



Linkage planning of QTL for agronomic and root qualities utilizing IB370 × MAS25 (*Oryza sativa* L.)-F₂ population developed under water restricted conditions

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Abstract

In the existing investigation, experiments were carried out to assess the F₂ population's resultant of crosses between improved Basmati 370 and MAS25 for various agronomical and root traits cultivated under aerobic water conditions. Large variations for grain yield, root length, root dry biomass, root thickness and length/breadth ratio of grain have been displayed in segregating F₂ populations. A close examination of phenotypic correlation exhibited that in the F₂ population, root length was certainly matched up ($r=0.496$) with root thickness. A DNA fingerprinting catalog for the currently studied F₂ generation was arranged using 61 polymorphic SSR markers. Composite Interval Mapping (CIM) inspection with WinQTL cartographer version 2.5 disclosed 13 putative QTL (Quantitative Trait Loci), out of which 6 QTL were for root characters 7 QTL for agronomical characters situated on 1, 2, 3, 8 and 10 chromosomes. The QTL documented in the above-said generation, some promising F₂ plants were also scrutinized and found in the homozygous or heterozygous state with high repetitions.

Keywords Aerobic rice · Linkage mapping · QTL · SSR markers

Introduction

Rice is a means of survival for the greater part of the earth's inhabitants (Zhu et al. 2018). Rice is an extravagant consumer of freshwater available for agriculture. Physical water insufficiency was observed in Asia on 17 million hectares of irrigated rice. Both freshwater requirement and rice consumption are expected to increase by 55% of water requirement and rice consumption shift from 8.8% in 2011–2020

to 32.75 by 2050 worldwide (Connor 2015; WWAP 2016). Water insufficiencies, climate change, and decreasing water-table levels are the significant reasons for less flooded rice production (Monaco et al. 2016). A significant part of the entire water is utilized for land preparation alone. If groundwater exploitation continues at the same pace in the next 20 years, there will be a loss of around 60% in water aquifers in India. There is a need to increase rice productivity to get maximum production with a single drop of water. The alternate strategy to combat water scarcity can be aerobic rice. Aerobic rice implies a cultivation system in which the rice crop is initiated by the direct-seeded method in non-puddle field conditions.

Aromatic Basmati rice serves as a prized commercial commodity because of its long grain and is famous for its fragrance, flavor and texture (Wakte et al. 2017). These are assessed as best in quality and get three times more worth than high-quality non-Basmati rice in the global trade. India has shipped 4.415 million tons (mt) of Basmati rice to the worldwide market with a total value of US\$ 4722 million (APEDA 2020). In the North-Western regions of India, drought and water availability are of great concern to the farmers. Basmati rice has confirmed difficulty in cultivation because of lessening combining ability and proportionate

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incompatibility with *indica/japonica* cultivars. It also bears more inter-grouping hybrid sterility, making growing it more difficult (Khush and Dela 2002). Introgression of required traits from *indica* and *japonica* rice varieties into Basmati rice confers many complications because it's very tough to keep all the Basmati traits intact during the selection procedure. Compared to non-aromatic rice types, the demand for aromatic rice has multiplied in recent years in the rice market. For sustainable Basmati rice production, if aerobic root traits could be integrated through the breeding program with the help of Marker Assisted Selection (MAS) in Basmati rice varieties, the problem of water crisis could decrease markedly.

In the present study, an effort has been made to cross the aerobic rice variety, MAS25 with the Basmati rice variety improved Basmati 370. Attempts have been made to scrutinize the presence of QTL using the SSR marker linked to them in the IB 370×MAS25 F₂ population. A little study has been done to evaluate Indian aromatic and quality rice (Nagaraju et al. 2002; Jain et al. 2004; Roy et al. 2015; Prasad et al. 2020; Yogi et al. 2020). Marker-assisted selection could assist researchers in better understanding the genetic structure of novel cultivars with improved root characteristics. Simple Sequence Repeats (SSRs) have a lot of promise, making it more desirable for genetic heterogeneity analysis, mapping of populations and marker-assisted plant upgrading strategies. The majority of SSR markers were connected with QTL for diverse root traits in direct-seeded rice or aerobic rice (Vikram et al. 2011; Sandhu et al. 2013; Dixit et al. 2014; Kharb et al. 2015; Zhao et al. 2018; Xu et al. 2020; Vinarao et al. 2021).

Rice has a considerable genetic variation in growth and development design, architecture and ecological adaptations due to its root growth. Several genes control root traits through QTL in most cases. Since Champoux et al. (1995) presented the first investigation to locate genes by regulating root qualities of rice with SSR markers, several QTL associated with rooting traits that significantly increase grain yield in water-limited conditions have been described (Dixit et al. 2012; Kharb et al. 2015; Shamsudin et al. 2016; Sandhu et al. 2018; Corales et al. 2020; Dhawan et al. 2021).

Materials and methods

Plant material

The study used improved Basmati-370, a scented high-yielding basmati rice cultivar intolerant of aerobic environments obtained from Punjab Agricultural University, Ludhiana, as the female parent and MAS25, an aerobic rice variety developed by the University of Agricultural Sciences, Bangalore, as the donor parent.

Field study

The crop was grown in the CCS Haryana Agricultural University's Rice Research Station in Kaul (Kaithal, Haryana, India) during the Kharif season. Seeds were taken from chosen IB370×MAS25-derived F₁ plants and sown in the field using the direct-seeded method under aerobic circumstances. All recommended agricultural practices were strictly followed to produce a high-quality crop. Seeds were direct sown in the field at 1–2 cm depth and 20 cm row spacing with a 15 cm spacing between plants was kept. Irrigation is done every 5 days until panicle emergence, then every 3 days after panicle emergence. The level of water was kept at 790–1430 mm/ha.

Data were kept for IB370×MAS25 derived 247 F₂ generation on diverse physio-morphological and root morphological traits, for instance, effective numbers of tillers, panicle length, plant height, 1000-grain weight, grain yield per plant, *L/B* (length to breadth) ratio of grain, root length, root thickness along with fresh and dry root weight appears in Table 2. Later, the data was analyzed to determine the frequency distribution curve, correlation coefficient analysis, and variability for the various agronomic and root attributes.

The CTAB method (Saghai-Marouf et al. 1984) was used to isolate genomic DNA from leaf samples of 15–21 day old young plants, to evaluate the quantity and quality of genomic DNA, agarose gel electrophoresis (AGE) was performed. By the electrophoresis unit system of C.B.S. Scientific Co., 4% polyacrylamide gels were utilized to resolve the amplified products (Wang et al. 2003). The QTL used in this investigation was located using Windows QTL Mapper 2.5. The Gramene website (<http://www.gramene.org>) was employed to get the map location (cM) of all the markers in the IB370×MAS25 derived F₂ generation used in the study. Parameters for scoring SSR allelic information can be found in the manual for the WinQTL cartographer.

