



Phenotyping, microsatellite marker analysis and linkage mapping of QTL for agronomic and root traits using IB370 × MAS-ARB25 F₂ rice (*Oryza sativa* L.) population grown under aerobic conditions

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Received 23 July 2020; revised & accepted 1 October 2020

Expanding water shortage, environmental change and decline water table level are the major hindrance for lowland basmati rice variety development in northern parts of India including Haryana. Rice is the absolute most client of fresh irrigated water as it devours 70% of the complete water accessible. More rice is needed to generate to take care of the expanding populace. The promising way to deal with battle the water shortage can be aerobic rice. Aerobic rice is a budding cultivation system that requires no puddling, no transplanting and without need of frequent irrigation than conventional flooded rice. The worth of basmati rice breeding can significantly improve through marker assisted selection (MAS). In the present study, experiments were conducted to assess F₂ generation obtained from IB370 × MAS-ARB25 for various agronomic qualities. Grain yield/plant indicated impressive positive relationship ($r = 0.25$) with root thickness in F₂ population. Fifty eight polymorphic SSR markers dispersed on the entire genome of rice were utilized for planning DNA fingerprint data set of the isolating IB370 × MAS-ARB25 F₂ population. Composite interval mapping analysis by WinQTL cartographer version 2.5 revealed a sum of 7 quantitative trait loci (QTLs) (three QTL for agronomic traits and four for root traits). The selected promising F₂ plants were additionally checked for these putative QTLs recognized in the F₂ populace, which were available in homozygous/heterozygous state in high frequencies.

Keywords: QTL, aerobic rice, molecular marker and water scarcity

Introduction

Rice (*Oryza sativa* L.) is prevalently known as worldwide grain since it serves for the greater part of the total populace¹. Water insufficiency, climate change, decreasing water-table level and rising manual work cost are the major causes of less production of irrigated rice². Rice production is becoming challenging due to these hurdles. By 2050, fresh water requirement is expected to increase by 55% worldwide³⁻⁴. There is a strong need to increase the food production by 60% globally and 100% in the developing countries of the world. Traditional rice production system utilized two to three times more fresh water in comparison to other cereals that leads to devastation of soil aggregates and reduction in macro pore volumes⁵. So there is strong necessity of switching from traditional way to produce rice to more suitable and better adopted approach. To save the

human being from hunger arising due to water shortage, only answer of this major challenge can be the concept of aerobic rice⁶. Aerobic rice is a water-saving rice growing system which is established by direct seeding grown in non-puddled and non-saturated soil conditions⁷. Aerobic rice has an efficient root system by means of root length, thickness and greater root penetration capacity and hence, can save half of irrigation water (up to 50%) as compared to lowland rice⁸. Apart from this, in aerobic rice flowering occurs prior resulting the lesser crop period and matures 7 - 10 days before the transplanted rice⁹.

During the path of evolution, the rice has differentiated into numerous varieties, but the consumer priority is basmati rice due to its aromatic nature and grain elongation after being cooked. The necessity for scented rice has been increasing in recent past years in rice producing countries due to reason of aroma, flavor and grain texture. It is upper most paid rice throughout the globe due to its better-quality grain. India exported an amount of 4.45 million tonnes of

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basmati rice with worth of US dollar 4.33 billion in financial year 2019-20. Basmati rice is cultivated at about 463.6 thousand hectares of land in year 2019 in the entire country which was 84% upper than the previous year, data provided by Agricultural and Processed Food Export Development Authority (APEDA). To achieve more production under water limited conditions it requires new varieties of aerobic rice combining with water stress / drought tolerance and high yielding basmati rice varieties. Marker-assisted selection may assist to figure out the genetic construction and to build up new cultivars with improved root characters. The potential of simple sequence repeats (SSRs) makes them preferable for QTL planning, genetic examination and MAS for plant improvement methodologies. Several QTLs governing root qualities across the populations have been reported in rice¹⁰⁻¹². Advancement in breeding program for water stress/drought tolerance is quite slow. So we need to identify more stable QTLs for various agronomic and root qualities that can boost the production of direct seeded rice cultivation under aerobic conditions. Keeping in mind the above points the current study was planned to introduce basmati rice aromatic character in the aerobic rice variety and to identify the QTLs linked to trait upgrading aerobic adaption in water limited field conditions.

Materials and Methods

Plant Material

Improved Basmati 370, lowland aromatic rice cultivars which is non-adapted to aerobic condition procured from Punjab Agricultural University (PAU), Ludhiana, India and MAS-ARB-25, aerobic rice variety, developed by International Rice Research Institute (IRRI), Manila, Philippines used in the present study.

Field Study

The crop was raised during kharif season in field at CCS Haryana Agricultural University, Rice Research Station, Kaul (Kaithal). Seeds harvested from the selected IB370 × MAS-ARB-25 derived F₁ plants were sown by direct seeded method under aerobic conditions in field. The recommended agronomic package and practices were followed for growing a good crop. In the field, seeds were direct seeded at 1 - 2 cm depth with a row spacing of 20 cm and plant to plant distance of 15 cm. Plants were irrigated at an interval of five days up to panicle emergence and after panicle emergence at an interval of three days.

Net House Study

An aggregate of 94 plants got from IB370 × MAS-ARB25 F₂ generation were grown in single plant per pot in the net house during season of kharif. The plants were irrigated on regular basis with one litre of water for first fifteen days after sowing the seeds and later on with same quantity after third day till appearance of panicle. The records for root morphological characters such as root length, root thickness, dry and fresh root weight was noted in the F₂ plants after attaining the maturity. For taking the root data the pots were flooded with water, roots were taken out without shattering the pot and washed carefully without damaging the root structure.

Data on various physio-morphological and root morphology traits such as plant height, effective number of tillers/ plant, panicle length, 1000 grain weight, grain yield/plant, l/b ratio of grain, root length, root thickness, fresh root weight and dry root weight was recorded for IB370 × MAS-ARB25 derived 230 F₂ plants. The data was subsequently analyzed to determine the variability, correlation coefficient analysis and frequency distribution curve for various physio-morphological and root traits. Genomic DNA was isolated from leaf samples of 2-3 week old seedlings using cetyl trimethyl ammonium bromide (CTAB) method¹³ and followed by agarose gel electrophoresis to check both quantity and quality of genomic DNA. Four percent polyacrylamide gels were used for the amplified products to resolve by electrophoresis¹⁴ WinQTL cartographer version 2.5 was used to map QTL in the present investigation. The map positions (cM) of all the SSR markers in IB370 × MAS-ARB25 derived F₂ plants under investigation were assessed from the website gramene (<http://www.gramene.org>). The scoring of SSR allelic data was done as per instructions given in the manual of WinQTL cartographer 2.5.

