.51 ^a	44.09 ^a	37.96 ^a	145.98 ^a	274.73 ^a	3017.03 ^a
		1.84 ^a	1.15	0.31	0.96	1.47	1.66 ^b	21.68 ^a

Means with similar subscript letter(s) are not significantly different at $p < 0.05$.

^a significance at 1 % level, ^b significance at 5 % level

genotypes at RRS Kaul, Haryana (North-western India) cultivated using traditional submerged as well as water-limited (aerobic) conditions. Under aerobic conditions, number of panicles per plant, number of grains per panicle and 1000 grain weight of lowland *indica* rice varieties decreased by 11–28 %, 5–37 % and 5–10 % respectively, whereas the aerobic rice varieties showed an increase of 9–23 %, 5–15 % and 15–25 %, respectively (Table 1). The aerobic rice genotypes had optimum yield under aerobic conditions, which declined by 13.4–20.1 % when cultivated under submerged conditions. On the contrary, lowland *indica* rice varieties had optimum yield under submerged conditions, which declined by 22.7 %–27.4 % under aerobic conditions. Under aerobic conditions, maximum yield (21.8 g) was recorded in MASARB25 and lowest yield in Pusa1121 (16.7 g) (Table 1). Under direct seeding cultivation conditions, yield of the aerobic rice genotypes was not at par with the lowland *indica* rice genotypes when grown under traditional submerged conditions. Result of Two-way ANOVA (Analysis of Variance) revealed significant difference between plant height and yield per plant of all the ten rice varieties when same variety was grown under both aerobic and submerged conditions separately (Table 1).

The above results were supported by the study of Bouman and Tuong (2001) which showed higher yield in lowland varieties under flooded conditions. Under aerobic conditions, the aerobic rice ‘Han Dao varieties’ yielded higher than lowland varieties (4.7–5.3 tha^{-1}). There is an interaction between variety and environment: the lowland varieties had strong phenological and productive traits under flooded conditions, but the aerobic varieties had higher yields under aerobic conditions (Yang et al. 2005). However, it must be noted that none of the aerobic rice genotypes under aerobic conditions showed higher yield per plant than lowland *indica* rice cultivars cultivated using traditional method (Table 1), indicating that there is a need to improve the yield potential of aerobic rice.

Variation in root and shoot growth under aerobic conditions in pots

To distinguish the variability in root and shoot morphology of aerobic and lowland *indica* rice genotypes, an experiment was conducted in pots under water limiting conditions. It revealed that the root length and biomass was significantly influenced by the rice varieties. The average root length of the aerobic and lowland *indica* rice varieties varied between 28.8–54.2 cm. MAS25 had maximum root length of 54.2 cm followed by MAS26 (48.8 cm) and lowland *indica* rice variety PAU201 (41.2 cm) and least in HKR47 (31.2 cm). The root length of MAS25 was found to be 31.6 to 88.2 % longer than lowland *indica* rice genotypes.

Among the aerobic rice genotypes, MAS-ARB25 (5.6 g plant⁻¹) and MAS25 (5.1 g plant⁻¹) recorded higher fresh root weight followed by MAS 26 (4.6 g plant⁻¹) and least in lowland aromatic *indica* (Basmati) Pusa1121 (3.2 g plant⁻¹). The dry root weight of MAS25 was 58.5 % and 78.7 % higher than aromatic *indica* (Taraori Basmati) and non aromatic *indica* (HKR47) respectively. While aerobic rice genotype, MAS-ARB25, had medium root length (35.5 cm) but had higher fresh (5.6 g) as well as dry root weight (2.17 g). Pusa Basmati 1460 revealed a maximum fresh shoot biomass of 29.9 g followed by Taraori Basmati (28.7 g) and MAS25 (24.0 g) (Table 2). It must be noted that root length of MAS-ARB25 was 35 % shorter than MAS25 whereas fresh and dry root biomass of MAS-ARB25 was 10 % and 64 % greater than MAS25.