Set of primer for rice aroma analysis

A specified set of primers were employed for evaluating the distinctness between Basmati and Non-Basmati rice cultivars for *BAD2A* (Betaine Aldehyde Dehydrogenase 2) genes (Bradbury et al. 2005) appears in Table 1.

Table 1 Primers for aroma

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 both agronomic and root traits in improved Basmati 370×MAS25 derived F₂ population raised under water-limited field and net house conditions

Trait	Improved Basmati 370	MAS25	IB370×MAS25 F ₂ population	
			Range	Mean
In field				
Plant height (cm)	108.5±0.56	92.0±0.48	80–136	108
Effective no. of tillers/plant	7.5±0.43	9.5±0.61	4.0–22	13.0
Panicle length (cm)	25.6±0.22	22.3±0.10	19.9–28.5	24.2
1000-grain weight (g)	20.5±0.12	20.3±0.19	17.9–26.6	22.2
Grain yield/plant (g)	8.8±0.34	11.3±1.20	3.9–40.9	22.4
<i>L/B</i> ratio	4.9±0.11	4.3±0.08	3.4–5.2	4.30
In pot house				
Plant height (cm)	86.5±0.37	80±0.32	63–106	84.5
Effective no. of tillers/plant	3.5±0.12	3.0±0.16	1.0–5.0	3.0
Panicle length (cm)	20.5±0.23	21.2±0.45	20.0–26.5	23.23
Grain yield/plant (g)	1.8±0.14	1.7±0.91	0.2–2.1	1.1
1000-grain weight (g)	13.9±0.54	12.9±0.47	9.9–13.1	11.5
<i>L/B</i> ratio	3.9±0.12	3.8±0.21	3.5–4.1	3.8
Root length (cm)	31.5±0.31	40.0±0.18	22–50	36.0
Root thickness (mm)	14.8±0.36	16.8±0.31	17.4–30.4	23.9
Fresh root weight (g)	3.1±0.22	3.6±0.06	2.2–27.4	15.1
Dry root weight (g)	1.0±0.28	1.1±0.33	0.7–9.3	5.0

Improved Basmati 370 (Yogi et al. 2021)

Results

Evaluation of IB370×MAS25 F₂ plants under the aerobic environment in both fields as well as net house

A vast disparity was noticed for different physio-morphological traits for IB370×MAS25 derived F₂ population (247 plants) and parental rice varieties grown under aerobic field conditions (Table 2). Phenotypic correlation coefficient scanning was implemented to evaluate the alliance between several traits in IB370×MAS25-derived F₂ plants under aerobic field conditions (Table 3) and pots in the net house (Table 4).

In the current population, a positive correlation was observed in grain yield/plant with plant height (0.576, $p=0.01$), effective no. of tillers per plant (0.696, $p=0.01$), panicle length (0.425, $p=0.01$) and 1000-grain weight

(0.283, $p=0.01$) under aerobic field conditions. While in pots under net house conditions, grain yield/plant also exhibits a notable affirmative alliance with panicle length (0.495, $p=0.05$) and 1000-grain weight (0.729, $p=0.01$) (Table 4). Root length, which is a significant trait of aerobic rice, found a remarkable positive connection with root thickness (0.496, $p=0.05$), fresh root weight (0.513, $p=0.05$) and dry root weight (0.463, $p=0.05$).

The frequency distribution curves of the IB370×MAS25-derived F₂ population and ancestral genotypes are shown for a variety of agronomic and root qualities in (Fig. 1). The plant height frequency distribution curve was skewed toward IB370. Effective number of tillers and grain yield per plant distribution curves were found to be illustrative and, to some extent, inclined towards MAS25. The curves for panicle length, grain weight per thousand grains, and *L/B* ratio were somewhat biased in favour of IB370. The MAS25-inclined frequency distribution curves for root length and root thickness.

Table 3 Phenotypic correlation coefficients among different agronomic traits of IB370×MAS25 derived F₂ population raised in water-restricted field conditions

	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	0.267**	1				
Panicle length (cm)	0.544**	0.193**	1			
1000-grain weight (g)	0.185**	0.160*	0.216**	1		
Grain yield/plant (g)	0.576**	0.696**	0.425**	0.283**	1	
L/B ratio	-0.221**	-0.030 ^{NS}	-0.045 ^{NS}	0.043 ^{NS}	-0.234**	1

*Significant at 5%

**Significant at 1% level

Allelic profile variation at the *BAD2A* locus for the fragrance characteristic

IB370 amplified two alleles of 585 and 257 base pairs because of Basmati characters', while MAS25, non-Basmati amplified 585 and 355 base pair size alleles. Fifty-eight selected improved Basmati 370×MAS25 derived F₂ Plants were divided into three groups: (i) plants having alleles of size 585 and 257 bp, (ii) plants having 585 and 355 bp alleles and (iii) plants having 585, 355 and 257 bp size alleles in heterozygous state at *BAD2A* locus. A sum of sixty-eight IB370×MAS25 derived F₂ populations from the field were sorted for *BAD2A* locus, with a proportion of 20:8:40 and *BAD2A* locus were also evaluated for 58-F₂ plants grown in net house, found a proportion of 12:18:28 when comparing plants with homozygous fragrant, heterozygous non-fragrant, and homozygous non-fragrant genotypes, respectively. A sum of 58 plants (homozygous fragrant, heterozygous non-fragrant). For further microsatellite marker study, the improved Basmati 370 specific allele at the *BAD2A* gene was chosen.

IB370×MAS25-derived F₂ populations showed the presence of three different fragments of 585 bp, 355 bp and 257 bp amplified using *BAD2A*-specific internal and external primers. These alleles (or fragments) were comparable to those reported earlier (Bradbury et al. 2005). Basmati rice variety, IB370, amplified 585 bp and 257 bp fragments, indicating aromatic (or fragrant) rice variety, while the non-aromatic aerobic rice variety, MAS25, showed amplification of 585 bp and 355 bp fragments.

Microsatellite marker analysis of IB370×MAS25 derived F₂ population

A sum of 93 SSR markers largely dispersed on all 12 rice chromosomes was utilized in the present investigation. Out of 93 SSR markers screened for polymorphism between two ancestor genotypes (IB370 and MAS25), 61 were found

polymorphic. Later on, a DNA fingerprint database of 58 IB370×MAS25 F₂ plants was produced with the help of 61 SSR markers (Fig. 2). A total of 125 alleles were recognized in the preferred 58 F₂ plants of IB370×MAS25 by using 61 SSR markers. Two of these 61 SSR markers (RM175 and RM21103) amplified recombinant (new) alleles, which were dissimilar to both ancestor varieties. Ten out of fifty-eight selected F₂ plants have recombinant alleles for more than one loci. On average, 50.4% of alleles came from IB370 and 49.6% were from MAS25 in all 58 F₂ plants.