Primers for Analysis of Aroma in Rice

An eight base pair deletion and three single nucleotide polymorphism (SNPs) in exon 7 of the gene encoding betaine aldehyde dehydrogenase (BAD) 2. Specific primers for *BAD2A* genes¹⁵ situated on chromosome 8 of *Oryza sativa* was found to be cause of fragrance in basmati rice were used for assessing the diversity between basmati and non-basmati rice varieties (Table 1). The polymorphism in this gene provides an opportunity to differentiate between aromatic and non-aromatic rice species. This quality makes it a perfect marker for fragrance in rice.

Results

Field Evaluation of IB370 × MAS-ARB25 F₂ Population Under Direct-Seeded Aerobic Field Conditions

Data was taken on various physio-morphological traits for IB370 × MAS-ARB25 derived F₂ population under direct-seeded aerobic field (230 plants) and water limiting net house conditions (94 plants) along with parental rice varieties. Enormous variation was observed for all the agronomic traits in F₂ population grown under direct-seeded aerobic field (Table 2) and net house (Table 3) conditions.

Correlation Coefficient Analysis Under Direct-Seeded Aerobic Field Conditions

Phenotypic correlation coefficient analysis of IB370 × MAS-ARB25 derived F₂ population showed

positive correlation between grain yield/ plant and plant height (0.313, p=0.01), effective number of tillers/ plant (0.657, p=0.01), panicle length (0.343, p=0.01) and 1000 grain weight (0.458, p=0.01) (Table 4). A positive correlation was also found between plant height with panicle length (0.403, p= 0.01) and 1000 grain weight (0.309, p=0.01), while negative correlation was observed between L/B ratio and plant height (-0.346, p=0.01).

Frequency Distribution for Agronomical and Root Traits

Figure 1 depicts the frequency distribution curves for various agronomical and root traits of IB370 × MAS-ARB25 derived F₂ population and parental rice genotypes. Frequency distribution curve for plant height, panicle length and grain length/breadth ratio was skewed towards IB370. Frequency distribution curves were parabolic and slightly tilted towards MAS-ARB25 for effective number of tillers and grain yield/plant. The 1000 grain weight, root length and root thickness frequency distribution curves, were slightly inclined towards MAS-ARB25.

Variation in Allelic Profile at *BAD2A* Locus

Improved basmati rice variety, 370 (IB 370) amplified two alleles of 585 and 257 bp, while MAS-ARB25 amplified 585 and 355 bp size alleles. Thirty

Table 1 — Primers for fragrance.

Primer name	Primer Sequence
External sense primer (ESP)	TTGTTTGGAGCTTGCTGATG
Internal fragrant antisense primer (IFAP)	CATAGGAGCAGCTGAAATATATACC
Internal non-fragrant sense primer (INSP)	CTGGTAAAAAGATTATGGCTTCA
External antisense primer (EAP)	AGTGCTTTACAAAGTCCCGC

Table 2 — Mean and range for agronomic traits in Improved Basmati 370 × MAS-ARB25 F₂ population grown under direct-seeded aerobic field conditions.

Trait	Improved Basmati 370	MAS-ARB25	IB370 × MAS-ARB25 F ₂ population (230 plants)	
			Range	Mean
Plant height (cm)	108.5 ± 0.56	90.5 ± 0.38	78 - 145	111.5
Effective no. of tillers/ plant	7.5 ± 0.43	10.0 ± 0.75	2.0 - 20	11.0
Panicle length (cm)	25.6 ± 0.22	23.1 ± 0.07	16.3 - 29.6	22.9
1000-grain weight (g)	20.5 ± 0.12	20.9 ± 0.18	17.2 - 26.1	21.6
Grain yield/ plant (g)	8.8 ± 0.34	9.0 ± 0.61	2.0 - 43.6	22.8
L/B ratio	4.9 ± 0.11	4.2 ± 0.06	3.5 - 5.5	4.5

Table 3 — Mean and range for agronomic traits and root traits in Improved Basmati 370 × MAS-ARB25 F₂ plants grown under aerobic conditions in net house.

Trait	Improved Basmati 370	MAS-ARB25	IB370 × MAS-ARB25 F ₂ plants (94 plants)	
			Range	Mean
Plant height (cm)	86.5 ± 0.37	85 ± 0.31	46 - 121	83.5
Effective no. of tillers/plant	3.5 ± 0.12	2.5 ± 0.14	1.0 - 9.0	5.0
Panicle length (cm)	20.5 ± 0.23	20.5 ± 0.16	15.1 - 30.6	22.9
Grain yield/ plant (g)	1.8 ± 0.14	1.2 ± 0.72	0.2 - 10.8	5.5
1000-grain weight (g)	13.9 ± 0.54	13.6 ± 0.23	9.0 - 17.4	13.2
L/B ratio	3.9 ± 0.12	3.9 ± 0.16	3.1 - 4.9	4.02
Root length (cm)	31.5 ± 0.31	38.5 ± 0.34	31 - 65	48.0
Root thickness (mm)	14.8 ± 0.36	16.6 ± 0.38	10.2 - 32.0	21.1
Fresh root weight (g)	3.1 ± 0.22	5.8 ± 0.44	2.1 - 33.1	17.6
Dry root weight (g)	1.0 ± 0.28	1.6 ± 0.80	0.7 - 11.8	6.3

Table 4 — Phenotypic correlation coefficients among agronomic traits of Improved Basmati 370 × MAS-ARB25 F₂ population grown under direct-seeded aerobic field conditions.

