RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
48 (4) 402-415, 2016

PRELIMINARY QTL DETECTION FOR IMPROVING BASMATI RICE IN A F_2 POPULATION DERIVED FROM THE CROSS BETWEEN KERNEL BASMATI AND pLIA-1 CARRYING *Oryza longistaminata* CHROMOSOME

E. GICHUHI[1], E. HIMI[2], N. AHMED[3], H. TAKAHASHI[4] and M. MAEKAWA[2*]

[1]Graduate School of Environmental and Life science, Okayama University, Okayama, Japan.
[2]Institute of Plant Science and Resources, Okayama University, Kurashiki, Japan.
[3]Centre of Agricultural Biochemistry and Biotechnology, University of Agriculture, Faisalabad, Pakistan.
[4]Graduate School of Bioresource Sciences, Akita Prefectural University, Akita 010-0195, Japan.
*Corresponding author's email: mmaekawa@rib.okayama-u.ac.jp
Email addresses of co-authors: gichuhiemily@gmail.com, eikohimi@okayama-u.ac.jp, nisar.ahmed@uaf.edu.pk, h-takahashi@akita-pu.ac.jp

SUMMARY

Basmati is a premium grade type of rice variety highly valued in the world rice market due to its characteristic aroma and slender grains. However it is low yielding and susceptible to lodging under highly applied fertilizers. At the Institute of Plant Science and Resources (IPSR), a potential Low Input Adaptable line (pLIA-1) derived from *Oryza longistaminata* and Taichung-65 was developed under non-fertilized conditions. The line was characterized by thick culms and large panicles with a large primary and secondary branch numbers and many spikelets per panicle. To improve the low yield of Basmati, the large panicle of pLIA-1 and thick culm-base diameter is considered to be useful. Hence, QTL analysis was conducted using 88 genome-wide SSR markers in F_2 of the cross between pLIA-1 and Kernel Basmati. In total 21 QTLs for yield-related traits were identified in 2012 and 2013. The QTL for primary branch number was detected in the same location on chromosome 8 in both years and a QTL cluster for secondary branch number and spikelet number per panicle was identified on chromosome 1 in 2013 and in the combined data. In addition, two QTLs for the culm-base diameter on chromosomes 4 and 11 and 4 and 8 were also identified in 2013 and in the combined data, respectively. These results suggest that it is possible to utilize the favorable QTLs from pLIA-1 to improve Kernel Basmati's yield and confer lodging resistance.

Key words: *Oryza longistaminata*, pLIA (potential Low input-adaptable), Basmati, QTL, yield-related trait

Key findings: In this study, new QTLs for yield-related traits were identified in a cross between an introgression line carrying *Oryza longistaminata* chromosome segments and Kernel Basmati. The results suggested that *Oryza longistaminata* might have the potential to improve Kernel Basmati's yield.

Manuscript received: May 1, 2016; Decision on manuscript: August 11, 2016; Manuscript accepted: October 8, 2016.

Communicating Editor: Bertrand Collard

INTRODUCTION

Recently food shortages have become a serious problem due to increased demand resulting from population growth (Brown and Funk, 2008; Takeda and Matsuoka, 2008) which is estimated to reach 9 billion by 2050 (Qi *et al*., 2012). Therefore, it is necessary to increase grain

production in order to meet the global food demand. Since rice is an important staple food and feeds more than half of the world's population (Jiang *et al*., 2013), increasing its yields is fundamental to meet food security in the world.

Through the green revolution, greater yield improvements among cereal crops including rice were obtained with the utilization of the semi-dwarf gene (*sd1*). The green revolution brought the high-yield crop varieties and high-input management practices currently utilized in agricultural systems, prevented mass starvation and improved living standards throughout the world (Brummer *et al*., 2011). However, these breakthroughs were not applicable to all commercial rice varieties. Basmati are premium grade rice varieties that are very popular in the world. It is highly preferred among the consumers due to its characteristic fragrance, superfine long grains and excellent elongation (Khush *et al*., 2002). It has a higher market price compared to other rice varieties. Hence, it is a preferred choice for production among farmers. However, it is tall, photosensitive and susceptible to biotic stresses. It has low yields and is unresponsive to fertilizer; increased supply of nitrogen fertilizer does not translate into increase in yield (Jhang *et al*., 2006). At maturity, it is very susceptible to lodging due to thin weak stems. Attempts to improve Basmati's yield have been unsuccessful due to the complex nature of Basmati rice grain quality traits and poor combining ability with other rice genotypes (Khush and dela Cruz, 1998). Therefore very few studies involving QTL analysis using Basmati crosses are available and the few available mainly focus on the superior grain qualities of aroma (Li *et al*., 2004; Wan *et al*., 2006; Singh *et al*., 2012) and kernel elongation (Xing *et al*., 2006; Govindaraj *et al*., 2005). However, improving Basmati's yields is still highly important and the genetics of key yield-related traits needs to be advanced extensively.

One of the main advantages of distant crosses is the possibility to introgress genetic variation. Introgression of favorable alleles from wild relatives of *Oryza sativa* to extend its genetic diversity has recently been attracting increasing attention due to the narrow genetic diversity of modern varieties (Fu *et al*., 2010). However, attempts to transfer the genes controlling quantitative traits from wild relatives to cultivated elite varieties of rice have been limited and unsuccessful. This is because such distant crosses usually bring about sterility problems and most importantly linkage drag-related problems. This makes it difficult to select and use superior phenotypes for breeding purposes (Brondani *et al*., 2002). To solve this problem, it is important to develop introgression lines carrying favorable traits in the progeny of the distantly remote cross between *O. sativa* and wild species. Gichuhi *et al*. (2012) reported that introgression lines having a thick culm and large spikelet number were selected in the progeny of the cross between *O. longistaminata* and T-65 under non-fertilized conditions. *Oryza longistaminata*; a wild rice species belonging to the AA genome and broadly distributed throughout tropical Africa, shows a vigorous biomass in low-input conditions (Sacks *et al*., 2003). *O. longistaminata* known as Mpunga wa Majani (MwM), collected at Mombasa, Kenya, was successfully crossed with *Oryza sativa*, Taichung-65, a japonica variety and large biomass lines selected under non-fertilized conditions. These lines were observed to have important traits for high productivity under low-input conditions. Their characterization under both fertilized and non-fertilized conditions showed that they have potential for low-input adaptability, hence they were named potential Low-Input Adaptable (pLIA) lines (Gichuhi *et al*., 2012). These lines exhibited important traits such as large panicles with many primary and secondary branches and spikelets per panicle and were resistant to lodging due to their very thick culms. To improve the low yield of Basmati, the large spikelet number per panicle and thick culm-base diameter of pLIA lines are considered to be useful. Therefore, this study focused on identifying the important QTLs for yield-related traits in the F_2 of the cross between pLIA-1 and Kernel Basmati that could be utilized to improve the low productivity of Basmati.