Similarity coefficient statistics derived from 61 SSR markers were employed to determine the coefficient numbers in the chosen 58 IB370×MAS25 F₂ plants and their parental varieties and exposed to UPGMA tree cluster examination. The allelic miscellany was executed to create a dendrogram (NTSYS-pc) that exhibits the genetic association amongst 58 chosen F₂ plants along with their parents. The selected F₂ plants at the similarity coefficient of 0.53 are bunched into two vital categories. Vital category I consisted of IB370, while category II had MAS25 and 58 F₂ plants. Two parental varieties, IB370 and MAS25, showed a little similarity coefficient and split at a coefficient value of 0.53. PCA analysis (NTSYS-pc) was used to examine the genetic links between these rice genotypes. According to two-dimensional PCA scaling, F₂ generation was dispersed amongst the two biological parent types with a preference for MAS25 (Fig. 3).

Discussion

The grain yield of the aerobic rice variety, MAS25, was considerably higher than that of the Basmati rice type, IB370, in studies done under the context of a direct-seeded aerobic field. According to the several root characters data obtained, the root length and root biomass in the aerobic rice, MAS25, were considerably better than the Basmati rice, IB370. The

Table 4 Phenotypic correlation coefficients among the agronomic traits of IB370×MAS25 F₂ plants raised under aerobic conditions in pots in the net house

	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)
Plant height (cm)	1									
Effective no. of tillers/plant	-0.067 ^{NS}	1								
Panicle length (cm)	0.185 ^{NS}	0.426 ^{NS}	1							
1000-grain weight (g)	-0.023 ^{NS}	-0.059 ^{NS}	0.148 ^{NS}	1						
Grain yield/plant (g)	-0.035 ^{NS}	0.135 ^{NS}	0.495 [*]	0.729 ^{**}	1					
L/B ratio	-0.281 ^{NS}	0.292 ^{NS}	-0.018 ^{NS}	-0.444 ^{NS}	-0.055 ^{NS}	1				
Root length (cm)	0.089 ^{NS}	-0.026 ^{NS}	0.181 ^{NS}	0.070 ^{NS}	0.112 ^{NS}	-0.396 ^{NS}	1			
Root thickness (mm)	0.178 ^{NS}	0.422 ^{NS}	0.262 ^{NS}	0.027 ^{NS}	0.055 ^{NS}	-0.149 ^{NS}	0.496 [*]	1		
Fresh root weight (g)	0.235 ^{NS}	0.171 ^{NS}	0.268 ^{NS}	0.069 ^{NS}	-0.015 ^{NS}	-0.404 ^{NS}	0.513 [*]	0.689 ^{**}	1	
Dry root weight (g)	0.205 ^{NS}	0.090 ^{NS}	0.316 ^{NS}	0.109 ^{NS}	0.091 ^{NS}	-0.385 ^{NS}	0.463 [*]	0.635 ^{**}	0.964 ^{**}	1

better yield may be due to the healthier and deeper-root structure developed in MAS25, which is an aerobic variety.

A sum of sixty-one discrete SSR markers scattered on the twelve rice chromosomes was used to locate the QTL linked with various traits by using 58 IB370×MAS25-derived F₂ plants. Composite Interval Mapping (CIM) study by Win-QTL Cartographer 2.5 exposed seven numbers of QTL for agronomical traits (Table 5, Fig. 4) and six QTL for root traits in rice (Table 5, Fig. 5).

One of these QTL was linked with plant height ($qPH_{2.1}$) at a map location of 95.9 cM on chromosome 2. The $qPH_{2.1}$ has beneficial worth for additive effect (14.78) suggesting the alleles that favour plant height were from the MAS25 donor parent. The $qPH_{2.1}$ had the greatest LOD score of 7.9 and accounted for 10.32% phenotypic variation. Two QTL linked with an effective number of tillers per plant ($qTP_{8.1}$ and $qTP_{10.1}$) at map positions of 98.2 and 27.0 cM on chromosomes 8 and 10, respectively. Kharb et al. (2015) identified three QTLs, $qTN_{8.1}$, $qTN_{8.2}$ and $qTN_{8.3}$ for tillers number/plant on chromosome number 8. Two QTL for an effective number of tillers per plant had positive worth for additive effect suggesting that the favoring alleles originated from the MAS25 parent. Two QTL correlated with grain yield per plant ($qGYP_{3.1}$ and $qGYP_{8.1}$) at map locations of 62.7 and 93.4 cM, on chromosomes 3 and 8, respectively. Dixit et al. (2014) observed a QTL, $qDTY3.1$ on chromosome 3 at a map location of 124.0 cM with a huge additive effect of 165.3 showed consistent effects under drought circumstances. Catolos et al. (2017) revealed a QTL, $qDTY8.1$ on chromosome 8 on a map location range 68.8–76.3 cM with a 34.17% additive effect. Xu et al. (2020) supported this result and reported three QTL, $qAER1$, $qAER3$, and $qAER9$ for aerobic adaptation in backcrossed inbred lines on chromosomes 1, 3 and 9. One QTL, $qAER3$ identified on chromosome 3 at a map position of 67.8–76.7 cM with the proportion of phenotypic variation is 19.19% at the marker interval of RM 218–RM232. Dhawan et al. (2021) introgressed a major QTL, $qDTY1.1$, for grain yield in water scarcity is about 12.6% to 16.9% of phenotypic variance), mapped on chromosome 1 into “Pusa Basmati 1” by marker-assisted backcrossing from drought-tolerant cultivars, Nagina 22. Baisakh et al. (2020) reported a total of 8 QTL for yield traits under drought stress, of which grain number per panicle, three QTLs were discovered out of which two QTLs located on chromosome 3 ($qGN3.1$ and $qGN3.2$) and single QTL located on chromosome 5 ($qGN5.1$). For grain yield in a water-limited environment, four QTLs, $qGY1.1$, $qGY7.1$, $qGY8.1$, and $qGY11.1$, were revealed on chromosomes 1, 7, 8, and 11, respectively, which all together control 45 percent of the phenotypic variance. Xu et al. 2020 identified QTL $qAER3$ on chromosome 3 in BC₄F₅ Back cross inbred populations under aerobic conditions. Corales et al. (2020) mapped 22 QTLs for root characteristics in a hybrid of the