Trait	Plant height (cm)	Effective no. of tillers/ plant	Panicle length (cm)	1000-grain weight (g)	Grain yield/ plant (g)	L/B ratio
Plant height (cm)	1					
Effective no. of tillers/ plant	-0.105 ^{NS}	1				
Panicle length (cm)	0.403 ^{**}	-0.044 ^{NS}	1			
1000-grain weight (g)	0.309 ^{**}	0.117 ^{NS}	0.311 ^{**}	1		
Grain yield/ plant (g)	0.313 ^{**}	0.657 ^{**}	0.343 ^{**}	0.458 ^{**}	1	
L/B ratio	-0.346 ^{**}	0.109 ^{NS}	-0.122 ^{NS}	0.009 ^{NS}	-0.118 ^{NS}	1

*Significant at 5%, ** Significant at 1% level

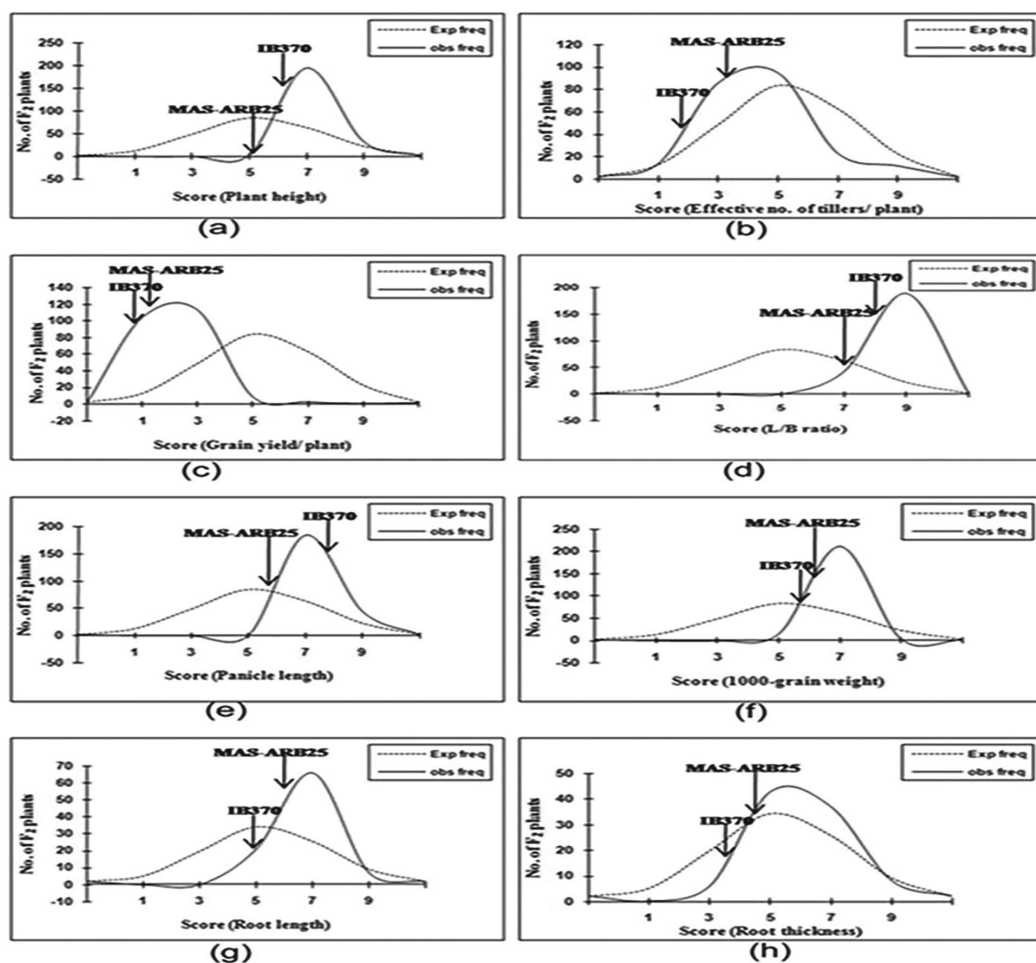


Fig. 1 — Frequency distribution curves for agronomic and root traits of IB370 × MAS-ARB25 F₂ population grown under aerobic condition in the field (a-f) and net house (g & h).

two selected IB 370 × MAS-ARB25 F₂ plants consisted of three types of plants: (i) plants with 585 and 257 bp alleles, (ii) plants with 585 and 355 bp alleles and (iii) plants heterozygous at *BAD2A* locus with 585, 355 and 257 bp alleles. A total of 67 IB370 × MAS-ARB25 F₂ plants from the net house were screened for *BAD2A* locus had a ratio of 9:23:35 for

homozygous fragrant, heterozygous non-fragrant and homozygous non-fragrant plants, respectively. A total of 32 plants (Table 5) having IB 370 specific allele at *BAD2A* locus (9 plants in homozygous and 23 plants in heterozygous condition for aromatic gene provides fragrance) were selected for further molecular marker analysis (Fig. 2).

Microsatellite Marker Analysis of IB370 × MAS-ARB25 F₂ Plants

A total of 109 microsatellite or SSR markers broadly dispersed on twelve chromosomes of rice were used in the present investigation. Total 109 SSR markers analyzed for polymorphism in IB370 and MAS-ARB25 (two parental rice genotypes), out of which only 58 were found polymorphic. Subsequently, a DNA fingerprint dataset of preferred aromatic 32 IB370 × MAS-ARB25 F₂ plants was prepared using 58 SSR markers. A total of 117 alleles were identified in the selected 32 F₂ plants by using 58 SSR markers. One of 58 SSR markers, RM334 amplified new (recombinant) allele which was not present in two parental rice varieties and was different. Sixteen out of 32 selected F₂ plants showed recombinant allele at one locus. Polyacryamide gels

displaying allelic polymorphism in IB370 × MAS-ARB25 F₂ plants for some SSR markers are shown in (Fig. 3). On an average, 50% alleles were from IB370 and 50% alleles were from MAS-ARB25 in all selected 32 F₂ plants.

Genetic Relationship Among IB370 × MAS-ARB25 F₂ Plants
Similarity coefficient data based on the 58 microsatellite markers was used to calculate the correlation coefficient values among the selected 32 IB370 × MAS-ARB25 derived F₂ plants and parental rice genotypes. Subsequently, the diversity data of allelic polymorphism was used to construct dendrogram (cluster tree analysis) through NTSYS-pc software to reveal the genetic relationship among selected 32 F₂ plants and the parents (Fig. 4). All the selected 32 F₂ plants forms two major groups at 0.58

Table 5 — Data on agronomic, root traits and allelic profile at *BAD2A* locus in Improved Basmati 370 × MAS-ARB25 F₂ plants selected for molecular analysis grown under aerobic conditions in pot house.