MATERIALS AND METHODS

Plant materials and trait measurement

Kernel Basmati was crossed with pLIA-1 line derived from F_2 of the cross between T-65 and MwM, *O. longistaminata*, under non-fertilized conditions. In 2012 and 2013, 55 F_2 plants and 80 F_2 plants of this cross, respectively, were grown and used for analysis of QTLs for several agronomic traits together with Kernel Basmati and pLIA-1. Sixteen plants of Kernel Basmati and pLIA-1 were grown with 2 replicates. The plants were planted with a spacing of 40 cm between rows and 15 cm between plants under non-fertilized conditions at the experimental field of the Institute of Plant Science and Resources, Okayama University, Japan. Flowering time was determined as from sowing date to emergence of the first panicle. At harvesting, culm length, panicle length, panicle number per plant, flag leaf length, fresh culm-base diameter at 5 cm above ground and panicle-base diameter were measured. After drying, primary branch number, secondary branch number and spikelet number per panicle were examined. Spikelet fertility in percentage was calculated by dividing the fertile spikelet number per panicle by the total spikelet number per panicle on the tallest tiller of each plant. To measure grain weight, 50 grains per plant were triplicated. Grain length and grain width were measured from a sample of 30 randomly selected hulled seeds from each plant by scanning the seeds and using Image/J (http://imagej.nih.gov/ij/) to analyze the grain shape.

DNA extraction

Leaf samples from seedlings of the F_2 plants were collected and dried at 50^{o}C overnight. The dried leaf samples were crushed by a Multi-beads shocker (YASUI KIKAI, Japan) and 400µl of extraction buffer (200 mM Tris-HCl (pH 7.5), 250 mM NaCl, and 25 mM EDTA, 0.5% SDS) (Maekawa *et al*., 2005) was added to each sample and then strongly mixed using a microtube mixer (MT-360; TOMY, Japan) at maximum speed for 15 minutes. The mixture was centrifuged at 15000 rpm at 4^{o}C for 10 minutes. After centrifugation, the supernatant transferred into a 1.5-ml tube was gently mixed with 300 µl of cold isopropanol. This mixture was then centrifuged at 15000 rpm at 4^{o}C for 10 minutes. The pellet was rinsed with 500 µl of 70% EtOH. After centrifugation at 15000 rpm at 4^{o}C for 5 minutes, the pellet was dried at room temperature and dissolved with 100 µl of TE.

QTL analysis

Genotyping of the F_2 plants was conducted using 88 markers out of 115 SSR markers distributed genome-widely which were found to be polymorphic between pLIA-1 and Kernel Basmati. The PCR reaction was prepared by mixing 3.5 µl of distilled water, 20 pmol of 0.5 µl forward primer, 20 pmol of 0.5 µl reverse primer, 5 µl of Quick Taq (TOYOBO, Japan) and 5ng of the extracted DNA. Amplification was performed as follows: the initial denaturing step at 95^{o}C for 7min, followed by 30 cycles for 45sec at 95^{o}C, 30 sec at 55^{o}C and 30 sec at 72^{o}C. Electrophoresis was performed using 3% agarose gel at 100 voltage for 90 minutes. The band pattern of the samples was observed under UV-lighting after staining with ethidium bromide.

A linkage map of 88 SSR markers was constructed using MAPMAKER 3.0. Composite Interval mapping (CIM) was performed for QTL analysis in F_2 populations using Windows QTL Cartographer 2.5 (Wang *et al*., 2007). As pointed out by Raghavan and Collard (2012), the F_2 population size in each year was small for reliability of QTLs obtained. To improve the defect due to small population size, the data obtained in 2012 and 2013 were standardized by applying the following equation:

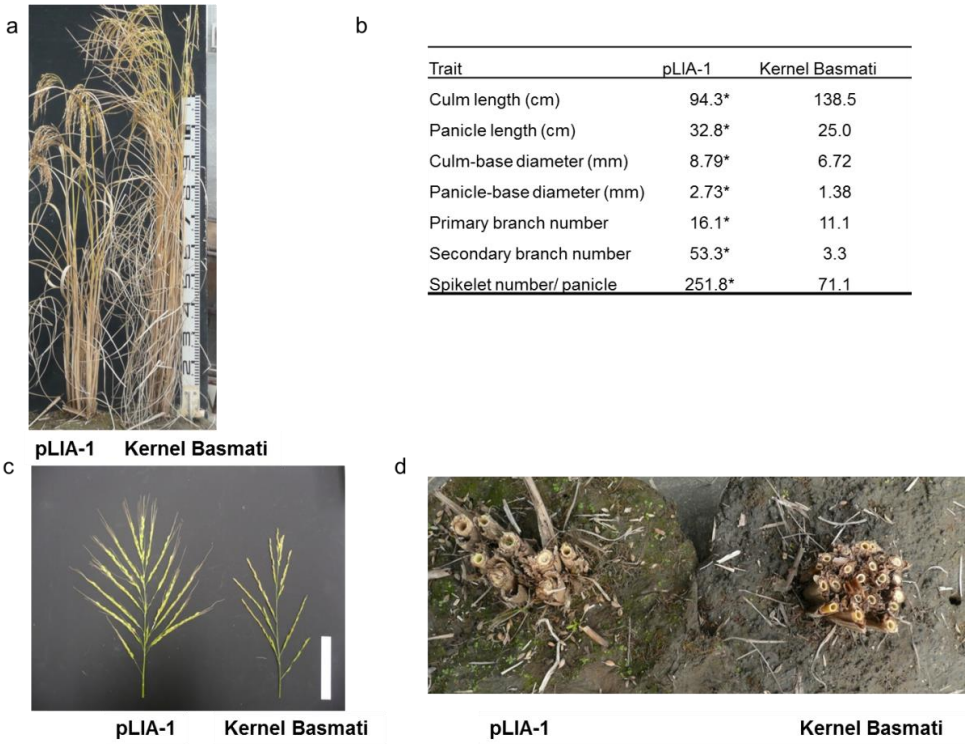
$$Z = \frac{\text{individual value-mean of the population}}{\text{standard deviation of the population}}$$

Then, each Z value for both years was added to the two-year population mean. After that, the combined data for 135 F_2 plants was subjected to QTL analysis. In all of the investigated traits, the LOD threshold was determined at the experiment-wise significant level of 5% by 1000 permutation test.