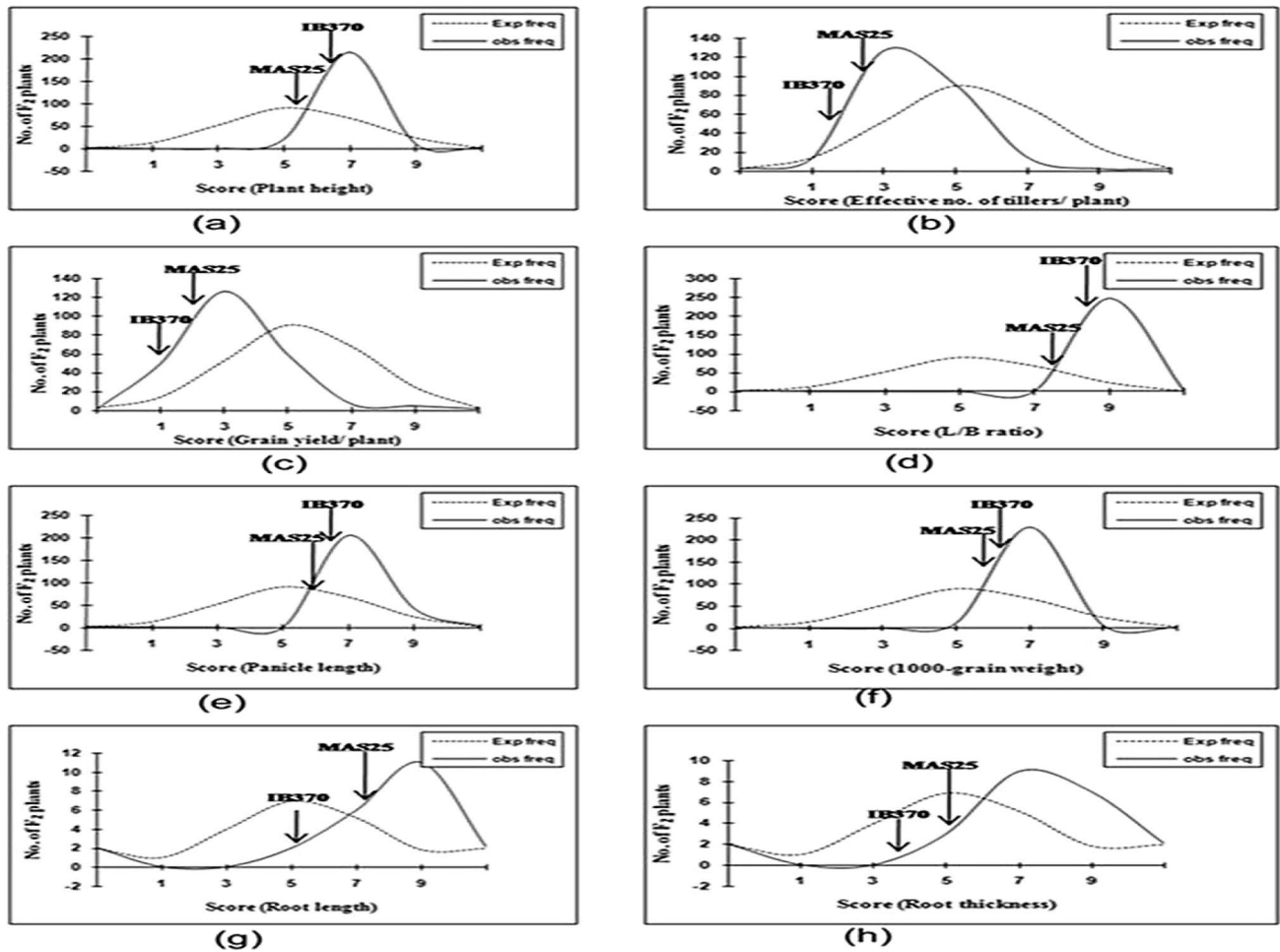


Fig. 1 IB370×MAS25 generated F₂ population frequency distribution curves for agronomic and root traits developed in water-limited field conditions (a–f) and net house (g, h)

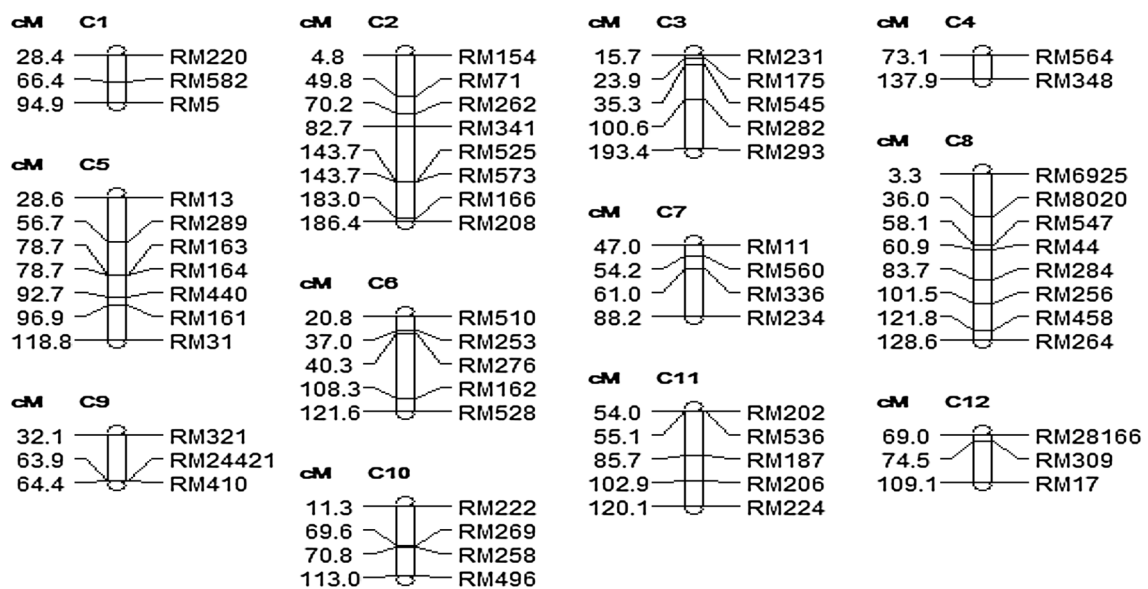


Fig. 2 Map the position of a total of sixty-one SSR markers situated on entire rice chromosomes in the whole genome