Plant no.	Plant height (cm)	Effective no. of tillers/plant	Panicle length (cm)	1000-grain weight (g)	Grain yield/plant (g)	L/B ratio	Root length (cm)	Root thickness (mm)	Fresh root weight (g)	Dry root weight (g)	Status at <i>BAD2A</i> locus
1	75	4	20.7	12.8	2.55	3.97	48	16.34	17.24	3.1	1
2	92	3	24.3	13.15	1.26	4.05	57	16.25	20.94	3.65	2
7	87	7	19.8	16.95	5.56	3.50	42	19.39	24	3.86	1
8	73	6	18.3	11.31	0.82	3.35	45	18.21	9.37	2.66	2
10	88	5	21.3	15.4	5.64	3.43	43	23.91	11.12	3.27	2
11	83	4	21.7	12.48	0.72	3.66	47	18.78	17.13	5.03	1
12	92	4	20.70	14.65	2.63	4.41	44	16.81	9.32	2.37	1
28	78	2	21.8	12.25	0.8	3.35	46	18.53	20.34	4.6	1
31	79	3	21.9	14.25	3.14	4.48	51	21.67	11.56	3.4	1
32	94	3	24.0	14.61	2.13	4.32	41	18.17	10.53	4.38	2
38	81	3	26.8	12.2	1.52	3.63	44	17.08	14.56	2.7	1
40	80	3	21.5	12.55	1.29	3.78	46	18.78	6.86	2.17	2
41	57	3	18.4	11.4	0.38	3.50	48	17.57	4.8	1.54	2
42	82	3	19.7	13.37	2.36	4.07	44	15.83	8.08	2.55	1
49	87	3	20.9	14.35	2.25	3.56	41	22.67	12.47	4.01	1
56	89	3	21.8	14.38	2.55	4.20	42	18.86	12.11	3.92	1
58	62	3	20.4	11	0.33	3.61	51	27.48	31.73	9.0	1
60	81	3	16.3	10.4	0.29	3.73	48	23.69	22.18	6.72	1
63	92	9	20.1	16.8	10.8	4.06	53	26.71	27.35	8.66	1
64	66	4	20.9	12.05	1.02	3.69	47	20.44	16.23	5.63	1
65	92	8	23.6	17.1	5.48	3.62	53	28.77	33.05	11.82	1
69	105	3	29.3	13.75	2.39	4.15	38	17.51	6.62	2.47	2
72	66	3	22.0	13.19	1.35	4.08	47	14	3.85	1.74	1
76	70	2	22.8	15.38	2.53	3.57	38	18.32	6.11	2.34	2
77	78	3	19.6	14	1.31	4.50	47	11.87	4.01	1.31	1
81	113	5	22.1	15.81	4.12	4.71	39.5	19.78	12.86	4.13	1
89	96	3	22.4	12.15	1.39	4.21	37.5	18.91	12.16	3.39	1
91	58	5	19.8	14.3	2.16	3.99	48	21.12	12.2	3.47	2
92	66	3	20.6	13.14	0.61	4.25	39	20.47	9.58	2.73	1
94	92	2	27.3	14.3	0.63	4.27	36	18.16	7.13	2.01	1
96	68	4	18.6	11.45	0.32	3.83	44	21.52	8.48	1.73	1
97	86	6	21.8	16.31	3.47	4.07	43	21.11	13.79	5.66	1

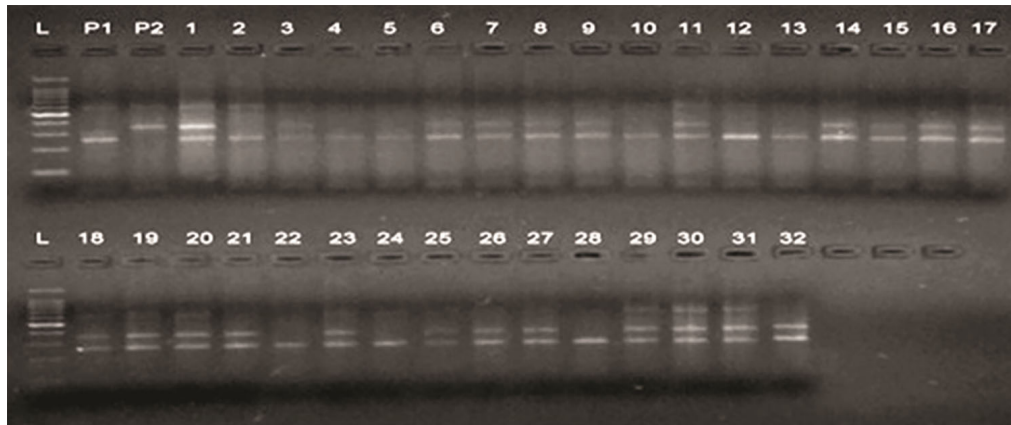


Fig 2 — Agarose gel displaying allelic status at BAD2A locus in IB370 × MAS-ARB25 derived 32 F₂ plants and parent rice varieties. Lane L-100 bp ladder, P1-IB370, P2-MAS-ARB25, 1-32: 32 selected F₂ plants.

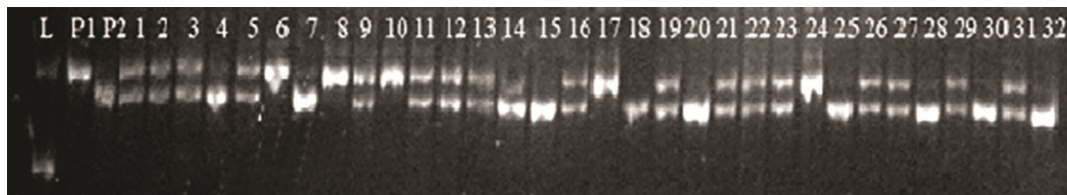


Fig 3 — Polyacrylamide gel showing allelic polymorphism among 32 selected F₂ plants and parental genotypes at RM84 locus. Lane L-20 bp ladder, P1-IB370, P2-MAS-ARB25, 1-32: 32 selected F₂ plants.

