



Microsatellite diversity analysis and QTL identification among progenies derived from aerobic × basmati rice (*Oryza sativa*) cross under direct-seeded conditions

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ABSTRACT

The present investigation was designed to identify QTL associated with various traits under aerobic condition using F₃ and F₄ population derived from the cross MASARB25 (aerobic rice) and IB370 (basmati rice). The phenotyping was done in both field and net house conditions during the *kharif* seasons of 2013-14 and 2014-15. The result indicated high variation among the population for studied traits and parabolic frequency distribution was recorded for panicle length, effective number of tillers/plant, 1000-grain weight while, for grain length/breadth ratio and root thickness, frequency distribution curve were skewed toward MASARB25. Composite interval mapping identified total 16 QTLs on chromosomes 1, 2, 3, 4, 6, 9, 10 and 12 during both the years. Maximum QTL were detected for grain length-breadth ratio. LOD score of these QTLs ranged from 2.88 (qENT12.1) to 5.51 (qLB3.1) and explained 61.63% and 69.04% variance, respectively. The QTL mapped for grain yield/plant (qGYP6.1) on chromosome 6 had LOD score of 2.90 and explained 28.4% phenotypic variation. The identified QTL in present investigation showed high phenotypic variation, hence after validation these QTLs could be used for the improvement of rice under aerobic condition.

Key words: Aerobic rice, Basmati, QTL, Root traits, SSR markers

Rice (*Oryza sativa* L. 2n=24), a member of the family *Poaceae*, is one of the oldest domesticated crops. It takes about 3000-5000 liters of water to produce 1 kg of rice. In Asia, 17 mha of irrigated rice areas may experience “physical water scarcity” and 22 mha “economic water scarcity” by 2025 (Bouman 2002). An increasing demand for food and a looming water crisis, a shortage of both may be on the horizon unless innovative technologies are developed for rice cultivation. Therefore, there is an urgent necessity to develop and implement solutions that have the potential

of increasing the efficiency, equity, and sustainability of water use.

Aerobic rice is considered to be one of the most promising strategies in terms of water-use efficiency (Tuong and Bouman 2002). Aerobic rice system is 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 (Kreye *et al.* 2009). Such ‘aerobic rice’ varieties combine the aerobic adaption of traditional upland varieties with the input responsiveness, yield potential of irrigated varieties (Atlin *et al.* 2006). The genomic locations associated with suitable agronomic aerobic traits such as plant height, effective no. of tillers, grain yield/plant and root parameters have been previously identified in various studies (Vikram *et al.* 2011; Dixit *et al.* 2012b; Sandhu *et al.* 2013; Sandhu *et al.* 2014). However, identification of genomic loci associated with aerobic conditions along with long grain and aroma of basmati rice would be beneficial, therefore keeping this in view, present study was conducted to identify genomic locations associated with basmati rice under aerobic conditions.

MATERIALS AND METHODS

Plant material

Total of 60 plants were selected from field for both F₃

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and F₄ generations derived from MASARB25 (aerobic rice variety) and IB370 (basmati rice) was used in the study.

Phenotyping

The cultivation practices and phenotyping was done in both field and net house conditions as described by Kumar *et al.* (2018) for two seasons, viz. 2013-14 and 2014-15. The data were recorded for Plant height (cm), Effective number of tillers per plant, Panicle length (cm), Grain yield per plant (g), 1000-grain weight (g), Length/Breadth ratio, Root length (g), Fresh and dry root weight (g) and Root thickness (cm). Data were subjected to statistical analysis using CROPSTAT 7.2 (available at <http://bbi.irri.org/products>) and SPAR 2.0.

Genotyping

Genomic DNA was isolated using CTAB method of Saghai-Marooof *et al.* (1984). 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.* (2006). A total of 604 SSR markers widely distributed on 12 rice chromosomes were used to find parental polymorphism and 60 were found to be polymorphic among the parents, were used to genotype the selected F₃ and F₄ plants. QTL analysis was performed using QTL Cartographer v2.5 (Wang *et al.* 2010). Simultaneously, these plants were evaluated for assessing the presence/absence of fragrance allele using specific primers for betaine aldehyde dehydrogenase 2 (BAD2A) genes (Bradbury *et al.* 2005). The population were divided into three types based on presence of BAD2A locus: (i) with 585 and 257 bp alleles, (ii) with 585 and 355 bp alleles and (iii) with 585, 355 and 257 bp alleles. The plants having positive allele for BAD2A were selected for further analysis.

