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Marker-assisted selection and QTL mapping for yield, root morphology and agronomic traits using MASARB25 (aerobic) × Pusa Basmati 1460 F_3 mapping populations

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Abstract

Increasing scarcity of water has threatened the sustainability of the irrigated rice production system and hence the food security and livelihood of rice producers. Experiments were conducted to study the correlation and QTL mapping for yield, root-related and agronomic traits under aerobic conditions using MASARB25 × Pusa Basmati 1460 F_3 mapping population. Yield of aerobic rice variety MASARB25 was 9-12.3% higher than Basmati rice variety Pusa Basmati 1460. MASARB25 had 9.32% higher root length, 11.76% higher fresh root weight, 19.98% higher dry root weight as compared to Pusa Basmati 1460. A total of 15 QTLs associated with 10 traits were mapped on chromosomes 2, 4, 6, 8, 9, and 11. $qGY_{8.1}$ with an R^2 value of 36.3% and $qGY_{2.1}$ with an R^2 value of 29% and $qRL_{8.1}$ with an R^2 value of 27.2% were identified for root length indicating the role of root traits in improving grain yield under water limited conditions. A positive correlation was found between root traits and yield under aerobic conditions. Breeding lines with higher yield per plant, root length, dry root biomass, length-breadth ratio, and with Pusa Basmati 1460-specific alleles in a homozygous or heterozygous condition at the *BAD2* locus were identified that will serve as novel material for the selection of stable aerobic Basmati rice breeding lines.

Key words: Aerobic, basmati, QTL, root traits, SSRs

Introduction

Increasing scarcity of water for agriculture, caused by increasing urbanization, industrialization, decreasing quality and resources of water, has threatened the sustainability of irrigated rice production [1, 2]. Irrigated rice is a profligate user of water. On a field basis, rice uses two to three times more water than other cereal crops such as wheat and maize [3]. Almost 70% of water from all the fresh water resources is used in agriculture and 50% of which is used only for rice cultivation. Overexploitation of groundwater has caused serious problems in many parts of India including Haryana and Punjab; groundwater table have dropped on average by 0.5-1.0 m y^{-1} in states of India. 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 experience "physical water scarcity," and most of the approximately 22 million ha of irrigated dry-season rice in South and Southeast Asia will suffer "economic water scarcity" [4]. If the issue of water scarcity is not dealt with soon, it may become a major problem for many other countries in the near future and consequences will be detrimental [5].

There are several actions that would be effective in preserving and conserving water. Rice is appropriate target for water conservation, which is cultivated on more than 30% of irrigated land and accounts for the use of 50% of irrigation water [6]. To strategically address the projected water scarcity, IRRI has also developed an "aerobic rice" technology that aims to significantly reduce the crop's water requirement below current levels. Aerobic rice is a water-saving rice production system in which potentially high yielding, fertilizer adapted rice varieties are grown in fertile aerobic soils that are non-puddled and have no standing water [7]. Such 'aerobic

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rice' varieties combine the aerobic adaptations of traditional upland varieties with the input responsiveness, lodging tolerance and yield potential of irrigated varieties [8]. Aerobic rice had about 51% less total water use and 32-88% higher water productivity, expressed as gram of grain per kilogram of water, than flooded rice [9]. In aerobic rice varieties, roots grow deeper and more profusely in comparison to shallow roots of 5 inches in conventional rice varieties, which help in better absorption of water thereby eliminating the need for water logging. Deep roots may provide a key to aerobic adaptation in rice because they contribute to water uptake from deeper soil layers during water deficit conditions. Root traits have been claimed to be critical for increasing yield under soil-related stresses [10]. Potential to grow deep roots is currently the most accepted target trait, and other traits will likely be elucidated as our understanding of plant-soil interactions improves [11].