RESULTS AND DISCUSSION

Phenotypic variations

The parental plants, pLIA-1 and Kernel Basmati have tall statures and Kernel Basmati was much taller than pLIA-1 (Figure 1a and 1b). Kernel Basmati is very susceptible to lodging at maturity. On the other hand, pLIA-1 is resistant to lodging due to a significantly thicker culm-base diameter compared to that of Kernel Basmati (Figure 1b and 1d). In comparison to pLAI-1, Kernel Basmati's panicle was smaller in size and had few primary and secondary branches which consequently led to a significantly lower spikelet number per panicle (Figure 1b and 1c). The above superior traits of pLIA-1 could be useful for improving Kernel Basmati's yield. Therefore, pLIA-1 was crossed with Kernel Basmati and the F_2 plants of this cross were evaluated for various agronomic traits and QTLs for yield-related traits that are important for improving Basmati's yield. Normal distribution patterns were observed in most of the traits measured in both years (Figures 2 and 3). Transgressive segregants were observed in all of the traits recorded except for spikelet number per panicle and grain width in 2012 (Figure 2) and secondary branch number and grain length in 2013 (Figure 3). Transgressive segregation is an important evidence of the favorable effect of such introgressions (Brondani *et al.*, 2002). In both years, culm-base diameter and panicle-base diameter were significantly positively correlated to panicle length, primary branch number, spikelet number per panicle and to each other. Panicle length was significantly positively correlated to panicle-related traits (primary branch number, secondary branch number and spikelet number per panicle). The panicle-related traits were also strongly correlated to each other in both years (Tables 1 and 2).



Trait	pLIA-1	Kernel Basmati
Culm length (cm)	94.3*	138.5
Panicle length (cm)	32.8*	25.0
Culm-base diameter (mm)	8.79*	6.72
Panicle-base diameter (mm)	2.73*	1.38
Primary branch number	16.1*	11.1
Secondary branch number	53.3*	3.3
Spikelet number/ panicle	251.8*	71.1

Figure 1. Morphological characteristics of pLIA-1 and Kernel Basmati. (a) pLIA-1and Kernel Basmati at maturity. (b) Agronomic performance of pLIA-1 and Kernel Basmati. * indicates significant difference at 5% level by *t*-test. (c) Panicles of pLIA-1 and Kernel Basmati. Bar = 10 cm. (d) Culm-base thickness of pLIA-1 and Kernel Basmati.

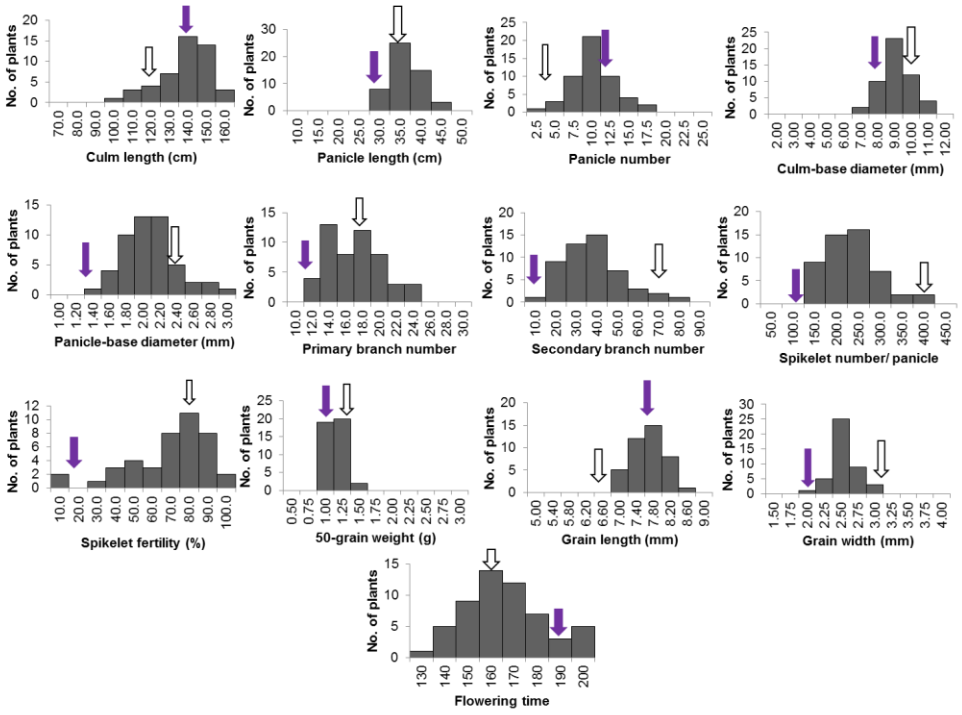


Figure 2. Frequency distribution of agronomic traits in F_2 of the cross between pLIA-1 and Kernel Basmati in 2012. White and purple arrows indicate mean values of pLIA-1 and Kernel Basmati, respectively.