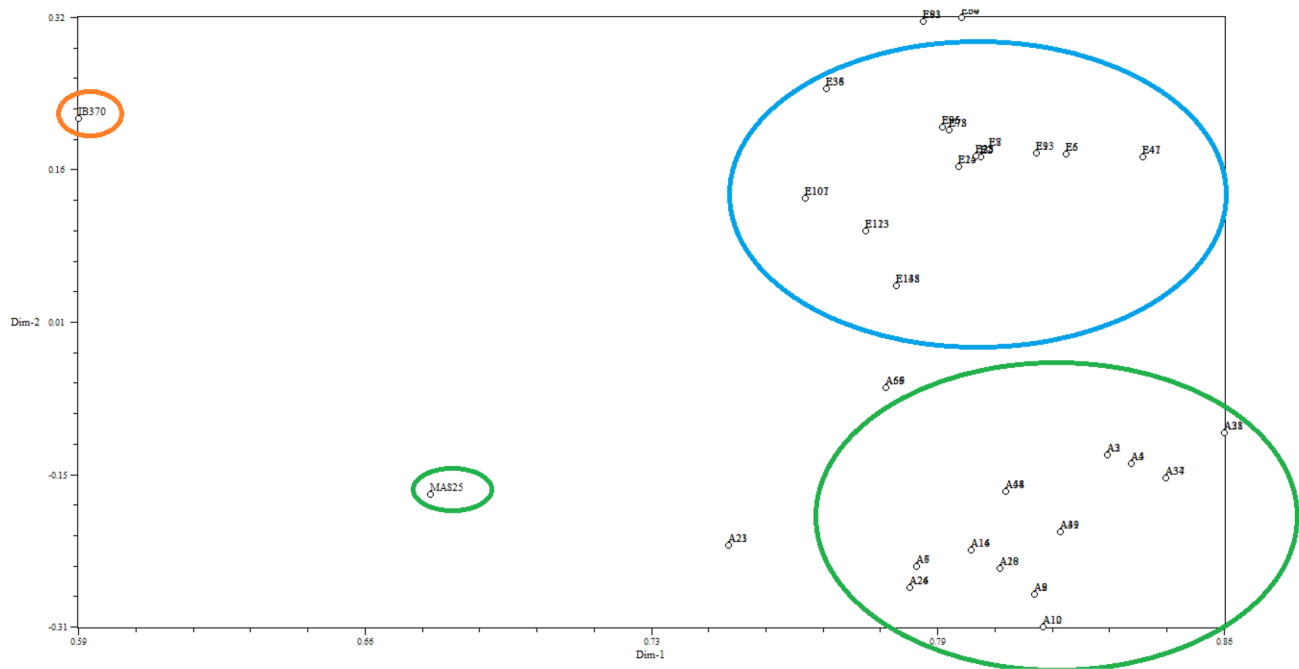


Fig. 3 Two-dimensional PCA scaling displaying diverseness in chosen 58 F_2 plants (IB370×MAS25) along with parents by applying allelic diversity data at sixty-one SSR loci

Table 5 QTL recognized for various agronomic and root characters by SSR marker analysis of IB370×MAS25 derived F_2 generation

Trait	QTL name	Chromosome number	Position (cM)	Flanking markers	Position of flanking markers (cM)	LOD	Additive effect	$R^2\%$	DPE
Plant height	<i>qPH</i> _{2.1}	2	95.9	RM341-RM525	82.7–143.7	7.9	14.78	10.32	M
Effective no. of tillers/plant	<i>qTP</i> _{8.1}	8	98.2	RM284-RM256	83.7–101.5	3.8	3.32	56.99	M
	<i>qTP</i> _{10.1}	10	27.0	RM222-RM269	11.3–69.6	5.8	3.39	11.26	M
Panicle length	<i>qPL</i> _{2.1}	2	31.0	RM154-RM71	4.8–49.8	3.3	0.24	41.78	M
1000-grain weight	<i>qTGW</i> _{2.1}	2	90.9	RM341-RM525	82.7–143.7	6.6	10.25	21.0	M
Grain yield/plant	<i>qGYP</i> _{3.1}	3	62.7	RM545-RM282	35.3–100.6	4.9	6.79	21.64	M
	<i>qGYP</i> _{8.1}	8	93.4	RM284-RM256	83.7–101.5	6.3	4.17	22.05	M
Root length	<i>qRL</i> _{2.1}	2	120.9	RM341-RM525	82.7–143.7	2.9	4.38	4.20	M
	<i>qRL</i> _{3.1}	3	143.8	RM282-RM293	100.6–193.4	4.2	−1.58	8.70	IB
Root thickness	<i>qRT</i> _{1.1}	1	57.1	RM220-RM582	28.4–66.4	3.6	1.96	21.60	M
Fresh root weight	<i>qFRW</i> _{10.1}	10	13.0	RM222-RM269	11.3–69.6	3.5	−3.27	23.62	IB
Dry root weight	<i>qDRW</i> _{1.1}	1	51.0	RM220-RM582	28.4–66.4	4.3	0.86	4.69	M
	<i>qDRW</i> _{3.1}	3	150.8	RM282-RM293	100.6–193.4	4.2	0.78	10.66	M

The direction of phenotypic effect (DPE); M situate for MAS25 and IB situate for improved Basmati 370, R^2 is for phenotypic deviation

Additive effect is half of the difference between the effect of MAS25 and IB370 alleles; its positive number specifies that the effect of the MAS25 genotype was in the direction of rising the trait value i.e., LOD-log10 of an odd rat

Japanese paddy rice Koshihikari and the Japanese upland rice Sensho, which grew in an aerobic environment.

Two QTL are associated with root length (*qRL*_{2.1} and *qRL*_{3.1}) at map locations of 120.9 and 143.8 cM on chromosomes 2 and 3, respectively (Table 5; Fig. 5). The *qRL*_{2.1} found positive numbers for additive effect (4.38) specified

that the recommending alleles were coming from donor parent, MAS25. At the same time, *qRL*_{3.1} had negative values for additive effect (−1.58), indicating that the favoring alleles were from a recurrent parent, IB370. One QTL associated with root thickness (*qRT*_{1.1}) at map location of 57.1 cM on chromosome 1. The *qRT*_{1.1} had the utmost LOD

Fig. 4 Chromosomal locations of QTL for agronomic traits recognized in selected F₂ generation obtained from mating between IB370×MAS25