RESULTS AND DISCUSSION

Phenotyping

In the present investigation, experiments were carried out to evaluate F₃ and F₄ populations derived from these crosses under direct-seeded water-limited aerobic conditions for various physio-morphological traits. Previously, increase in performance of aerobic rice under water limited conditions were reported by (Sandhu *et al.* 2012; Promila *et al.* 2015).

Parents as well as F₃ and F₄ populations showed high variations for all the physio-morphological traits under aerobic field conditions (Supplementary file T1). All the physio-morphological traits were showing immense variation in both seasons. Frequency distribution curves for various physio-morphological and root traits of MASARB25 × IB370 derived F₃ population and parental rice genotypes are shown (supplementary file Figure S1A and S1B). For panicle length, effective number of tillers/plant, 1000-grain weight, frequency distribution curves were parabolic while for grain length/breadth ratio and root thickness, frequency

distribution curve were skewed toward MASARB25. Frequency distribution curve for grain yield/plant and plant height were parabolic and tilted toward IB370.

In field phenotypic correlation coefficient analysis of MASARB25 × IB370 derived F₃ and F₄ population showed grain yield/plant has positive correlation between plant height (0.545 and 0.465) and grain length/breadth ratio (0.863 and 0.394) during both the seasons however, during the crop season 2014-15 grain yield showed significant positive correlation with effective number of tillers/plant (0.658), 1000-grain weight (0.934), panicle length (0.517) and root length (0.476) (Supplementary file T2 and T3). Root dry weight showed positive significant correlation with root length (0.456 and 0.513) during both the cropping seasons. Likewise, fresh root weight showed significantly positive correlation with root thickness (0.570 and 0.428) during both the crop seasons. During crop season 2015-16 effective tillers showed significant positive correlation with root length (0.482) and root thickness (0.772) (Supplementary file T2 and T3).

Significant positive correlation between grain yield and plant height, panicle number and effective number of tillers and thousand grain weights was identified under aerobic condition (Mirza *et al.* 1992; Girish *et al.* 2006; Ramesha *et al.* 2010; Nagaraju *et al.* 2013; Reddy *et al.* 2013; Ramanjneyulu *et al.* 2014; Kumar *et al.* 2014). Plant height had registered positive and significant association with grain yield per plant (Akhtar *et al.* 2011; Yadav *et al.* 2011; Seyoum *et al.* 2012). Significant and positive correlation between some root traits (root hair density, and lateral root and nodal root number) and grain yield under dry direct-seeded conditions was also reported (Henry *et al.* 2011; Sandhu *et al.* 2014; Kumar *et al.* 2014; Kharb *et al.* 2016; Kumar *et al.* 2016; Rani *et al.* 2017; Meena *et al.* 2018).

Genotyping

In present investigation allelic polymorphism in MASARB25 × IB370 F₃ populations for SSR markers showed 49.1% alleles under field and 53.3% alleles under net house were recorded in IB370 and 50.9% and 46.8% alleles were recorded for MASARB25 under field and net house, respectively during F₃ generation. In F₄ generation average, 49.6% alleles were from IB370 and 50.4% alleles from MASARB25 recorded under field condition while, in net house plants, 50.8% alleles were from MASARB25 and 49.1% were from IB 370 were recorded, respectively. In most cases, populations were skewed towards the respective aerobic rice parent and which is also clear from frequency distribution curves for the majority of agronomic and root traits. Clustering of genotypes of basmati variety was in a different group than lowland and aerobic indica rice. The similar results were also previously reported (Jain *et al.* 2004; Kovach *et al.* 2009; Sandhu *et al.* 2012). It indicated that aerobic rice varieties were developed from different indica × upland rice crosses and may have differential level of genetic content from the recurrent indica and donor upland rice varieties.