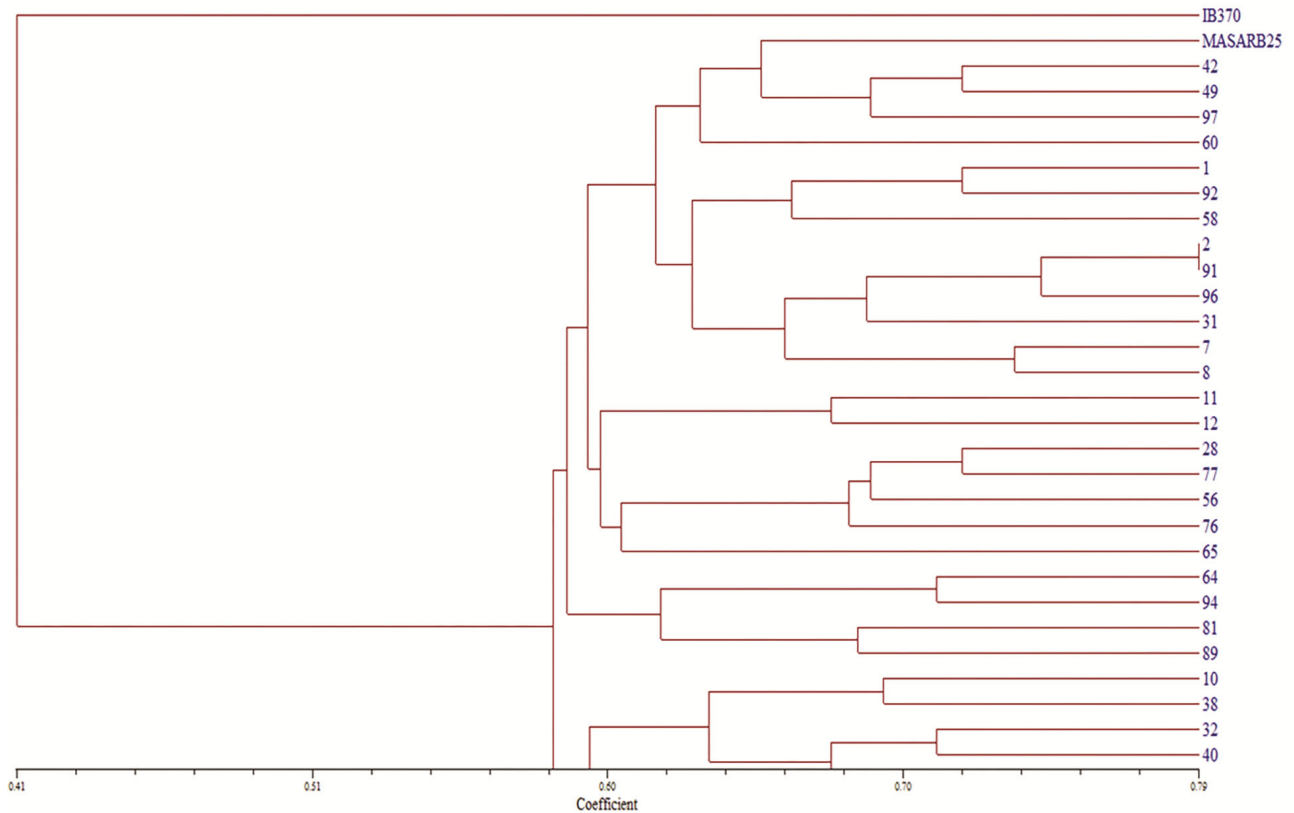


Fig. 4 — Dendrogram (NTSYS-PC) display diversity among 32 selected F₂ plants (IB370 × MAS-ARB25) and parental genotypes, using allelic diversity data at 58 SSR loci.

similarity coefficient value. Major group I consisted of IB370 while the major group II had MAS-ARB25 and selected 32 F₂ plants. The parent rice varieties, IB370 and MAS-ARB25 had low similarity coefficient and bifurcate at 0.58 similarity coefficient. Genetic relationships among these rice genotypes were also assessed by principal component analysis (PCA) using NTSYS-pc software. Two dimensional PCA scaling exhibited that F₂ population was interspersed between the two parental lines with inclination towards MAS-ARB25 (Fig. 5).

QTL Mapping for Agronomic Traits

Total 58 polymorphic SSR markers dispersed on twelve chromosomes of rice were used to map the QTL associated with agronomic traits in rice using selected 32 IB370 × MAS-ARB25 F₂ plants (Table 6). Composite interval mapping (CIM) analysis by

WinQTL cartographer version 2.5 revealed three QTL for agronomic traits in rice. In cross IB370 × MAS-ARB25, though size of the population was small, QTL identified in the present study should be considered as putative QTL and need to be confined using a large population.

One QTL associated with 1000 grain weight (*qTGW_{4.1}*) at map position of 15.0 cM, on chromosome 4. The *qTGW_{4.1}* had negative values for additive effect (-0.40) indicated that the favouring alleles for 1000 grain weight were from IB370 parent. Two QTL associated with grain yield/plant (*qGYP_{2.1}* and *qGYP_{5.1}*) at map positions of 127.6 and 91.7 cM, on chromosomes 2 and 5 respectively. Both the QTLs had negative values for additive effect (-2.34 and -0.40) indicated that the favouring alleles for grain yield/plant were from the parent IB370 (Fig. 6).