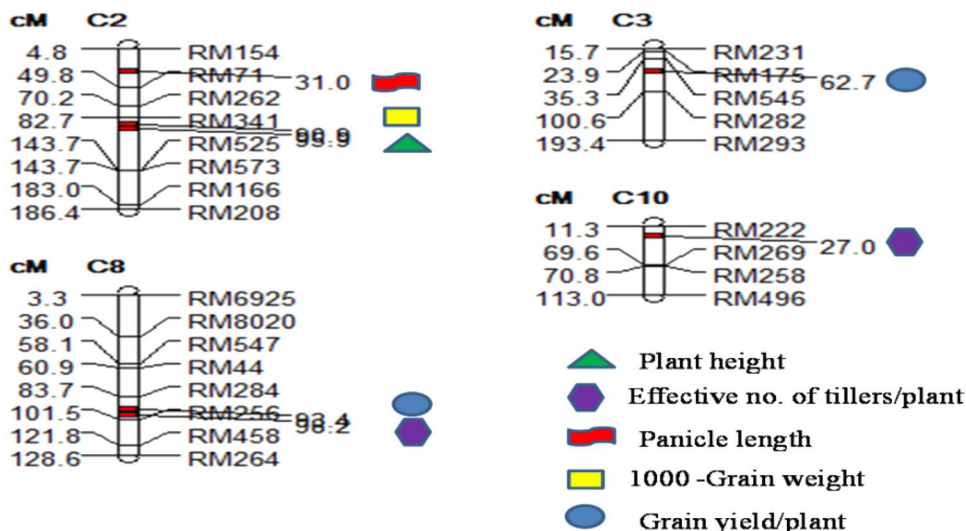
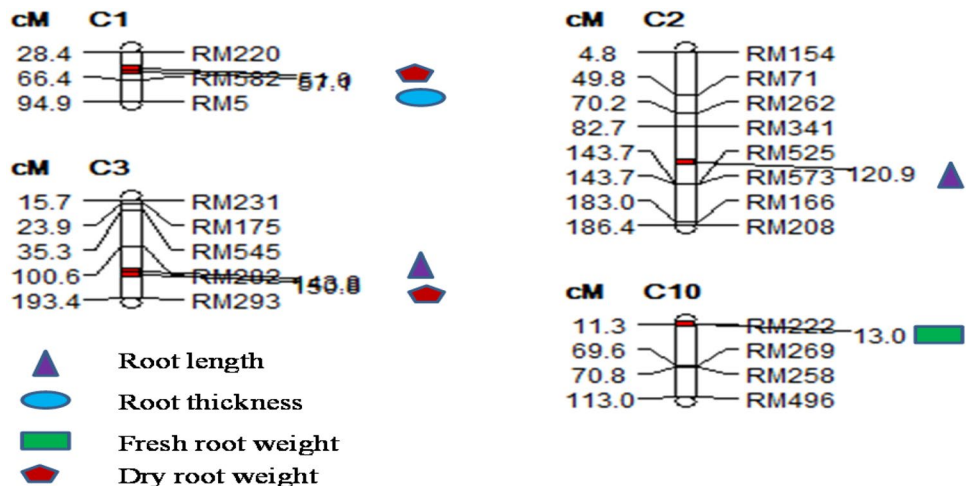


Fig. 5 Chromosomal locations of QTL for different root traits recognized in selected F₂ generation obtained from mating between IB370×MAS25



(LOD- \log_{10} of an odds ratio) score of 3.6 considered for 21.6% phenotypic deviation and found affirmative numbers for additive effect (1.96) specified that the favoring alleles were coming out from MAS25 genotype (Table 5, Fig. 5). Corales et al. (2020) mapped a QTL for root length on chromosome 4 at a map position of 4.6 cM with 18.80 phenotypic variations for huge additive effects (177.12). Yogi et al. (2021) located a QTL for root length on chromosome 5 at a map location of 118.8 cM in IB370×MAS-ARB25 derived F₂ generation with an inclination towards MAS-ARB25, donor parent.

One QTL linked with fresh root weight (*qFRW_{10.1}*) at map site of 13.0 cM on chromosome number 10. The *qFRW_{10.1}* had depressing numbers for additive effect (-3.27) suggesting that the recommending alleles originated from IB370 parents (Table 5, Fig. 5). Two QTL are associated with dry root weight (*qDRW_{1.1}* and *qDRW_{3.1}*) at map locations of 51.0 and 150.8 cM, on chromosomes 1 and 3, respectively. Both

the QTL had affirmative numbers for additive effect (0.86 and 0.78) specifying that the recommending alleles were coming from the donor parent, MAS25 (Table 5, Fig. 5). Similar results were perceived by Corales et al. (2020) who mapped 1 QTL for root dry weight on chromosome 1 at a position of 113.5 cM with phenotypic variations of 11.66% in aerobic field conditions. Yogi et al. (2021) observed 2 QTL at the map location of 97.7 and 94.8 cM on chromosome 5 in IB370×MAS-ARB25 derived F₂ generation under water-limited situations.

In the case of IB370×MAS25 derived F₂ plants, six QTL for root characters and seven QTL for agronomic characters were recognized by using the CIM WinQTL Cartographer 2.5. Notably, four QTL out of a total of six putative QTL for root traits recognized in this study were coming out from the second parent, MAS25.

Two QTL for root length (*qRL_{2.1}* and *qRL_{3.1}*) were depicted on chromosomes 2 and 3 at map positions 120.9

and 143.8 cM with a phenotypic variance of 4.2 and 8.7%, respectively. One QTL for root thickness ($qRT_{1,1}$) was traced on chromosome number 1 at map site 57.1 cM with a phenotypic variance of 21.6%. Liu et al. (2008) reported basal root thickness correlated QTL traced on chromosome number 1 (brt1.a) with a phenotypic variance of 4.6%. Li et al. (2015) revealed a QTL, $qRT9$ located on chromosome 9 controlling root length and root thickness using $F_{2,3}$ populations obtained by crossing, Yuefu and IL392 upland rice genotypes. Zhao et al. (2018) recognized 44 and 97 major QTL genes connected with root length and root thickness, and five QTL for root length were authenticated with T-DNA insertional mutation; Two QTL for dry root weight ($qDRW_{1,1}$ and $qDRW_{3,1}$) were mapped on chromosomes 1 and 3 at map locations 51.0 and 150.8 cM with phenotypic variances of 4.7 and 10.7%, respectively. One QTL for fresh root weight ($qFRW_{10,1}$) was mapped on chromosome 10 at map position 13.0 cM with a phenotypic variance of 23.6%. On chromosome 2 at a map location of 95.9 cM, one QTL ($qPH_{2,1}$) associated with plant height was identified, with a phenotypic variance of 10.3%. One QTL ($qPH_{2,1}$) for plant height was found at chromosome 2 by Lin et al. in (2007), exhibiting a phenotypic variance of 13.9%.