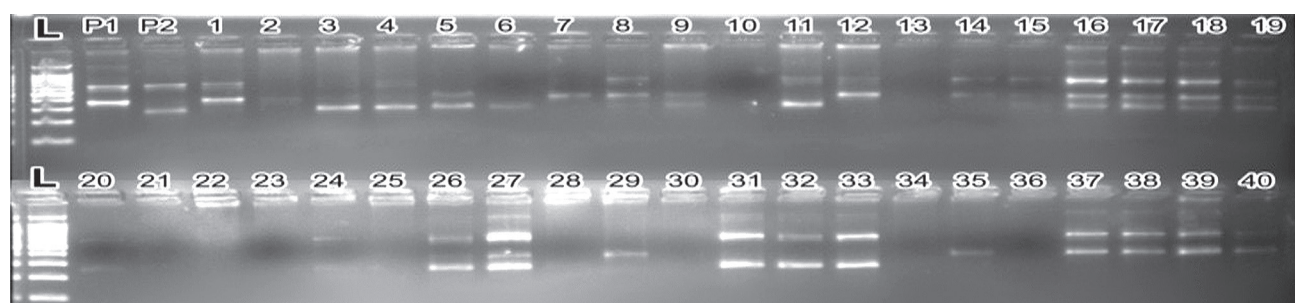


Fig 1 Agarose gel showing allelic status at *BAD2A* locus of MASARB25 × IB370 60 F_3 plants and parental rice varieties. Lane L-100 bp ladder, P1: MASARB25, P2: IB370, 1-60: selected F_3 plants.

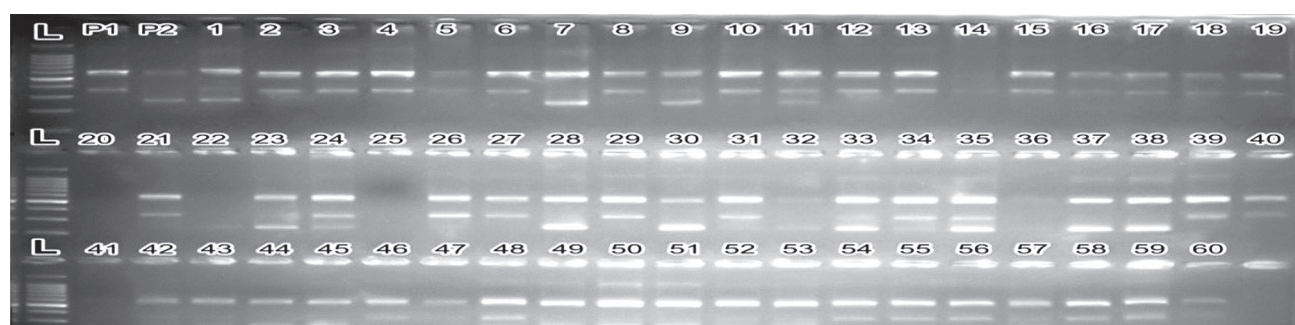


Fig 2 Agarose gel showing allelic status at *BAD2A* locus of MASARB25 × IB370 60 F_4 plants and parental rice varieties. Lane L-100 bp ladder, P1: IB370, P2: MASARB25, 1-60 F_4 progenies.

Basmati × aerobic indica derived F_3 and F_4 populations, being segregating populations, showed the presence of either two or three fragments, representing the homozygous for fragrance (585 and 257 bp alleles) or non-fragrance allele (585 and 355 bp alleles) and heterozygous state (585, 355 and 257 bp alleles), respectively. Under net house and field plants has the allelic profile at *BAD2A* locus in above three categories in the ratio 4:2:7 and 4:4:8 in F_3 and F_4 generations, respectively (Fig 1, 2 and supplementary file S2, S3). It shows perfect co-segregation with the trait of fragrance in the mapping population (Sakthivel *et al.* 2006; Kovach *et al.* 2009; Sandhu *et al.* 20014). Similarly variation for *BAD2* locus among fragrance and non-fragrance rice was also identified in various studies (Bradbury *et al.* 2005; Li *et al.* 2006; Bourgis *et al.* 2008; Fitzgerald *et al.* 2008; Kovach *et al.* 2009; Sandhu *et al.* 2012; Yeap *et al.* 2013). Plants having positive alleles were selected for further SSR marker genotyping for both generations. The chromosome wise positions of all of the markers used is provided (supplementary file S4).