In India, development of aerobic rice varieties was initiated at the University of Agricultural Sciences, Bangalore in 1980 using the available upland paddy and high-yielding rice germplasm [12-13]. Aerobic rice varieties MAS 946-1 and MAS 26 were bred and released in 2007 and 2008 respectively for South Eastern Dry Zone of Karnataka following farmer participatory breeding and selection. These varieties have a maturity period of 115 to 120 days and grain yield 5.5 tons ha^{-1} and fodder yield of 6 tons ha^{-1} [14]. In this paper, we report the evaluation of MASARB25 x Pusa Basmati 1460 F_3 plants for root traits, yield and yield components under aerobic conditions to identify QTLs conferring a potential yield advantage, yield-related agronomic traits and root traits having a positive correlation with grain yield under aerobic conditions using marker-assisted breeding to develop rice varieties better adapted to aerobic conditions.

Materials and methods

Plant material

The experimental material comprised of seed harvested from of MASARB25 x Pusa Basmati 1460 F_2 plants where MASARB25 and Pusa Basmati 1460 was used as a male and female parent, respectively. MASARB25 (developed at IRRI, Manila, Philippines) is a drought tolerant rice genotype developed from IR64/-Azucena/ xx IR64 crosses. Pusa Basmati 1460 is a semi dwarf type plant developed by IARI, New Delhi.

Evaluation of rice genotypes in field and nethouse under aerobic conditions

The crop was raised during kharif season (2012-13) in the net house of Department of Molecular Biology and Biotechnology, CCS Haryana Agricultural University, Hisar and field at CCS HAU, Rice Research Station (RRS), Kaul (Kaithal). F_2 plants were selected on the basis of root and grain yield traits. Seed harvested from the MASARB25 x Pusa Basmati 1460 selected F_2 plants were raised to maturity in pots (25 cm diameter × 25 cm height) with one plant/pot in a net house under aerobic conditions in 2012. The pots were irrigated with 1.0 litres of water after every two days for the first 10 days, and then with 750 ml after every fifth day up to panicle emergence. After every tenth day, the pots were irrigated with full-strength nutrient solution for the first 20 days and with half-strength nutrient solution thereafter.

Seeds obtained from F_2 plants were evaluated in fields at the Rice Research Station, Kaul, during 2012 under dry direct-seeded aerobic cultivation practices. Dry seeding was carried out at approximately 2-cm depth in dry-ploughed and harrowed aerobic plots with row spacing of 30 cm resulting in a seed rate of approximately 342 seeds m^{-2}. The seeds obtained from each selected F_2 plant (10% best and 10% worst (52 plants) were grown in 2-m single-row plots with two replications using a seeding density of 2 g per linear meter of row to record observations for the root study, grain yield, agronomic traits, and genotyping. For the yield trial, the plots were randomized by using Crop Stat version 7.2. Aerobic fields were irrigated for about 1 week with a 2-3-cm water layer to facilitate crop establishment; thereafter, the fields were re-irrigated once at a 10-day interval. Nitrogen was applied at 60-60- 60 kg ha^{-1} in three splits. In addition, 30 kg P ha^{-1}, 40 kg K ha^{-1}, and 5 kg Zn ha^{-1} were applied as basal and 30 kg P ha^{-1} at 25 days after sowing. Manual weeding was done to keep the field weed free. At physiological maturity data were recorded on agronomic traits, plant height (PH, cm), panicle length (PL, cm), effective numbers of tillers per plant (TN), seeds per panicle (S/P), percent seed setting (PSS), GY (Kg ha^{-1}), grain length-breadth ratio (L/B) and 1000 grain weight (GW, g). The data on root morphological traits-root length (RL, cm), root thickness (RT), and fresh and dry root weight (FRW and DRW in g), fresh and dry shoot weight from six plants from each line at maturity were recorded and analyzed. For the measurement of root traits, plants were removed from the soil. 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. For fresh root weight, the roots were blotted gently with a soft paper towel to remove any free surface moisture. Then, the roots were weighed immediately (plants have a high composition of water, so waiting to weigh them may lead to some drying and therefore produce inaccurate data).Then the roots were dried at 70°C for 72 h before weighing for measuring dry biomass.

Genotyping

Genomic DNA was extracted from young leaf samples using the modified CTAB method [15]. DNA quantity was estimated by ethidium bromide staining on 1% agarose gels using a standard containing 100 ng/µl genomic DNA. PCR amplification, denaturing polyacrylamide gel electrophoresis, and silver staining were essentially carried out as described earlier by Jain *et al*. [16].