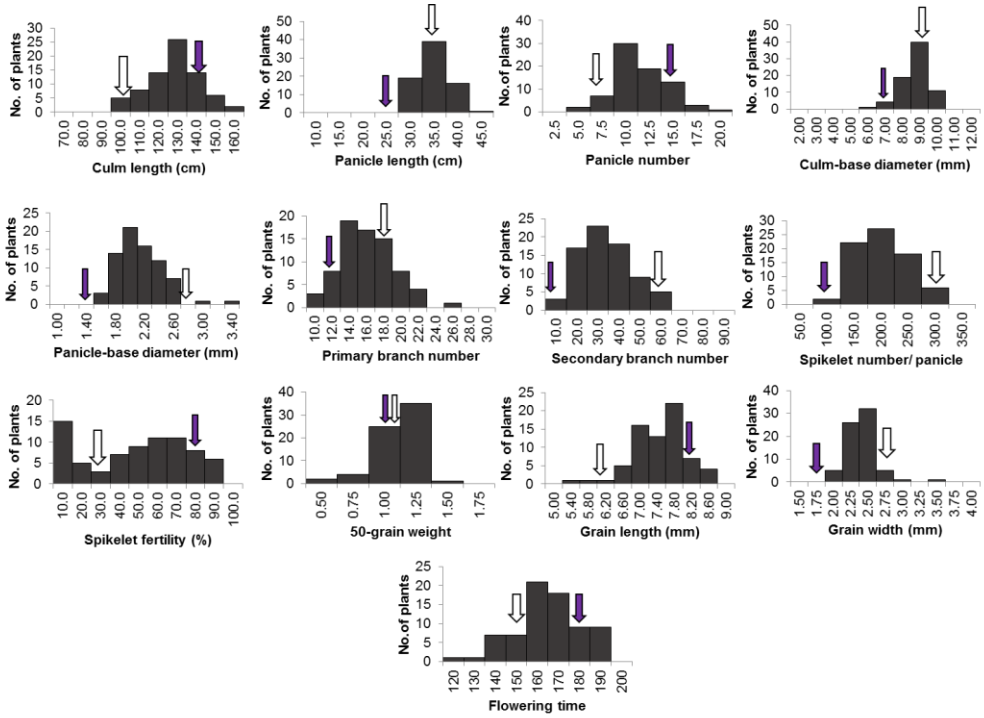


Figure 3. Frequency distribution of agronomic traits in F_2 of the cross between pLIA-1 and Kernel Basmati in 2013. White and purple arrows indicate mean values of pLIA-1 and Kernel Basmati, respectively.

Table 1. Correlation coefficient of yield-related traits in F_2 of the cross between pLIA-1 and Kernel Basmati in 2012.

	Culm length (cm)	Panicle length (cm)	Panicle number	Culm-base diameter (mm)	Panicle-base diameter (mm)	Flag leaf length (cm)	Primary branch number	Secondary branch number	Spikelet number/ panicle	Spikelet fertility (%)	50-grain weight (g)	Grain length (mm)	Grain width (mm)
Panicle length (cm)	0.334*												
Panicle number	0.157	-0.068											
Culm-base diameter (mm)	0.186	0.329*	0.051										
Panicle-base diameter (mm)	0.147	0.466*	0.009	0.663*									
Flag leaf length (cm)	-0.015	0.063	0.013	0.136	0.180								
Primary branch number	0.142	0.295*	0.021	0.542*	0.606*	0.203							
Secondary branch number	0.148	0.393*	-0.116	0.430*	0.674*	0.291*	0.637*						
Spikelet number/ panicle	0.170	0.406*	-0.058	0.493*	0.716*	0.245	0.753*	0.967*					
Spikelet fertility (%)	-0.075	0.049	-0.023	0.089	0.114	0.031	0.110	0.035	0.052				
50-grain weight (g)	0.002	-0.028	-0.073	0.210	0.038	-0.194	0.120	0.092	0.103	0.083			
Grain length (mm)	0.050	-0.063	-0.017	0.139	-0.076	0.023	-0.003	0.183	0.139	-0.031	0.562*		
Grain width (mm)	-0.014	-0.039	-0.071	0.100	-0.011	-0.186	-0.045	-0.175	-0.133	0.084	0.737*	0.030	
Flowering time	-0.295*	-0.595*	0.043	-0.048	-0.200	0.024	-0.394*	-0.389*	-0.445*	-0.090	-0.096	0.050	-0.002

*Significant at 5% level.

Table 2. Correlation coefficient of yield-related traits in F_2 of the cross between pLIA-1 and Kernel Basmati in 2013.

	Culm length (cm)	Panicle length (cm)	Panicle number	Culm-base diameter (mm)	Panicle-base diameter (mm)	Flag leaf length (cm)	Primary branch number	Secondary branch number	Spikelet number/ panicle	Spikelet fertility (%)	50-grain weight (g)	Grain length (mm)	Grain width (mm)
Panicle length (cm)	0.025												
Panicle number	0.339*	0.139											
Culm-base diameter (mm)	-0.020	0.276*	-0.012										
Panicle-base diameter (mm)	-0.109	0.449*	-0.110	0.453*									
Flag leaf length (cm)	0.105	0.200	-0.197	-0.099	0.234*								
Primary branch number	-0.155	0.275*	0.025	0.349*	0.657*	0.151							
Secondary branch number	0.068	0.328*	-0.048	0.211	0.757*	0.257*	0.455*						
Spikelet number/ panicle	-0.008	0.390*	-0.020	0.319*	0.839*	0.222*	0.677*	0.943*					
Spikelet fertility (%)	0.534*	0.103	0.281*	-0.128	-0.027	0.024	-0.039	-0.015	-0.016				
50-grain weight (g)	0.183	0.154	0.123	-0.037	-0.043	-0.045	0.097	0.067	0.039	0.021			
Grain length (mm)	0.152	0.045	0.040	0.080	0.076	-0.115	0.029	0.214	0.177	-0.001	0.429*		
Grain width (mm)	-0.006	0.099	-0.100	-0.007	0.030	0.251*	0.145	-0.076	-0.019	0.064	0.021	-0.387*	
Flowering time	-0.108	-0.147	0.010	0.142	-0.059	-0.354*	-0.139	-0.078	-0.113	-0.049	-0.106	0.028	-0.258*

*Significant at 5% level.

QTLs for yield-related traits

In order to introduce the favorite characteristics of pLIA-1 into Kernel Basmati, QTL analysis for yield-related traits was conducted in F_2 of the cross between pLIA-1 and Kernel Basmati in 2012 and 2013. However, the F_2 population size was small in both years. The accuracy and reliability of QTLs detected in the small population size were reduced as Raghavan and Collard (2012) reported. Further, Collard and Mackill (2008) intensively reviewed that the accuracy and reliability of QTLs are inevitable for marker-assisted selection. Therefore, to complement the defect due to the small population size in each year, combined data derived from standardization values of individual plants in each year was also used for QTL analysis.