Under field condition genetic diversity analyses of F_3 population were clustered in three groups at similarity coefficient of 0.49. All the progenies were placed in the same group while both parents were quite distinct and placed in different sub group. NTSYS-pc scaling exhibited in F_3 population was interspersed between the two parental lines with the distribution of most plants towards MASARB25 (Fig 3 and supplementary file S5). Likewise, in F_4 population three major groups were identified with similarity coefficient of 0.49. Major group, I consisted of MASARB25 and major

group III contained IB370 (Fig 4 and supplementary file S6). In field house plants genetic relationships and PCA scaling among this rice genotype exhibited that F_3 and F_4 population was interspersed between the two parental lines with distribution of most plants towards MASARB25. While, under net house condition F_3 and F_4 plant were clustered with the similarity coefficient of 0.48 and 0.43 and showed three and two major groups, respectively. The 13 net house F_3 plants were clustered in three major groups at the similarity coefficient of 0.48. Similarly most of the F_3 plants twelve out of thirteen F_3 plants from the net house experiments were place in same group other two group had MASARB25 and IB370 along with one F_3 plants respectively.

QTL analysis

In the present study, though size of four populations was small but several QTL were mapped for various agronomic traits promoting adaptation for aerobic conditions. These QTL should be considered as putative QTL and need to be confirmed using the large/stable populations derived from the respective crosses. Composite interval mapping identified total 16 QTLs on chromosomes 1, 2, 3, 4, 6, 9, 10 and 12 during both the years (Table 1).

In F_3 generation 10 QTLs were mapped and explained 1.7 to 72.2% phenotypic variation with the LOD score ranging from 2.88 to 5.51 (Fig 5). Two QTL for effective no. of tillers/plant were mapped on chromosome 12 (*qENT_{12.1}*) and 4 (*qENT_{4.1}*), explaining 63.63 % and 61.74% phenotypic variation which were linked with markers *RM415-RM512* and *RM551-RM3042*, respectively. Panicle length was