Statistical methods

Mean and standard error were calculated by using standard procedure. Correlation coefficient was calculated by Pearson Correlation Matrix.

Genetic analysis

A total of 125 mapped SSR and aroma gene-specific *BAD2* markers based on published rice genome maps [17] were screened for polymorphism between the parents. The physical position (Mb) on the *indica* genome (www.gramene.org) was used as a reference. A total of 75 markers showed polymorphism and were run on 50 plants. QTL analysis was performed using Q gene [18]. For interval mapping (IM) analysis, an LOD threshold score of 2.5 was selected. The proportion of the total phenotypic variation explained by each QTL was calculated as R^2 value (R^2 = ratio of the sum of squares explained by the QTL to the total sum of squares). To more accurately determine QTL positions, composite interval mapping (CIM) were performed with default parameters permutation time 5000, significance level 0.05, forward and backward method, walks speed 2 cM, etc.).

Results and discussion

The experiment was carried out to study the field performance of MASARB25 x Pusa Basmati 1460 F_3 plants for various agronomic traits at RRS Kaul, Haryana (North-western India). Experiments conducted under aerobic condition clearly showed that grain yield in aerobic rice variety MASARB25 was significantly higher as compared to the Basmati rice variety Pusa Basmati 1460. In Pusa Basmati 1460 x MASARB25 F_3 population, grain yield ranged between 310-2916 kg ha^{-1} (Pusa Basmati 1460-593 kg ha^{-1} and MASARB25-760 kg ha^{-1}) (Table 1). The above results were supported by Xiaoguang *et al*. [19] which showed that lowland rice variety JD305 (8.8 t ha^{-1}) yielded more than the aerobic rice varieties HD502 (6.8 t ha^{-1}) and HD297 (5.4 t ha^{-1}) under flooded conditions. But, under aerobic conditions, the aerobic varieties yielded higher than the lowland variety.

Table 1. Range for various physio-morphological traits in MASARB25 x Pusa Basmati 1460 F_3 population grown under aerobic conditions in the field

Trait	Pusa Basmati 1460		MASARB25		MASARB25 x Pusa Basmati 1460	
	Mean	Range	Mean	Range	Mean	Range
PH (cm)	92.2±0.70	82.0-102.5	79.3±1.05	64.0-92.0	85.7±0.84	63.0-115.0
TN	13.0±0.71	8.0-16.0	16.0±0.58	13.0-19.0	12.9±0.72	4.0-35.0
PL (cm)	25.0±0.33	23.0-27.8	21.5±0.38	18.8-24.0	24.6±0.55	19.0-29.0
SPP	96.0±3.80	45.0- 160.0	82.3±2.73	54.0-127.0	85.7±0.88	52.0-145.0
PSS	68.4±1.50	43.0-83.3	84.1±0.88	70.0-97.3	76.0±0.65	58.1-90.1
L/B	5.09±0.22	4.02-5.69	4.08±0.12	3.72-4.51	4.47±0.72	3.9-6.0
1000 GW(g)	23.0±0.49	19.0-25.5	25.4±0.61	17.5-28.0	25.1±0.56	19.0-32.5
GY (kg ha^{-1})	593±90.0	383 - 793	760±100.7	536-887	830±81.7	310-2916

PH=Plant height, TN=Effective number of tillers, PL=Panicle length, SPP=Seeds per panicle, PSS=Percent seed setting, L/B=Length/breadth ratio, GW=Grain weight and GY=Grain yield

Results clearly showed that root length and biomass in aerobic rice variety MASARB25 was significantly higher compared to the Basmati rice variety Pusa Basmati 1460. Aerobic rice variety MASARB25 had 9.32% higher root length, 11.76% higher fresh root weight, 19.98% higher dry root weight as compared to Pusa Basmati 1460 under aerobic conditions (Table 2). Fukai and Cooper [20] reported that under low-moisture stress, root characters are considered to be a vital component to fight against mechanism since they contribute to regulation of plant growth and extraction of water and nutrients from deeper layers. Several components of root morphology contributing to drought tolerance have been identified [21].