One QTL for culm length was identified on chromosome 8 in 2013 and combined data. The Kernel Basmati allele at this QTL increased culm length (Table 3). Although two QTLs for number of panicles per plant were identified on chromosomes 2 and 3 in 2013, two QTLs were found on chromosomes 3 and 8 in the combined data. The QTL found on chromosome 3 was located around RM55 in 2013 and in the combined data. The pLIA-1 allele for the QTL on chromosome 3 decreased the trait. Two QTLs for culm-base diameter were mapped on chromosomes 4 and 11 in 2013 and two QTLs were estimated on chromosomes 4 and 8 in the combined data. One QTL was detected around RM252 on chromosome 4 in 2013 and combined data. QTLs observed in the combined data showed higher LOD scores and phenotypic variances than those in 2013. The pLIA-1 allele at QTLs increased culm-base diameter (Table 3). Although two QTLs for panicle-base diameter were identified on chromosomes 1 and 11 in 2012, only one QTL was found on chromosome 11 in the combined data. The QTL that showed high phenotypic variation on chromosome 1 in 2012 was not observed in combined data. Thus, it was likely that the QTL located on chromosome 11 controlled panicle-base diameter. The pLIA-1 allele at the QTL on chromosome 11 had a positive contribution to the trait (Table 3). For primary branch number, a single QTL was detected around RM3634 on chromosome 8 in both years. In the combined data, two QTLs were observed on chromosomes 8 and 11. The QTL found in the vicinity of RM6976 on chromosome 8 might be the same as that in each year since RM6976 was closely located to RM3634. The QTL on chromosome 8 was detected in both years and in the combined data with high LOD scores and high phenotypic variances, suggesting that this QTL might govern primary branch number. The pLIA-1 allele at QTL on chromosome 8 increased primary branch number (Table 3). Previously a QTL (*WFP*) encoding *OsSPL14* gene was identified near the same location and was found to regulate panicle branching (Miura *et al*., 2010). Large panicles containing high primary branch number are important for increasing rice yields. On the other hand, only one QTL was estimated around RM6324 on chromosome 1 in 2013 and combined data with very high phenotypic variances. It was found that the pLIA-1 allele at this QTL increased secondary branch number (Table 3). Two QTLs for spikelet number per panicle were detected on chromosomes 1 and 7 in 2013 and a QTL was found on chromosome 1 in the combined data. Because RM6324 was closely linked to RM1331 on chromosome 1, the QTL detected on chromosome 1 was likely important for spikelet number per panicle. A QTL for secondary branch number was also presumed to be located around RM6324 on chromosome 1. Since a high correlation between secondary branch number and spikelet number per panicle was observed in both years, it is therefore possible that spikelet number per panicle was controlled by secondary branch number. Actually, Ashikari *et al*. (2005) reported that *Gn1a* which is responsible for secondary branch number was located around this region. These results suggested that the large spikelet number of pLIA-1 which is an important parameter for high yield might be caused by two QTLs at the distal end of chromosomes 1 and 8.

Table 3. QTLs for yield-related traits detected in F_2 of the cross between pLIA-1 and Kernel Basmati in 2012, 2013 and combined data from both seasons.

Trait	Year	Chr.	Marker	Position (cM)	LOD	Additive effect	Dominance effect	r^2
Culm	2013	8	RM3634	+0.0	5.2	-9.04	4.98	0.22
length	2012 & 2013 combined	8	RM3634	+0.0	5.1	-0.47	0.31	0.13
Panicle	2013	2	RM1385	+0.0	3.5	0.94	1.79	0.14
number		3	RM55	+48.0	4.1	-1.46	1.15	0.20
	2012 & 2013 combined	3	RM55	+5.0	4.1	-0.60	0.07	0.14
		8	RM6976	+0.0	4.0	-0.27	0.60	0.11
Culm-	2013	4	RM252	+21.0	4.4	0.49	0.08	0.21
base		11	RM286	+18.0	4.0	0.17	0.54	0.13
diameter	2012 & 2013 combined	4	RM252	+15.0	6.8	0.65	0.03	0.23
		8	RM1345	+0.0	6.6	0.54	0.28	0.16
Panicle-	2012	1	RM23	+36.0	4.0	0.32	0.05	0.37
base		11	RM21	+0.0	3.8	0.10	-0.31	0.20
diameter	2012 & 2013 combined	11	RM21	+0.0	4.1	0.19	-0.62	0.11
Primary	2012	8	RM3634	+18.0	4.2	2.85	0.97	0.37
branch	2013	8	RM3634	+0.0	7.7	2.52	-0.39	0.32
number	2012 & 2013 combined	8	RM6976	+5.0	9.8	0.72	0.05	0.27
		11	RM21	+5.0	3.9	0.32	-0.54	0.11
Secondary	2013	1	RM6324	+27.0	5.7	14.28	-0.36	0.65
branch number	2012 & 2013 combined	1	RM6324	+20.0	6.4	0.99	-0.21	0.47
Spikelet	2013	1	RM1331	+0.0	5.6	31.01	16.28	0.24
number/		7	LM7-2	+21.0	4.5	-35.05	-40.33	0.40
panicle	2012 & 2013 combined	1	RM6324	+25.0	5.8	1.10	-0.01	0.57
Spikelet fertility	2012	6	LM6-7	+8.0	6.1	-26.09	14.02	0.50
50-grain	2013	6	RM204	+0.0	11.5	0.17	0.14	0.48
weight	2012 & 2013 combined	6	RM204	+0.0	7.6	0.71	0.51	0.25
Grain	2012	6	P06	+16.0	5.2	0.42	0.06	0.49
length	2013	3	RM231	+15.0	3.5	-0.32	0.22	0.14
		6	RM3	+18.0	4.9	-0.57	0.14	0.36
		6	RM204	+0.0	3.7	0.31	0.23	0.14
	2012 & 2013 combined	6	RM3	+25.0	6.6	-0.90	0.41	0.32
		6	P06	+5.0	4.1	0.50	0.32	0.14
Grain	2013	1	RM9	+21.0	6.4	-0.54	-0.57	0.33
width		12	RM27970	+3.0	8.7	-0.54	-0.57	0.33
	2012 & 2013 combined	3	RM55	+10.0	4.3	-0.41	0.38	0.13
		5	LM5-3	+10.0	4.7	0.67	-0.24	0.23
		8	RM1345	+15.0	3.5	0.57	-0.17	0.18
		9	RM1328	+20.0	4.2	1.61	-1.61	0.26
Flowering	2012	6	RM190	+26.0	8.9	-14.88	-7.08	0.46
time	2013	6	RM190	+3.0	12.8	-17.28	-8.66	0.50
	2012 & 2013 combined	6	RM204	+0.0	20.5	-1.06	-0.59	0.46

A single QTL for spikelet fertility was detected on chromosome 6 in 2012. Although several LOD score peaks were observed in 2013 and in the combined data, no significant peaks were obtained (data not shown). The complicated segregations for spikelet fertility in F_2s were shown in Figures 2 and 3, suggesting that several genes with minor effects might have segregated.