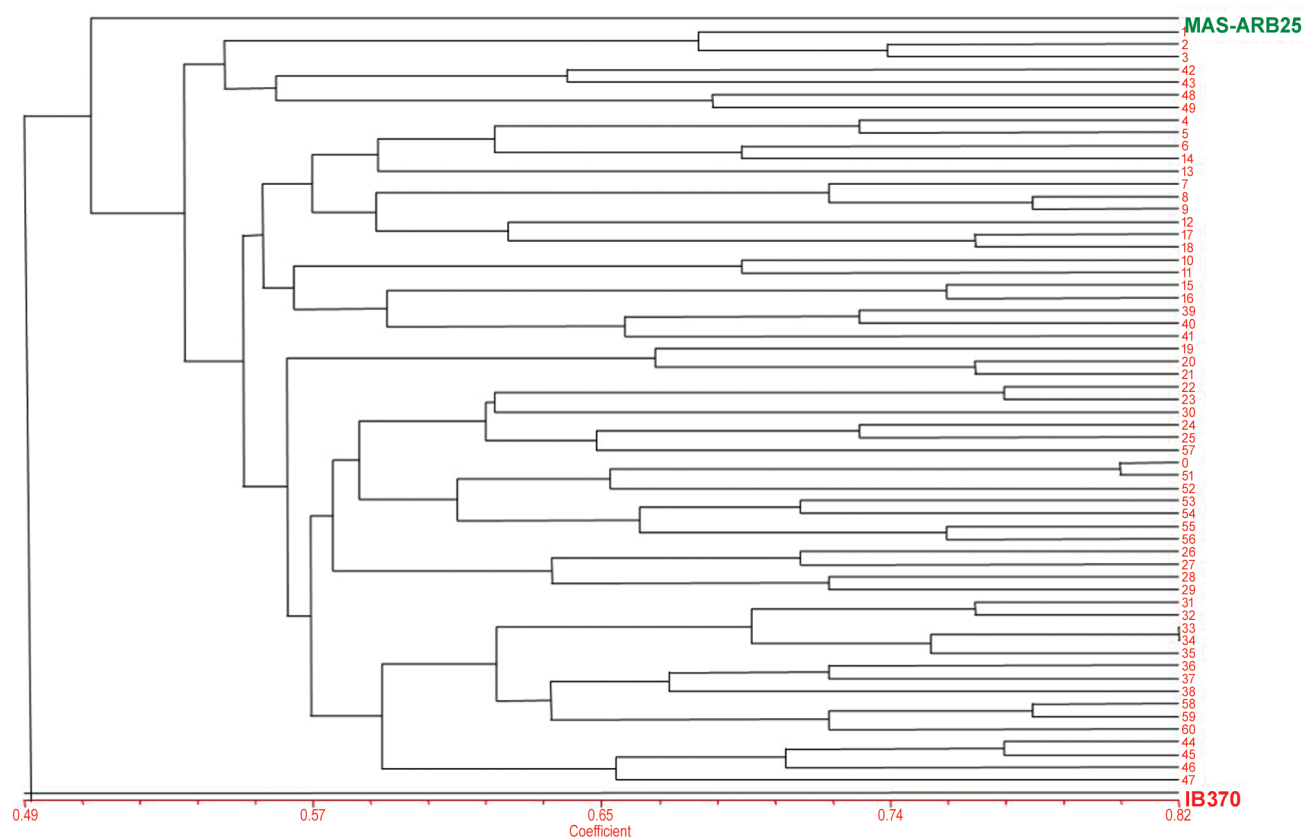


Fig 3 Dendrogram (NTSYS-pc) displaying diversity among 60 F₃ plants (MASARB25 × IB370) using allelic diversity data at 59 SSR loci.

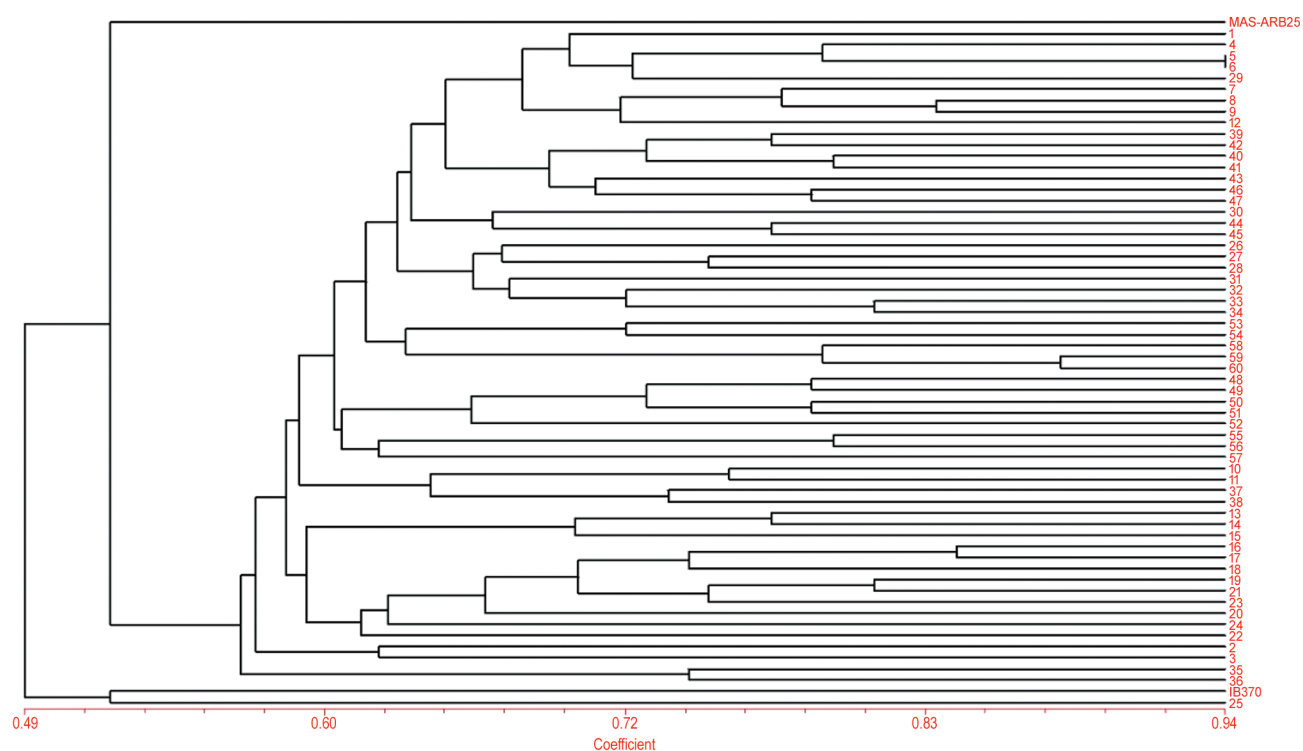


Fig 4 Dendrogram (NTSYS-pc) displaying diversity among 60 F₄ plants (MASARB25 × IB370) (grown in field) using allelic diversity data at 59 SSR loci.