For an effective number of tillers/plant, two QTL ($qTP_{8,1}$ and $qTP_{10,1}$) were located on 8 and 10 chromosomes at map locations of 98.2 and 27.0 cM with a phenotypic variance of 57.0 and 11.3%, respectively. Sandhu et al. (2013) described two QTL ($qTN_{8,1}$ and $qTN_{8,3}$) for tiller number at chromosome 8 at map location 15.7 and 123.2 cM with a phenotypic variance of 29.3 and 26.4%, respectively, of HKR47 \times MAS26 F_2 population.

One QTL associated with panicle length ($qPL_{2,1}$) was situated on chromosome number 2 at a map location of 31.0 cM with a phenotypic variance of 41.8%. One QTL associated with 1000-grain weight ($qTGW_{2,1}$) was situated on chromosome number 2 at a map location of 90.9 cM with a phenotypic variance of 21.0%. Lin et al. (2007) reported one QTL ($TSW_{2,1}$) for 1000-seed weight at chromosome 2 with a phenotypic variance of 10.1%. Two QTL associated with grain yield/plant ($qGYP_{3,1}$ and $qGYP_{8,1}$) were portrayed on chromosomes 3 and 8 at map locations of 62.7 and 93.4 cM with a phenotypic variance of 21.6 and 22.1%, respectively.

IB370 \times MAS25 derived F_2 plants were examined for an allelic profile at *BAD2A* locus and 61 SSR markers dispersed on the whole genome of rice. A population of 126 IB370 \times MAS25 F_2 plants was screened for an allelic profile at *BAD2A* locus with a ratio of 32:26:68 for homozygous fragrant, heterozygous non-fragrant and homozygous non-fragrant plants, respectively. The NTSYS-pc UPGMA tree cluster analysis and two-dimensional PCA scaling revealed that both parental genetic makeup were

fairly divergent, whereas 58 IB370 \times MAS25 F_2 plants scattered in between the two parental rice genotypes. SSR scrutiny also displayed that the F_2 population is tilted towards MAS25, confirmed in frequency distribution curves for most agronomic and root traits. Based on field and net house assessment of IB370 \times MAS25 F_2 plants, 20 plants were chosen based upon grain yield/plant, grain *L/B* ratio, Basmati specific allele at *BAD2A* locus (homozygous or heterozygous states) and root length (in case of net house evaluation). Composite Interval Mapping scanning exposed a total of 13 putative QTL (six for root traits and seven for agronomical traits). Out of which one QTL $qRL_{2,1}$ for root length with 4.2 $R^2\%$, one QTL $qRT_{1,1}$ for root thickness with 21.60 $R^2\%$ and two QTL for dry root weight $qDRW_{1,1}$ and $qDRW_{3,1}$ with 4.69 and 10.66 $R^2\%$, respectively, further the direction of the phenotypic effect of these QTL are towards MAS25 so can be utilized to upgrade the root characters' in future.

Conclusion

Composite Interval Mapping shows a total of 13 putative QTL (six for root traits and seven for agronomical traits). The probable QTLs for aerobic adaptation revealed in the F_2 generation were examined in the selected promising F_2 plants, and they were shown to be present in homozygous or heterozygous states at high frequencies. This study aids in the identification of promising genotypes in the rice breeding program for aerobic system adaptability. Identification of optimal aerobic genotypes with QTL can give improved adaptability and water intake in locations where water scarcity and labor shortages are a problem. After additional validation, the potential plants with documented putative QTL could be used as donors in upcoming marker-assisted breeding programs.

Author contribution statement RY and NK conducted the field trials, RY performed the molecular marker analysis, RY, RK and MK performed the statistical analysis.

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Data availability The datasets generated and analysed during current study are available from corresponding author on reasonable request.

Declarations

Conflict of interest We declare that the authors of this paper have no conflicting interests.