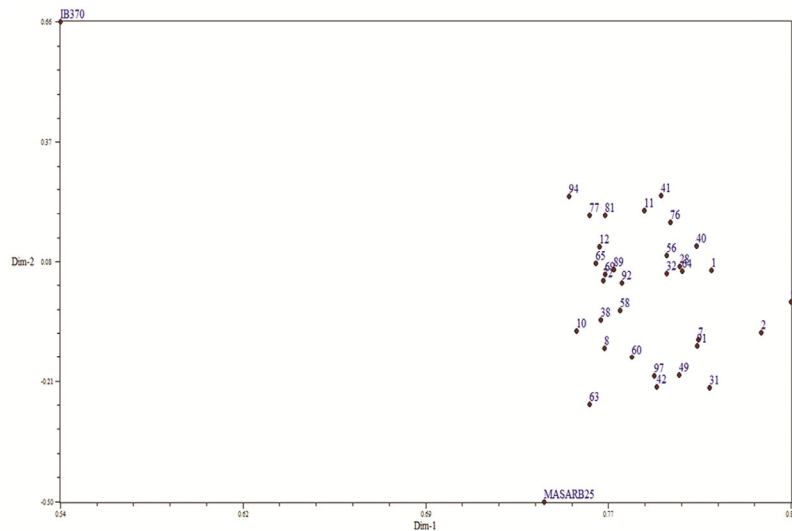


Fig. 5 — Two dimensional PCA scaling display diversity among 32 selected F₂ plants (IB370 × MAS-ARB25) and parental genotypes, using allelic diversity data at 58 SSR loci.

Table 6 — QTL identified for agronomic and root traits by microsatellite marker analysis of IB370 × MAS-ARB25 F₂ population.

Trait	QTL name	Chromosome number	Position (cM)	Flanking markers	Position of flanking markers (cM)	LOD	Additive effect	R ² %	DPE
1000 grain weight	<i>qTGW_{4.1}</i>	4	15.0	RM307 - RM177	0.0 - 50.8	2.9	-0.40	12.49	IB
Grain yield/ plant	<i>qGYP_{2.1}</i>	2	127.6	RM211 - RM213	14.4 - 186.4	4.8	-2.34	12.86	IB
	<i>qGYP_{5.1}</i>	5	91.7	RM291 - RM440	78.7 - 92.7	3.2	-0.40	19.05	IB
Root length	<i>qRL_{5.1}</i>	5	118.8	RM31	118.8	3.9	0.98	4.22	MA
Fresh root weight	<i>qFRW_{5.1}</i>	5	89.8	RM291 - RM440	78.7 - 92.7	2.8	2.35	2.02	MA
	<i>qFRW_{5.2}</i>	5	97.7	RM440 - RM31	92.7 - 118.8	2.6	2.69	2.11	MA
Dry root weight	<i>qDRW_{5.1}</i>	5	94.8	RM440 - RM31	92.7 - 118.8	3.5	0.21	2.85	MA

DPE (direction of phenotypic effect); MA and IB indicate “MAS-ARB25” and “Improved Basmati 370” alleles, respectively. R² is phenotypic variation, Additive effect is half the difference between effect of “MAS-ARB25” allele and “Improved Basmati 370” allele; its positive value indicates that the effect of genotype “MAS-ARB25” was in the direction of increasing the trait value, LOD-log₁₀ of an odd ratio.

QTL Mapping for Root Traits

Fifty eight polymorphic SSR markers distributed on twelve rice chromosomes were used to map the QTL associated with root traits using selected 32 IB370 × MAS-ARB25 derived F₂ plants (Table 6). CIM analysis by WinQTL cartographer version 2.5 exposed four QTL for root traits on chromosome number 5 in rice (Fig. 7).

Monitoring of Putative QTL Promoting Aerobic Adaptation in Selected F₂ Plants

The putative QTL identified in F₂ populations of IB370 × MAS-ARB25, for various agronomic and root traits in the present study, were also checked

in the selected promising F₂ plants. QTL for grain yield/plant (*qGYP_{5.1}*), fresh root weight (*qFRW_{5.1}* and *qFRW_{5.2}*) and dry root weight (*qDRW_{5.1}*) present in four promising IB370 × MAS-ARB25 F₂ plants (homozygous state) selected on the basis of evaluation of plants grown in net house, were contributed by donor parent MAS-ARB25, while QTL for root length (*qRL_{5.1}*) in four promising F₂ plants were governed by both the parents (heterozygous state) (Table 7).

Discussion

Water particularly, is quick turning into a valuable ware, as an ever increasing number of individuals

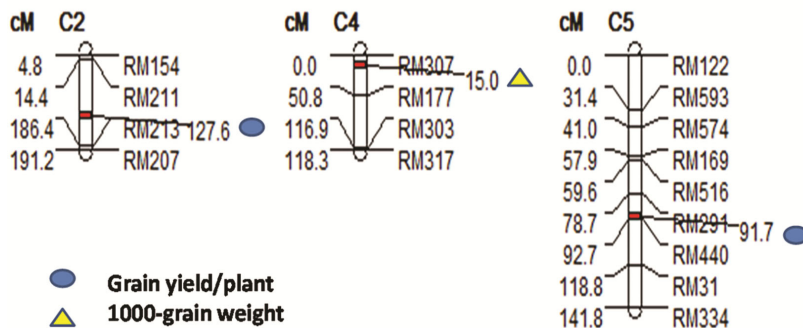


Fig. 6 — Chromosomal locations of quantitative trait loci (QTL) for grain yield and 1000-grain weight per plant identified in F₂ population.

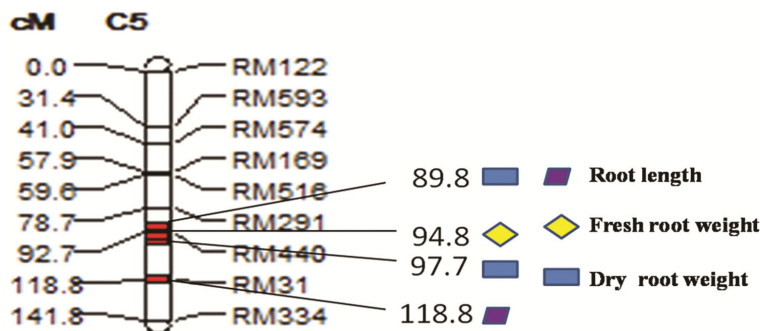


Fig. 7 — Chromosomal locations of quantitative trait loci (QTL) for root traits identified in F₂ population derived from a cross between IB370 × MAS-ARB25.

Table 7 — Monitoring of putative QTL promoting aerobic adaptation in selected IB370 × MAS-ARB25 F₂ plants.