Correlation analysis was carried out to identify how root morphological characters influence the grain yield and yield morphological traits under aerobic conditions. Phenotypic correlation coefficient analysis (Table 3) of MASARB25 x Pusa Basmati 1460 F_3 population revealed a positive correlation between grain yield with fresh root weight (0.266, p=0.05), dry root weight (0.282, p=0.01), root thickness (0.321, p=0.01). It indicates the role of root traits for improving yield under aerobic situations possibly through improved water and nutrient uptake. This shows that a well developed root system will help the plant in maintaining high plant water status which ultimately leads to increase in yield potential under water deficit conditions.

Genotypic analysis of mapping population

Several markers have been identified to be linked with aerobic root traits/drought tolerance in rice [22]. The marker used in the present investigation RM302 on chromosome 1 and RM256, RM310 and RM547 on chromosome 8 and RM205 on chromosome 9, this region earlier has been found to be associated with biomass, deep root mass, leaf drying, relative water content, osmotic adjustment, basal root thickness, tiller number and deep root to shoot ratio, grain yield, panicle length, canopy temperature and root length [23-26].

Several QTLs associated with root traits and agronomically important traits in the mapping population were detected in the MASARB25 x Pusa Basmati 1460 (Table 4) population. A total of 15 QTLs associated with 10 traits were mapped on chromosomes 2, 4, 6, 8, 9, and 11. $qGY_{8.1}$ with an R^2 value of 36.3% and $qGY_{2.1}$ with an R^2 value of 29% (Fig. 1) and $qRL_{8.1}$ with an R^2 value of 27.2% were identified for root length under aerobic conditions. For the MASARB25 × Pusa Basmati 1460 population on chromosome 8 in a region of 60.4-68.8 cM, QTLs for root length and GY and in a region of 100-102 cM QTLs were reported to be significantly associated with different root traits and agronomically important traits (RT, FRW, DRW and 1000 GW). These

Table 2. Variation for root morphology and physio-morphological traits in MASARB25 x Pusa Basmati 1460 F_3 plants grown under aerobic conditions in pots in the net house

Trait	Pusa Basmati 1460		MASARB25		MASARB25 x Pusa Basmati 1460	
	Mean	Range	Mean	Range	Mean	Range
PH (cm)	90.2±0.23	88.0-94.0	78.0±0.12	77.0-81.0	84.9±0.78	51.0-107.0
TN	13.0±0.40	12.0-16.0	15.0±0.38	13.0-17.0	9.4±0.56	6.0-14.0
PL (cm)	23.3±0.45	21.0-24.5	19.3±0.23	17.0-20.2	23.8±0.77	18.0-31.0
SPP	86.4±0.34	85.0-89.2	77.4±0.14	75.0-79.5	90.5±0.88	52.0 -157.0
PSS	66.9±0.41	64.0-71.0	82.2±0.28	79.0-85.0	74.5±0.44	60.0-89.8
L/B	4.90±0.11	5.03-5.78	3.92±0.07	3.70-4.06	4.62±0.23	3.69-5.78
1000 GW (g)	19.5±0.28	17.8-21.2	21.8±0.38	18.5-24.4	22.7±0.60	17.0-30.0
GY (kg ha^{-1})	506±90.0	13.9-17.3	607±100	17.2-21.2	480±71.0	110-967
RL (cm)	33.6±1.68	24.0-51.0	41.5±0.66	40.0-54.0	31.2±1.20	15.0-56.0
RT (mm)	21.7±1.13	14.3-28.4	23.4±0.35	23.0-30.5	25.3±0.77	11.1-36.7
FRW(g)	20.5±1.97	7.3-34.2	26.1±0.64	24.1-35.4	28.1±2.01	4.6-88.1
DRW (g)	8.2±1.32	2.5-4.5	12.3±0.31	11.1-16.3	9.7±2.20	1.6-43.8

PH=Plant height, TN=Effective number of tillers, PL=Panicle length, SPP=Seeds per panicle, PSS=Percent seed setting, L/B=Length/breadth ratio, GW=Grain weight, GY=Grain yield, RL=Root length, RT=Root thickness, FRW=Fresh root weight and DRW=Dry root weight