One QTL for 50-grain weight was identified on chromosome 6 in 2013 and in the combined data (Table 3). The pLIA-1 allele for this QTL increased the grain weight with a high phenotypic variance.

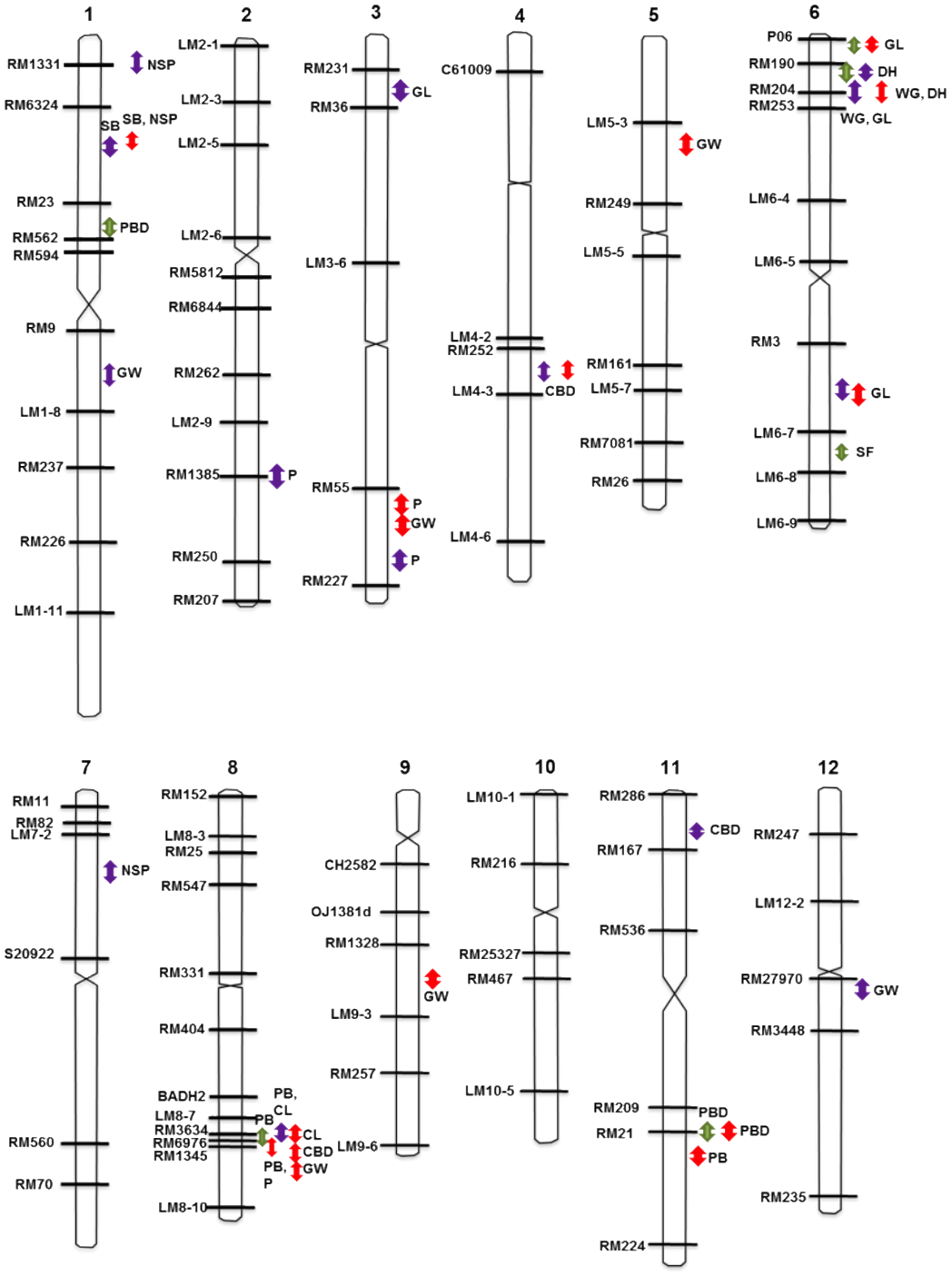


Figure 4. QTLs for the yield-related traits detected in F_2 of the cross between pLIA-1 and Kernel Basmati. CL; culm length. P; panicle number. CBD; culm-base diameter. PBD; panicle-base diameter. PB; primary branch number. SB; secondary branch number. NSP; spikelet number per panicle. SF; spikelet fertility. WG; 50-grain weight. GL; grain length. GW; grain width. DH; flowering time. Two headed green, purple and red arrows indicate tentative position of QTLs detected in 2012, 2013, and in combined data from both seasons, respectively.