References

- APEDA (2020) Agricultural & processed food products export development authority, Government of India, New Delhi. https://apeda.gov.in/apedawebsite/six_head_product/cereal.htm
- Baisakh N, Yabes J, Gutierrez A, Mangu V, Ma P, Famoso A, Pereira A (2020) Genetic mapping identifies consistent quantitative trait loci for yield traits of rice under greenhouse drought conditions. *Genes* 11:62. <https://doi.org/10.3390/genes11010062>
- Bradbury LMT, Henry RJ, Jin Q, Reinke F, Waters DLE (2005) A perfect marker for fragrance genotyping in rice. *Mol Breed* 16:279–283. <https://doi.org/10.1007/s11032-005-0776-y>
- Catolos M, Sandhu N, Dixit S, Shamsudin NAA, Kumar A et al (2017) Genetic loci governing grain yield and root development under variable rice cultivation conditions. *Front Plant Sci* 8:1763. <https://doi.org/10.3389/fpls.2017.01763>
- Champoux MC, Wang G, Sarkarung S, Mackill DJ, O'Toole JC, Huang N, McCouch SR (1995) Locating genes associated root morphology and drought avoidance in rice via linkage to molecular markers. *Theor Appl Genet* 90:969–981. <https://doi.org/10.1007/BF00222910>
- Connor R (2015) The United Nations world water development report 2015: water for a sustainable world, vol 1. UNESCO Publishing, Paris
- Corales M, Nguyen NTA, Abiko T, Mochizuki T (2020) Mapping quantitative trait loci for water uptake of rice under aerobic conditions. *Plant Prod Sci* 23(4):436–451. <https://doi.org/10.1080/1343943X.2020.1766361>
- Dhawan G, Kumar A, Dwivedi P, Krishnan SG et al (2021) Introgression of *qDTY1.1* governing reproductive stage drought tolerance into an elite basmati rice variety “Pusa Basmati 1” through marker assisted backcross breeding. *Agronomy* 11:202. <https://doi.org/10.3390/agronomy11020202>
- Dixit S, Swamy BPM, Vikram P, Bernier J, Sta Cruz MT, Kumar A (2012) Increased drought tolerance and wider adaptability of *qDTY12.1* conferred by its interaction with *qDTY2.3* and *qDTY3.2*. *Mol Breed* 30:1767–1779. <https://doi.org/10.1007/s11032-012-9760-5>
- Dixit S, Singh A, Cruz MTS, Maturan PT, Amante M, Kumar A (2014) Multiple major QTL lead to stable yield performance of rice cultivars across varying drought intensities. *BMC Genet* 15:16. <https://doi.org/10.1186/1471-2156-15-16>
- Jain S, Jain RK, McCouch SR (2004) Genetic analysis of Indian aromatic and quality rice (*Oryza sativa* L.) germplasm using panels of fluorescently-labeled microsatellite markers. *Theor Appl Genet* 109:965–977. <https://doi.org/10.1007/s00122-004-1700-2>
- Kharb A, Sandhu N, Jain S, Jain R (2015) Linkage mapping of quantitative trait loci for traits promoting aerobic adaptation on chromosome 8 in *indica* rice (*Oryza sativa* L.). *Rice Genom Genet* 6:1–5. <https://doi.org/10.5376/rgg.2015.06.0006>
- Khush GS, Dela CN (2002) Developing Basmati rices with high yield potential. In: Duffy R (ed) *Speciality rices of the world: breeding, production and marketing*. Science Pub, Inc, Enfield, pp 15–18
- Li J, Han Y, Liu L, Chen Y, Du Y, Zhang J, Sun H, Zhao Q (2015) *qRT9*, a quantitative trait locus controlling root thickness and root length in upland rice. *J Exp Bot* 66:2723–2732. <https://doi.org/10.1093/jxb/erv076>
- Lin MH, Lin CW, Chen JC, Lin YC, Cheng SY, Ku HM et al (2007) Tagging rice drought-related QTL with SSR DNA markers. *Crop Environ Bioinform* 4:65–76
- Liu L, Mu P, Li X, Qu Y, Wang Y, Li Z (2008) Localization of QTL for basal root thickness in *japonica* rice and effect of marker-assisted selection for a major QTL. *Euphytica* 164:729–737
- Monaco F, Sali G, Ben Hassen M, Facchi A, Romani M, Valè G (2016) Water management options for rice cultivation in a temperate area: a multi-objective model to explore economic and water saving results. *Water* 8:336–355. <https://doi.org/10.3390/w8080336>
- Nagaraju J, Kathirvel M, Rameshkumar R, Siddiq EA, Hasnain SE (2002) Genetic analysis of traditional and evolved Basmati and non-Basmati rice varieties by using fluorescence-based ISSR-PCR and SSR markers. *Proc Natl Acad Sci USA* 99:5836–5841
- Prasad GSV, Padmavathi G, Suneetha K, Madhav MS, Muralidharan K (2020) Assessment of diversity of Indian aromatic rice germplasm collections for morphological, agronomical, quality traits and molecular characters to identify a core set for crop improvement. *CABI Agric Biosci* 1:13. <https://doi.org/10.1186/s43170-020-00013-8>
- Roy S, Banerjee A, Mawkhlieng B, Misra AK, Pattanayak A, Harish GD, Singh SK, Ngachan SV, Bansal KC (2015) Genetic diversity and population structure in aromatic and quality rice (*Oryza sativa* L.) landraces from north-eastern India. *PLoS ONE* 10(6):e0129607. <https://doi.org/10.1371/journal.pone.0129607>
- Saghai-Marouf MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal spacer length polymorphism in Barley: Mendelian inheritance, chromosomal location and population dynamics. *PNAS* 81:8014–8019
- Sandhu N, Jain S, Kumar A, Mehla BS, Jain RK (2013) Genetic variation, linkage mapping of QTL and correlation studies for yield, root, and agronomic traits for aerobic adaptation. *BMC Genet* 14:104–120. <https://doi.org/10.1186/1471-2156-14-104>
- Sandhu N, Dixit S, Swamy BPM, Vikram P, Venkateshwarlu C, Catolos M et al (2018) Positive interactions of major-effect QTLs with genetic background that enhances rice yield under drought. *Sci Rep* 8:1626. <https://doi.org/10.1038/s41598-018-20116-7>
- Shamsudin NA, Swamy BM, Ratnam W, Cruz MTS, Raman A, Kumar A (2016) Marker assisted pyramiding of drought yield QTLs into a popular Malaysian rice cultivar, MR219. *BMC Genet* 17:30. <https://doi.org/10.1186/s12863-016-0334-0>
- Vikram P, Swamy BPM, Dixit S, Ahmed HU, Sta Cruz MT, Singh AK, Kumar A (2011) *qDTY1.1*, a major QTL for rice grain yield under reproductive-stage drought stress with a consistent effect in multiple elite genetic backgrounds. *BMC Genet* 12:89–104. <https://doi.org/10.1186/1471-2156-12-89>
- Vinarao R, Proud C, Zhang X, Snell P, Fukai S, Mitchell J (2021) Stable and novel quantitative trait loci (QTL) confer narrow root cone angle in an aerobic rice (*Oryza sativa* L.) production system. *Rice* 14:28. <https://doi.org/10.1186/s12284-021-00471-2>
- Wakte K, Zanan R, Hinge V, Khandagale K, Nadaf A, Henry R (2017) Thirty-three years of 2-acetyl-1-pyrroline, a principal basmati aroma compound in scented rice (*Oryza sativa* L.): a status review. *J Sci Food Agric* 97(2):384–395. <https://doi.org/10.1002/jsfa.7875>
- Wang D, Shi J, Carlson SR, Cregan PB, Ward RW, Diers BW (2003) A low cost, high throughput polyacrylamide gel electrophoresis system for genotyping with microsatellite DNA markers. *Crop Sci* 43:1828–1832. <https://doi.org/10.2135/cropsci2003.1828>
- WWAP (2016) The United Nations world water development report (2016): water and jobs. United Nations World Water Assessment Programme. UNESCO, Paris
- Xu P, Yang J, Ma Z, Yu D, Zhou J, Tao D, Li Z (2020) Identification and validation of aerobic adaptation QTLs in upland rice. *Life* 10:65. <https://doi.org/10.3390/life10050065>

- Yogi R, Kumar N, Kumar R, Jain RK (2020) Genetic diversity analysis among important rice (*Oryza sativa* L.) genotypes using SSR markers. *Adv Bioresour* 11(2):68–74. <https://doi.org/10.15515/abr.0976-4585.11.2.6874>
- Yogi R, Naveen Kumar N, Meena RK, Jain RK (2021) 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. *Indian J Biotechnol* 20:91–100
- Zhao Y, Zhang H, Xu J, Jiang C, Yin Z, Xiong H, Xie J, Wang X, Zhu X, Li Y, Zhao W, Rashid R, Li J, Wang W, Fu B, Ye G, Guo Y, Hu Z, Li Z (2018) Loci and natural alleles underlying robust roots and adaptive domestication of upland ecotype rice in aerobic conditions. *Genet* 14(8):e1007521. <https://doi.org/10.1371/journal.pgen.1007521>
- Zhu C, Kobayashi K, Loladze I, Zhu J, Jiang Q, Xu X, Liu G, Seneweera S, Ebi KL, Drewnowski A, Fukagawa NK, Ziska LH (2018) Carbon dioxide (CO₂) levels this century will alter the protein, micronutrients, and vitamin content of rice grains with potential health consequences for the poorest rice-dependent countries. *Sci Adv* 4(5):eaaq1012. <https://doi.org/10.1126/sciadv.aaq1012>

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