Table 1 Identified QTL for physio-morphological traits promoting aerobic adaptation of MASARB25 × IB370 F³ and F⁴ population grown under aerobic conditions

Trait	QTL name	Chromosome number	Position (cM)	Flanking markers	Position of flanking markers (cM)	LOD	Additive effect	R ² %	DPE
<i>F₃ generation</i>									
Effective no. of tillers/plant	qENT _{12.1}	12	17.01	RM415-RM512	0.0-43.2	2.88	-1.6803	61.639	IB
	qENT _{4.1}	4	50.31	RM551-RM3042	20-69.8	4.49	-1.2609	61.744	IB
Panicle length	qPL _{4.1}	4	68.11	RM551-RM3042	20-69.8	4.08	-1.2152	71.045	IB
1000-grain weight	qTGW _{10.1}	10	25.91	RM239-RM294	25.2-87.1	4.41	-2.1859	72.731	IB
Grain length-breadth ratio	qLB _{1.1}	1	115.51	RM5-RM1088	94.9-150.7	3.98	0.4056	31.290	M
	qLB _{3.1}	3	78.91	RM426-RM503	27.6-153.9	5.51	-0.2836	69.048	IB
	qLB _{3.2}	3	101.91	RM426-RM503	27.6-153.9	5.13	-0.2822	1.758	IB
	qLB _{9.1}	9	73.41	RM434-RM205	57.7-114.7	3.60	-0.2596	66.546	IB
	qLB _{9.2}	9	82.41	RM434-RM205	57.7-114.7	3.31	-0.2445	3.150	IB
	qLB _{11.1}	11	31.01	RM4-RM208	5.2-102.9	3.27	-0.372	72.255	IB
<i>F₄ generation</i>									
Grain yield/plant	qGYP _{6.1}	6	61.21	RM217-RM345	26.2-123.9	2.90	2.8344	4.22	M
Grain length-breadth ratio	qLB _{2.1}	2	92.11	RM327-RM318	72.6-152.8	4.75	0.2842	0.091	M
	qLB _{6.1}	6	61.21	RM217-RM345	26.2-123.9	4.10	-0.4867	79.46	IB
	qLB _{6.2}	6	79.21	RM217-RM345	26.2-123.9	4.87	0.2905	61.47	M
	qLB _{6.3}	6	94.21	RM217-RM345	26.2-123.9	5.09	0.4841	61.25	M
	qLB _{9.1}	9	29.01	RM444-RM24093	3.3-42.9	3.80	0.0189	71.87	M

associated with QTL *qPL_{4.1}* located in the chromosome 4 with the linkage of markers *RM551-RM3042* explained 71.04 % phenotypic variation. The *qTGW10.1* in chromosome 10 was associated thousand grain weight explained 72.7 % phenotypic variation and linked with markers *RM239-RM294*. In *F₃* generation QTLs for grain length-breadth ratio were identified on chromosomes 1 (*qLB1.1*), 3 (*qLB3.1* and *qLB3.2*), 9 (*qLB9.1* and *qLB9.2*) and 11 (*qLB11.1*). Leila Bazrkar-Khatibani *et al* (2019) also reported seven QTLs for various traits such as grain length and quality traits in rice with phenotypic variance ranging up to 44%. Solis *et al.* (2018) also high phenotypic variance ranging between 30-34% in rice while mapping various yield related traits under drought conditions.

In *F₄* generation 6 QTLs for various phenotypic traits

analyzed were identified on chromosomes 2 (*qLB2.1*), 6 (*qLB6.1*, *qLB6.2*, *qLB6.3* and *qGYP6.1*) and 9 (*qLB9.1*) respectively. The phenotypic variation explained was ranged from 1.89 % to 48.67 % with the LOD score ranging from 2.90-4.87 and explained 1.7 to 72.2% phenotypic variation. The QTL identified in chromosome 9 for grain length-breadth ratio in *F₃* and *F₄* generation were different. QTL for grain length have been reported earlier on chromosomes 1, 2, 3 and 6 (Aluko *et al.* 2004; Fan *et al.* 2006; Lou *et al.* 2009). Wan *et al.* (2006) reported the six main-effects QTL for grain length in four environments on five rice chromosomes (2, 3, 5, 7 and 9). Among them, *qGL-3a*, was consistently detected in these four environments and mapped to the interval C80–C1677 in the centromeric region of chromosome 3. On the other hand, a loss of function