Growth of the rice root, in terms of total dry matter, maximum root depth, and root length density, increases until flowering stage and then decreases sharply to maturity (Yoshida and Hasegawa 1982). Kawata and Soejima (1974) indicated that roots produced after flowering may play an important role during the grain-filling period. Root traits have been claimed to be critical for increasing yield under soil-related stresses. Regression analysis showed that the grain yield was positively correlated with the root length ($r=0.7324$) (Fig. 1) and biomass ($r=0.8401$) (Fig. 2), suggesting that root length and biomass may be the limiting factor for grain yield under water-saving conditions.

The ANOVA for root and shoot growth showed significant difference for all lowland *indica* and aerobic rice genotypes. Variability analysis revealed that there is significant difference observed among *indica* and aerobic rice varieties for root length, fresh shoot weight and dry root weight (Table 2).

In the present study, aerobic rice genotypes performed better under water limited conditions, which could be attributed to their relatively better root length and/or biomass. Root system is considered as one of the important component to fight against the water scarce conditions. The improvement of upland rice through a deeper root system is thought by many to be a promising way to increase water uptake, and ultimately grain yield under water stress conditions. Our results are

consistent with Martin et al. (2007), who reported that the rice varieties (ADT 39 and PMK 3) that were suitable for cultivation under aerobic conditions, had longer and deeper root system compared to the other rice varieties; a strong and positive correlation existed between root length, biomass and yield. A deeper and thickened root system having large xylem vessels has been shown to allow upland rice varieties to extract more water from the soil, resulting in a higher yield potential under water scarce conditions (Nguyen et al. 1997).

Effect of PEG-6000 induced water stress at vegetative stage

PEG-6000 induced stress resulted in the reduction of seedling length (root and shoot length), biomass (root and shoot fresh biomass) and relative water content; the inhibition rate being significantly lower in aerobic rice genotypes as compared to lowland *indica* rice varieties (Table 3). Under severe stress (−3 bar), two (MAS26 and MASARB25) of the aerobic rice genotypes recorded minimum decrease in root length (16.7 and 17.7 %) as compared to lowland *indica* rice genotypes (26.4–34.6 %). The inhibitory effect of PEG (−3 bar) on fresh root biomass was relatively lower in MAS26 (7.2 %) compared to that in lowland *indica* rice genotypes (10.9–16.0 %). Notably, dry root as well as shoot biomass of the seedlings invariably increased under PEG-6000 induced water stress. At −3 bar, % increase in dry root weight was relatively higher in case of aerobic rice genotypes (52.5–70.3 %) compared to that recorded in lowland aromatic (38.4–47.9 %) and non aromatic (39.2–44.3 %) *indica* rice genotypes.

Relative water content (RWC) is indicative of a relationship between physiological traits and level of drought tolerance that tends to integrate the water balance of plant tissues over days or may be weeks (Sinclair and Ludlow 1985). The relative water content of leaves was significantly lower for water-stressed plants compared to control. RWC of rice seedlings declined progressively with increased concentration of PEG (Table 3). At −3 bar PEG treatment, % decrease in RWC was significantly higher in lowland *indica* rice varieties (e.g. 52.4 % in Pusa1121) as compared to aerobic rice varieties (e.g. 25.7 % in MASARB25) (Table 2). Percent decrease in RWC of leaves (under −3 bar) in lowland

Fig. 3 Scoring of ten rice genotypes for PEG-6000 tolerance on the basis of mean average score for seedling growth and biomass and relative water content

Fig. 4 SSR primers linked to drought resistance traits already identified in the various studies used in the present investigation

indica rice genotypes was found to be equivalent to % decrease in aerobic rice genotypes under -1 bar. The lowland *indica* were more sensitive to PEG-stress with a score of 5.9–7.6 for Basmati and 6.1–6.7 for *indica* rice varieties, than the aerobic rice genotypes (score 2.7–3.3) (Fig. 3).