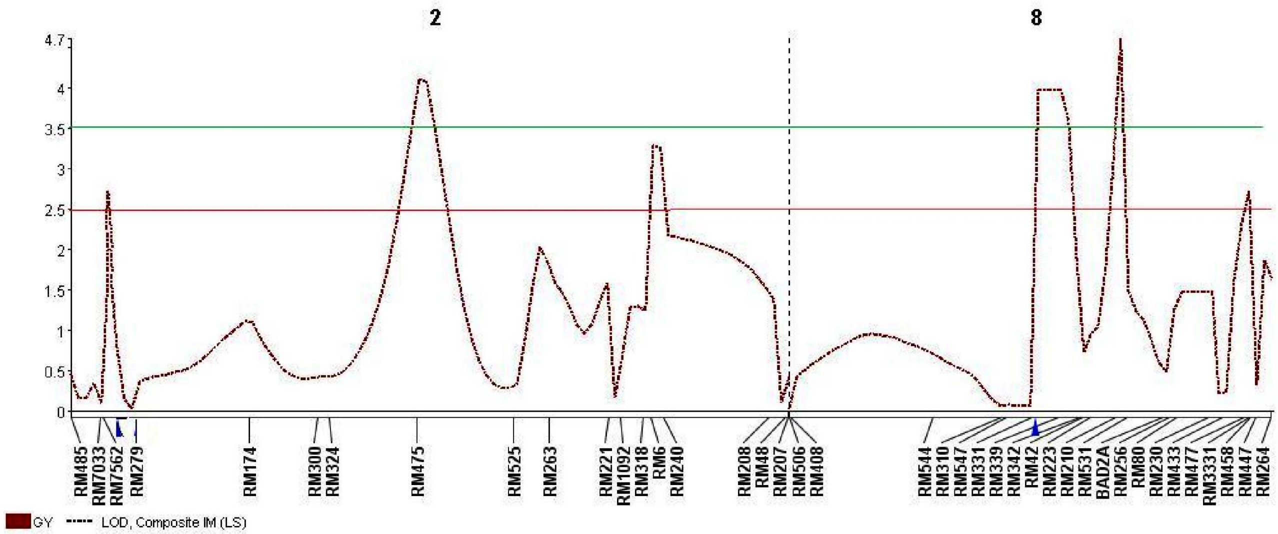

Fig. 1. QTL likelihood curves of LOD score of GY QTL on chromosome 2 and 8 in MASARB25 × Pusa Basmati 1460 F_3 population

coexisting chromosomal regions governing root and yield traits provide a unique opportunity for breeders to introgress such regions together as a unit into lowland varieties through MAS/MAB and to develop cultivars possessing higher yield and increased adaptation to aerobic conditions. Qu *et al*. [22] reported QTL for fresh root weight and root number coinciding with $qRL_{8.1}$ and $qGY_{8.1}$ linked to marker RM331. These QTL may confer a grain yield advantage under direct-seeded conditions and this is supported by the earlier reported large-effect consistent QTL $qDTY_{8.1}$ in Basmati334/Swarna [27] for grain yield under drought and for relative yield and drought susceptibility index in IR64 × Binam-derived NILs under drought stress [28].

In the present study, we reported QTLs associated with 1000 grain weight and root length on chromosome 6 between RM204 and RM527, indicating that this is an important region in governing root traits and improving yield under aerobic conditions. In this region a large-effect QTL ($qDTY_{6.1}$) associated with grain yield in aerobic environments was identified in a total of 20 hydrological environments over a period of five seasons and in five populations in three genetic backgrounds using bulk-segregant analysis by Venuprasad *et al*. [29].

Our study identified a number of promising lines with higher yield, better root traits and with Pusa Basmati 1460-specific alleles in homozygous or heterozygous condition at the *BAD2* locus. These lines will serve as novel materials for the selection of stable aerobic Basmati rice varieties. Although the effect of the identified QTL needs to be validated in different genetic backgrounds, under the present scenario, successful introgression of the identified QTL following marker-assisted backcrossing can be beneficial for increasing productivity and aerobic adaptation of rice.

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