QTL	Chr. No.	Flanking markers (position cM)	QTL present in F ₂ plants					
			IB370	MAS-ARB25	2	31	63	65
<i>qTGW_{4.1}</i> *	4	RM307-RM177 (0.0 - 50.8)	+ -	- +	- +	+ -	- +	- +
<i>qGYP_{2.1}</i> *	2	RM211-RM213 (14.4 - 186.4)	+ -	- +	++	+ -	+ -	+ -
<i>qGYP_{5.1}</i> *	5	RM291-RM440 (78.7 - 92.7)	+ -	- +	- +	- +	- +	- +
<i>qRL_{5.1}</i> **	5	RM31 (118.8)	+ -	- +	++	++	++	++
<i>qFRW_{5.1}</i> **	5	RM291-RM440 (78.7 - 92.7)	+ -	- +	- +	- +	- +	- +
<i>qFRW_{5.2}</i> **	5	RM440 - RM31 (92.7 - 118.8)	+ -	- +	- +	- +	- +	- +
<i>qDRW_{5.1}</i> **	5	RM440 - RM31 (92.7 - 118.8)	+ -	- +	- +	- +	- +	- +

* QTL from IB370, ** QTL from MAS-ARB25; the status of allelic profile at this marker (bold one) was used to access presence of respective putative QTL

keep utilizing water for the family, industry, and farming. Expanding water shortage is the serious issue that restricts the adoption of high-producing rice varieties (HYVs) in rain-fed environments. The purposes behind water shortage are different and region specific, which incorporates diminishing water quality, diminishing resources and expanded rivalry from metropolitan users. To handle the present circumstance, it becomes very essential to create substitute methods of rice development that require less water with no decrease in grain yield and quality. Aerobic rice is an arising agronomical creation that utilizes less water than ordinary irrigated rice. In aerobic rice development, where fields stay unsaturated all through the season like an upland crop, offers a chance to create more rice with less water¹⁷.

Assessment of IB370 × MAS-ARB25 F₂ Plants Under Direct Seeded Aerobic Field and Net House Conditions

Huge variety was seen among IB370 × MAS-ARB25 resultant F₂ populations for various agronomic and root qualities. The trial led under direct seeded aerobic field conditions clearly indicated that grain yield in aerobic rice variety; MAS-ARB25 was significantly higher as compared to basmati rice variety, IB370. The various root morphological traits data collected clearly showed that root length and biomass in aerobic rice variety, MAS-ARB25 were significantly higher when contrasted with basmati rice variety, IB370. The outcomes were supported by Xiaoguang and coworkers¹⁶ which showed that under aerobic field conditions, the aerobic varieties yielded better than the lowland rice variety. Bouman¹⁷ also observed 'Han Dao' varieties the aerobic rice yield better than lowland varieties grown in water limited conditions.

SSR Marker Investigation and Linkage Planning of QTL for Agronomic and Root Characteristics

The root development of rice presents a significant genetic assortment in terms of architecture, growth and development patterns and environmental adaptations. Root traits are generally controlled by many genes through quantitative trait loci. Beginning from the first study to situate genes by Champoux and coworkers¹⁸, controlling root traits of rice with molecular markers, various QTL linked to root traits which significantly increases grain yield under drought conditions have been reported¹⁹⁻²³.

In the current study, microsatellite marker information bases of IB370 × MAS-ARB25 got F₂ populations indicating the deviation for different

agronomic and root qualities utilizing 58 SSR markers which were polymorphic. The tree cluster analysis and two dimensional PCA scaling through NTSYS-pc software showed that the parent genotypes were quiet distinct and diverse whereas IB370 × MAS-ARB25 derived F₂ plants interspersed between the two parent rice genotypes. SSR marker analysis data showed that F₂ populations were skewed towards MAS-ARB25, which is also depicted from majority of agronomical and root traits frequency distribution curves. Four QTL for root traits and three QTL for agronomic traits were identified using microsatellite fingerprint database of IB370 × MAS-ARB25 derived F₂ population. One QTL for root length (*qRL_{5.1}*) was mapped on chromosome 5 at map position 118.8 cM with phenotypic variance of 4.2%. One QTL for total root length on chromosome 5 (*qRL₅*) with phenotypic variance of 24.2% was reported by Jiang and coworkers²⁴. Two QTL for fresh root weight (*qFRW_{5.1}* and *qFRW_{5.2}*) were mapped on chromosome 5 at map positions 89.8 and 97.7 cM with phenotypic variance of 2.0 and 2.1% respectively. One QTL for dry root weight (*qDRW_{5.1}*) was mapped on chromosome 5 at map position 94.8 cM with phenotypic variance of 2.9%. Two QTL for grain yield/ plant (*qGYP_{2.1}* and *qGYP_{5.1}*) were mapped on chromosomes 2 and 5 at map positions 127.6 and 91.7 cM with phenotypic variance of 12.9 and 19.1%, respectively. One QTL for 1000 grain weight (*qTGW_{4.1}*) was mapped on chromosome 4 at map position 15.0 cM with phenotypic variance of 12.5%. Ishimaru and coworkers²⁵ reported three QTLs each for number of panicles/plant on chromosome 4, 7 and 8; and grain yield/plant on chromosome 3 and 5; and nine QTLs for plant height on chromosome 1, 2, 3, 7 and 8 using backcross inbred lines of *japonica* Nipponbare × *indica* Kasalath in rice. Wang and coworkers²⁶ observed a major QTL, qRL7 on chromosome 7 linked with root length in hydroponic conditions. Large variation was also observed among IB370 × MAS-ARB25 derived F₂ population (230 plants) for plant height (78–145 cm; IB370 - 108.5 ± 0.56 cm and MAS-ARB25 - 90.5 ± 0.38 cm), effective number of tillers/ plant (2 - 20; IB370 - 7.5 ± 0.43 and MAS-ARB25 - 10 ± 0.75) and grain yield per plant (2.0 - 43.6 g; IB370 - 8.8 ± 0.34 g and MAS-ARB25 - 9 ± 0.61 g). F₂ plant number "188" had exceptionally high grain yield/ plant (43.6 g).

CIM analysis revealed seven QTLs (four QTLs for root traits and three for agronomic traits) on chromosomes 2 (*qGYP_{2.1}*), 4 (*qTGW_{4.1}*) and 5 (*qGYP_{5.1}*, *qRL_{5.1}*, *qFRW_{5.1}*, *qFRW_{5.2}*, and *qDRW_{5.1}*).