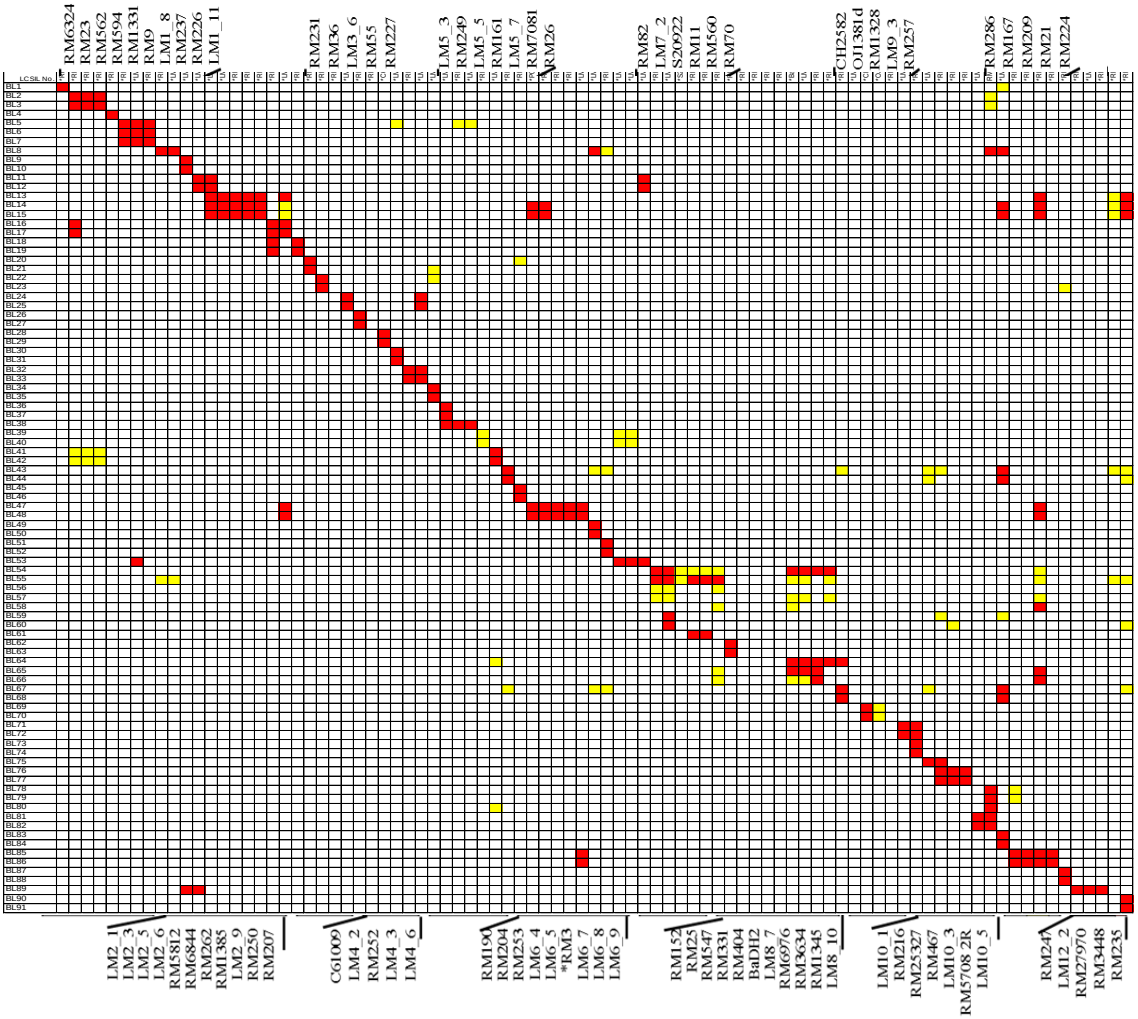


Figure 5. Longistaminata Chromosome Segment Introgression lines (LCSILs) carrying pLIA-1 chromosome segments in Kernel Basmati background. Red and yellow parts represent pLIA-1 genotype and heterozygote in Kernel Basmati genetic background, respectively.

For grain length, one QTL and 3 QTLs were detected at the distal region of short arm of chromosome 6 in 2012 and chromosomes 3 and 6 in 2013, respectively. In the combined data, 2 QTLs were mapped around P06 and RM3 on chromosome 6. The QTL located around RM3 showed higher phenotypic variation, with negative additive effect to grain length contributed by pLIA-1 allele, than that around P06. On the other hand, the QTL around P06 was likely the same as that around RM204 found in 2013 because P06 was closely linked to RM204 at the distal end of chromosome 6. The allele of pLIA-1 at this QTL elongated the grains (Table 3). At the distal end of chromosome 6, a QTL for grain length was detected and it was presumed to have some effect to grain weight. Actually, a strong positive correlation between 50-grain weight and grain length was observed in 2012 and 2013 (Tables 1 and 2). On the other hand, two major QTLs for grain width were detected on chromosomes 1 and 12 in 2013. However, QTLs in the combined data were found to be mapped on chromosomes 3, 5, 8 and 9. Although small LOD peaks were observed around RM1345 and RM1328 of chromosomes 8 and 9, respectively, in 2013, different QTLs detection in 2013 and in the combined data

might be caused by small variation in grain length (Figure 2) and poor maturation of grains due to late heading.

A QTL for flowering time was mapped around RM190 and RM204 on chromosome 6 in both years and in the combined data, respectively. Since RM190 was closely linked to RM204, a single QTL for flowering time was presumed to be detected at the distal end of short arm of chromosome 6. The pLIA-1 allele at the detected QTL shortened flowering time (Table 3). This QTL region is near the *Hd3a* (Kojima *et al.*, 2013) and *RFT1* genes that control flowering in rice (Ogiso-Tanaka *et al.*, 2013). It is likely that the detected QTL was related to *Hd3a* and *RFT1*.

Through QTL analysis for agronomic traits in the F_2 of the cross between pLIA-1 and Kernel Basmati, important QTLs were found to be localized at the distal ends of short arm of chromosome 1, short arm of chromosome 6 and long arm of chromosome 8 (Figure 4).

Improving Kernel Basmati's yield is important since breeding high yielding varieties carrying Basmati's grain qualities has been unsuccessful (Vemireddy *et al*., 2015). Therefore, important QTLs for yield-related traits that lead to high yields should be introduced into Kernel Basmati. The QTLs for thick culms and large panicles identified in this study can be utilized to improve Kernel Basmati. Through QTL pyramiding using a large population, it is possible to introduce these QTLs into Kernel Basmati for developing higher yielding Kernel Basmati that is resistant to lodging. Therefore, we have been developing Longistaminata Chromosome Segment Introgression lines (LCSILs) in Kernel Basmati background as shown in Figure 5. These lines could be used for fine mapping of individual QTLs and pyramiding of important QTLs to improve Kernel Basmati as Ramos *et al*. (2016) pointed out that CSSLs developed using *Oryza longistaminata* present the potential for new sources of genes/QTLs for valuable agronomic traits.

CONCLUSION

The pLIA-1 developed from the distantly remote cross between *Oryza sativa* and *O. longistaminata* is characterized of thick culm and large spikelet number under non-fertilized conditions. QTL analysis for agronomic traits in F_2 of the cross between pLIA-1 and Kernel Basmati revealed that some important QTL clusters for yield-related traits were located at the distal end of the short arm of chromosome 1, the short arm of chromosome 6 and the long arm of chromosome 8. These QTLs could be useful for improving the defective traits of Kernel Basmati.

ACKNOWLEDGEMENT

This research was funded by the Japan Science and Technology Agency (JST)/Japan International Cooperation Agency (JICA), the Science and Technology Research Partnership for Sustainable Development (SATREPS) and the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

REFERENCES

Ashikari M, Sakakibara H, Lin S, Yamamoto T, Takashi T, Nishimura A, Angeles ER, Qian Q, Kitano H, Matsuoka M (2005). Cytokinin oxidse regulates rice grain production. *Science* 309: 741-745.

Brondani C, Rangel PHN, Brondani RPV, Ferreira ME (2002). QTL mapping and introgression of yield-related traits from *Oryza glumaepatula* to cultivated rice (*Oryza sativa*) using microsatellite markers. *Theor. Appl. Genet.* 104:1192–1203.