Under control conditions, acceleration of root growth (length and biomass) in aerobic rice genotypes was relatively higher, which might have resulted in the rapid establishment of seedlings and avoidance of water stress (Konwar 2009). Asch et al. (2005) reported greater reduction in root length, plant height and total dry matter production in lowland rice varieties as compared to upland varieties under drought stress. Report suggests that the decrease in fresh root weight may be due to presence of O_2 in the aerenchyma tissue whereas increase in dry root weight may be due to increase in root aerenchyma tissue due to cortical disintegration which leads

to increase in the lateral roots production (Lasalita et al. 2008). The results observed in present study strongly support the earlier observations that deep-rooting rice varieties with wide root distribution might provide greater resistance to drought (Yoshida and Hasegawa 1982). RWC has been widely used to quantify the water deficits in leaf tissues. RWC may be attributed to differences in the ability of the variation to absorb more water from the soil and/or the ability to control water loss through stomata and RWC parameter can be used to select high yielding genotypes that maintain cell turgor under water stress environment to give relative high yield. It measures the energetic status of water inside the leaf cells; higher the leaf water potential and RWC, the higher was the photosynthetic rate. Our findings are in consonance with Choudhury et al. (2005) who reported that decrease in relative water content of tolerant genotypes of rice was gradual but it was drastic in susceptible ones.

Allelic profile of the markers associated with drought resistance traits

A small set of three microsatellite DNA markers (RM212, RM302 and RM3825), which have been shown earlier to be associated with drought resistance (Kanagaraj et al. 2010) were used to amplify the SSR regions in ten rice genotypes. These three markers were located on chromosome 1 of rice between 135.8 and 143.7 cM (McCouch et al. 2002) (Fig. 4); this region has been reported to be linked with several drought resistance traits including plant height, biomass, deep root mass, leaf drying, relative water content, osmotic adjustment, basal root thickness, tiller number and deep root to shoot ratio, grain yield and panicle length, canopy temperature in IR20/Nootripathu RI lines under drought stress (Beena 2005). Remarkably, two of these markers (RM212 and RM302) amplified a specific allele (112 and 190 bp, respectively) in all the aerobic rice genotypes which were absent in lowland *indica* rice genotypes while the third marker showed monomorphic pattern. Thus, SSR allelic profile of these markers could be used to differentiate between lowland *indica* and aerobic rice genotypes (Fig. 5).

Fig. 5 A silver stained gel showing allelic polymorphism among parental rice varieties at RM212, RM302 and RM3825 locus (from left side) respectively. Lane L –20 bp ladder, lanes 1 to 10 represents Taraori

Basmati, Pusa1121, PB1460, HKR47, PAU201, MAS25, MAS26, MAS109, MASARB25, MASARB868 varieties for each RM locus respectively

RM212 has shown linkage to the deep root dry weight and deep root mass in CT9993/IR62266 DH lines (Kamoshita et al. 2002) and a QTL for osmotic adjustment was reported in the vicinity of this region in IR62266/IR60080 backcross progenies (Robin et al. 2003). Hittalmani et al. (2003) reported that a genomic region of 7.9 cM (from 135.8 to 143.7 cM) on chromosome 1 was associated with drought resistance traits such as leaf rolling, number of spikelets, heading date and harvest index in IR64/Azucena rice DH lines. It is plausible that aerobic rice varieties used in the present investigation have 7.9 cM RM212-RM302 genomic region on chromosome 1, which has been reported earlier to be linked to drought resistance in rice (McCouch et al. 2002).

The present study clearly outline the large differences among aerobic and lowland *indica* rice genotypes for root traits, yield and yield components, PEG-induced drought stress and 7.9 cM RM212-RM302 genomic region on chromosome 1. It is apparently clear that none of the aerobic rice genotypes under water-limited conditions had the capability to yield, which is at par with that of lowland *indica* rice cultivars cultivated using traditional method, indicating the need to improve the yield potential of aerobic rice. The five aerobic rice genotypes used in this study possessed deeper and denser root system and performed better compared to lowland *indica* rice varieties under water-limited cultivation conditions in pots. The grain yield was positively correlated with the root length and biomass suggesting that root length and biomass might be the key factor for improving grain yield under water-saving conditions. In fact, the study also showed that aerobic rice genotypes were endowed with the greater tolerance to PEG induced drought stress. Thus, it should be feasible to combine drought tolerant, water-efficient and high-yielding traits from crosses between existing aerobic and lowland *indica* rice genotypes. This study shows the possible linkage between 7.9 cM RM212-RM302 genomic region on chromosome 1 to be associated with drought resistance. But, further genetic analysis is required using mapping populations derived from aerobic x lowland *indica* rice crosses.

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