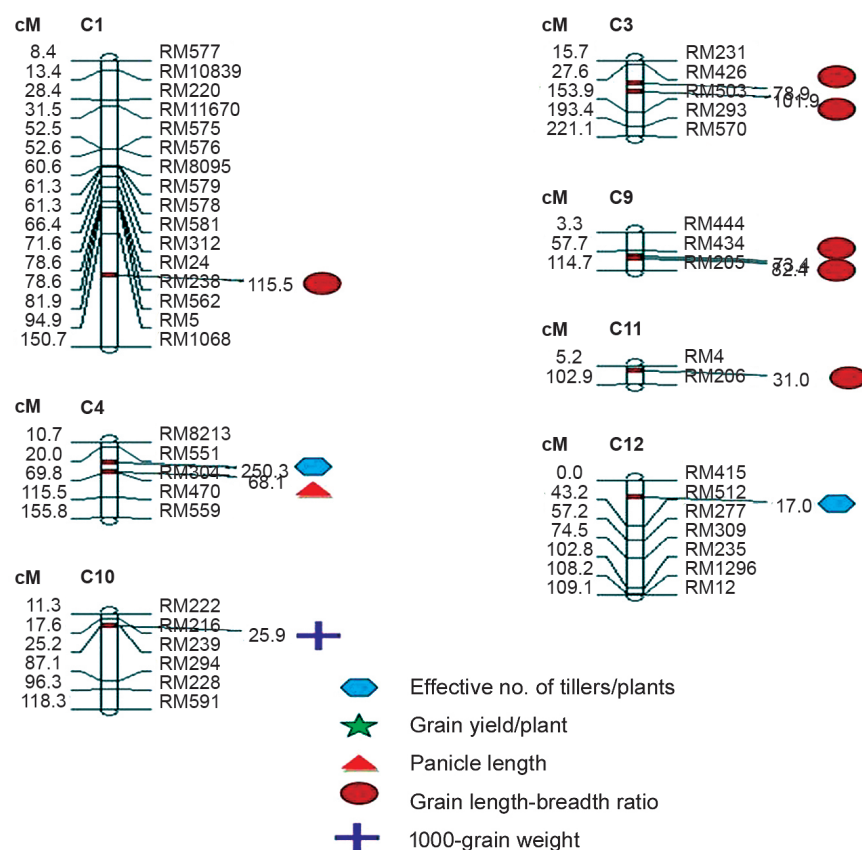


Fig 5 Chromosomal locations of QTL for physio-morphological traits identified in MASARB25 × IB370 F3 population via microsatellite marker analysis.

mutation in GW2, a QTL located on chromosome 2, affects the grain width and weight (Song *et al.* 2007). Sixteen QTL were mapped on Chromosome 1, 2, 3, 6, 7 and 9 for grain quality in RIL populations derived from indica × japonica crosses (Lou *et al.* 2009). For grain yield/plant, a QTL was detected on chromosome 6 (qGYP6.1) linked with markers RM217-RM345 explained 28.44% phenotypic variation. QTL mapping of drought resistance traits in the background of locally adapted indica/Basmati rice lines have been reported by several research groups in rice (Gomez *et al.* 2010; Sandhu *et al.* 2014; Venuprasad *et al.* 2009). Large-effect QTL for grain yield under drought has been identified (Bernier *et al.* 2007 and Venuprasad *et al.* 2009) and their successful introgression has established yield-advantage under drought (Swamy *et al.* 2013). Earlier, QTL for grain yield in rice have been reported on chromosome 2, 3, 5, 7 and 9 (Wan *et al.* 2006; Song *et al.* 2007; Weng *et al.* 2008). Sandhu *et al.* (2015, 2016) reported QTL for grain yield per plant (qGY8.1 at 56.3 cM) on chromosome 8 in the Aus276/3*IR64 BC2F4 population. Many SSR markers have been reported to be linked to QTL promoting aerobic adaptation in rice such as yield under drought (Venuprasad *et al.* 2009; Vikram *et al.* 2011), root length (Steele *et al.* 2007), basal root thickness (Qu *et al.* 2008) and root dry weight (Kanbar and Shashidhar 2004).

The identified QTL in present investigation showed high phenotypic variation however, the population size

was limited therefore, after validation of these QTLs could be used marker assisted selection for the improvement of rice under aerobic condition with keeping the basmati traits.

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