Brown ME, Funk CC (2008). Food security under climate change. *Science* 319: 580-581.

Brummer CE, Barber WT, Collier SM, Cox TS, Johnson R, Murray SC (2011). Plant breeding for harmony between agriculture and the environment. *Front. Ecol. Environ.* 9:561–568.

Collard BCY, Mackill DJ (2008). Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Phil. Trans. R. Soc. B* 363: 557-572.

Fu Q, Zhang P, Tan L, Zhu Z, Ma D, Fu Y, Zhan X, Cai H, Sun C (2010). Analysis of QTLs for yield-related traits in Yuanjiang common wild rice (*Oryza rufipogon* Griff.). *J. Genet. Genomics* 37: 147−157.

Gichuhi EW, Himi E, Takahashi H, Maekawa M (2012). *Oryza longistaminata*'s chromosome segments are responsible for agronomically important traits for environmentally smart rice. In: Proc. 2012 JKUAT Scient., Technol. and Indust. Conf., November 15-16, 2012, Nairobi, Kenya. Jomo Kenyatta University of Agriculture and Technology, Research Production and Extension Division, Nairobi. pp. 713-719.

Govindaraj P, Arumugachamy S, Maheswaran M (2005). Bulked segregants analysis to detect main QTL associated with grain quality parameters in Basmati 370/ASD 16 cross in rice (*Oryza sativa* L.) using SSR markers. *Euphytica* 144: 61-68.

Jhang T, Vikal Y, Singh K, Dhaliwal HS, Sidhu JS (2006). Molecular identification and introgression of QTLs for yield components from temperate japonica rice in Basmati 370. *SABRAO J. Breed. Genet.* 38: 83-92.

Jiang SH, Zhou H, Lin DZ, Dong YJ, Ye SH, Zhang XM (2013). Identification and gene mapping of a thermo-sensitive leaf-color mutant at seedling stage in rice. *Chin. J. Rice Sci*. 27: 359–364.

Khush GS, N dela C, (2002). Developing Basmati rice with high yield potential. In: R. Duffy, ed., Speciality rices of the world: Breeding, production and marketing. Science publisher, Enfield, USA, pp. 15-18.

Khush GS, N dela C. (1998). Developing Basmati rice with high yield potential. Cahiers Options Mediterraneennes 24: Rice quality. A pluridisciplinary approach. (CD-ROM computer file) CIHEAM, paris.https://www.agronomy.org/publications/cs/pdfs/39/6/1715.

Li J, Xiao J, Grandillo S, Jiang L, Wan Y, Deng Q, Yuan L, McCouch SR (2004). QTL detection for rice grain quality traits using an inter-specific backcross population derived from cultivated Asian (*O. sativa* L.) and African (*O. glaberrima* S.) rice. *Genome* 47:697-704.

Kojima S, Takahashi Y, Kobayashi y, Monna L, Sasaki T, Araki t, Yano M (2002). *Hd3a*, a rice ortholog of the *Arabidopsis FT* gene, promotes transition to flowering downstream of *Hd1* under short-day conditions. *Plant Cell Physiol.* 43: 1096-1105.

Maekawa M., Takamure I., Ahmed N., Kyozuka J. (2005) Bunketsu-waito, one of tillering dwarf, is controlled by a single recessive gene in rice (*Oryza sativa* L.). *Breed. Sci.* 55: 193-196.

Miura K, Ikeda M, Matsubara A, Song X, Ito M, Asano K, Matsuoka M, Kitano H, Ashikari M (2010). *OsSPL14* promotes panicle branching and higher grain productivity in rice. *Nat. Genet.* 42: 545-549.

Ogiso-Tanaka E, Matsubara K, Yamamoto SI, Nonoue Y, Wu J, Fujisawa H, Ishikubo H, Tanaka T, Ando T, Matsumoto T, Yano M (2013). Natural variation of the *RICE FLOWERING LOCUS T 1* contributes to flowering time divergence in Rice. *PLoS ONE* 8(10): e75959.

Qi P, Lin YS, Song XJ, Shen JB, Huang W, Shan JX, Zhu MZ, Jiang L, Gao JP, Lin HX (2012). The novel quantitative trait locus *GL3.1* controls rice grain size and yield by regulating Cyclin-T1; 3. *Cell Research* 22:1666–1680.

Raghavan C, Collard BCY (2012) Effect of small mapping population sizes on reliability of quantitative trait locus (QTL) mapping. *African J. Biotech*. 11: 10661-10674.

Ramos JM, Furuta T, Uehara K, Chihiro N, Angeles-Shim RB, Shim J, Brar DS, Ashikari M, Jena KK (2016). Development of chromosome segment substituteon lines (CSSLs) of *Oryza longistaminata* A. Chev. & Rohr in the background of the elite *japonica* rice cultivar, Taichung 65 and their evaluateon for yield traits. *Euphytica* 210: 151-163.

Sacks EJ, Roxas JP, Cruz MTS (2003). Developing perennial upland rice II: Field performance of S_1 families from an intermated *Oryza* sativa*/O. longistaminata* population. *Crop Sci.* 43:129–134.

Singh V, Gupta KR, Singh N, Jain RK (2012). Genetic analysis of Basmati RILs and characterization of major fragrance gene by microsatellite markers. *Vegetos* 25: 266-272.

Takeda S, Matsuoka M (2008). Genetic approaches to crop improvement: Responding to environmental and population changes. *Nat Rev Genet*. 9: 444-457.

Vemireddy LR, Noor S, Satyavathi W, Srividhya A, Kaliappan A, Parimala SRN, Bharathi PM, Deborah DA, Rao KVS, Shobharani N, Siddiq EA, Nagaraju J (2015). Discovery and mapping of genomic regions governing

economically important traits of Basmati rice. *BMC Plant Biology* 15:207.

Wan XY, Wan JM, Jiang L, Wang JK, Zhai HQ, Weng JF, Wang HL, Lei CL, Wang JL, Zhang X, Cheng ZJ, Guo XP (2006). QTL analysis for rice grain length and fine mapping of an identified QTL with stable and major effects. *Theor. Appl. Genet.* 112: 1258-1270.

Wang S, Basten CJ, Zeng ZB (2007). Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, USA. http://statgen.ncsu.edu/qtlcart/WQTLCart.htm.

Xing YZ, Tan YF, Xu CG, Hua JP and Sun XL (2001). Mapping quantitative trait loci for grain appearance traits of rice using a recombinant inbred line population. *Acta. Bot. Sin.* 43:840-845.