The putative QTLs promoting aerobic adaptation reported in the F₂ population were also checked in the selected promising F₂ plants; these QTLs were present in homozygous or heterozygous state in high frequencies. This research helps us to identify promising genotypes in rice breeding program for adaptation to aerobic systems. The areas where water scarcity and labor shortage is a problem, identification of suitable aerobic genotypes with QTL can provides a better adaptation and water uptake. The promising plants with identified putative QTL may serve as donor's in future marker-assisted breeding program after further validation.

References

- Kahani F & Hittalmani S, Genetic analysis and traits association in F₂ inter varietal populations in rice under aerobic condition, *J Rice Res*, 3 (2015) 152.
- Monaco F, Sali G, Ben Hassen M, Facchi A, Romani M *et al*, Water management options for rice cultivation in a temperate area: A multi-objective model to explore economic and water saving results, *Water*, 8 (2016) 336-355.
- Connor R, The United Nations World Water Development Report 2015: Water for a sustainable world (UNESCO Publishing) 2015, Vol. 1.
- WWAP, The United Nations World Water Development Report 2016: Water and Jobs. United Nations World Water Assessment Programme. (Paris: UNESCO) 2016.
- Pascual V J & Wang Y, Utilizing rainfall and alternate wetting and drying irrigation for high water productivity in irrigated lowland paddy rice in southern Taiwan, *Plant Prod Sci*, 20 (2017) 24-35.
- Kumari M, Suresh B G & Jyothi T, Evaluation of rice *Oryza sativa* L. germplasm for yield and yield component traits under aerobic condition, *The Ecoscan*, 9 (2015) 867-872.
- Kreye C, Bouman B A M, Faronilo J E & Llorca L, Causes for soil sickness affecting early plant growth in aerobic rice, *Field Crops Res*, 114 (2009) 182-187.
- Parthasarathi T, Vanitha K, Lakshmanakumar P & Kalaiyarasi D, Aerobic rice-mitigating water stress for the future climate change, *Int J Agron & Plant Prod*, 3 (2012) 241-254.
- Farooq M, Basra S M A & A, Wahid, Priming of field-sown rice seed enhances germination, seedling establishment, allometry and yield, *Plant Growth Regul*, 49 (2006) 285-294.
- Venuprasad R, Bool M E, Quiatchon L & Atlin G, A QTL for rice grain yield in aerobic environments with large effects in three genetic backgrounds, *Theor Appl Genet*, 124 (2012) 323-332.
- Lartaud M, Perin C, Courtois B, Thomas E, Henry S *et al*, PHIV-Root Cell, a supervised image analysis tool for rice root anatomical parameter quantification, *Frontiers in Plant Sci*, 5 (2014) 1-7.
- Kharb A, Jain S & Jain R, Phenotypic analysis, correlation studies and linkage mapping of QTL for traits promoting cultivation under dry direct seeded aerobic conditions for the development of water-efficient high yielding rice lines, *Cereal Res Commun*, 44 (2016) 658-668.
- Saghai-Marouf M A, Soliman K M, Jorgensen R A & Allard R W, Ribosomal spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics, *PNAS*, 81 (1984) 8014-8019.
- Wang D, Shi J, Carlson S R, Cregan P B, Ward R W *et al*, A low cost, high throughput polyacrylamide gel electrophoresis system for genotyping with microsatellite DNA markers, *Crop Sci*, 43 (2003) 1828-1832.
- Bradbury L M T, Henry R J, Jin Q, Reinke F & Waters D L E, A perfect marker for fragrance genotyping in rice, *Mol Breed*, 16 (2005) 279-283.
- Xiaoguang Y, Bouman B A M, Huaqi W, Zhimin W, Junfang Z *et al*, Performance of temperate aerobic rice under different regimes in North China, *Agric Water Manage*, 74 (2005) 107-122.
- Bouman B A M, Aerobic rice (Han Dao): A new way of growing rice in water short areas, *Mol Gen Genet*, 4 (2002) 53-61.
- Champoux M C, Wang G, Sarkarung S, Mackill D J, O'Toole J C *et al*, Locating genes associated root morphology and drought avoidance in rice via linkage to molecular markers, *Theor Appl Genet*, 90 (1995) 969-981.
- Dixit S, Swamy B P M, Vikram P, Bernier J, Sta-Cruz M T *et al*, Increased drought tolerance and wider adaptability of *qDTY_{12.1}* conferred by its interaction with *qDTY_{2.3}* and *qDTY_{3.2}*, *Mol Breed*, 30 (2012) 1767-1779.
- Sandhu N, Jain S, Kumar A, Mehla B S & Jain R K, Genetic variation, linkage mapping of QTL and correlation studies for yield, root and agronomic traits for aerobic adaptation, *BMC Genet*, 14 (2013) 104-120.
- Kharb A, Sandhu N, Jain S & Jain R, Linkage mapping of quantitative trait loci for traits promoting aerobic adaptation on chromosome 8 in *indica* rice (*Oryza sativa* L.), *Rice Genomics & Genet*, 6 (2015) 1-5.
- Shamsudin N A, Swamy B M, Ratnam W, Cruz M T S, Raman A *et al*, Marker assisted pyramiding of drought yield QTLs into a popular Malaysian rice cultivar, MR219, *BMC Genet*, 17 (2016) 1-14.
- Sandhu N, Dixit S, Swamy B P M, Vikram P, Venkateshwarlu C *et al*, Positive interactions of major-effect QTLs with genetic background that enhances rice yield under drought, *Sci Rep*, 8 (2018) 1626.
- Jiang S, Zhang X, Jiang H, Wang T, Ding G *et al*, Identification of QTLs formed root architectural traits in rice (*Oryza sativa* L.) after transplantation, *Plantomics*, 7 (2014) 410-414.
- Ishimaru K, Yano M, Aoki N, Ono K, Hirose T *et al*, Toward the mapping of physiological and agronomic characters on a rice function map: QTL analysis and comparison between QTLs and expressed sequence tags, *Theor Appl Genet*, 102 (2001) 793-800.
- Wang H, Xu X, Zhan X, Zhai R, Wu W *et al*, Identification of *qRL₇*, a major quantitative trait locus associated with rice root length in hydroponic conditions, *Breed Sci*, 63 (2013) 267-274.