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Genetic Mapping of Grain Nutritional Profile in Rice using Basmati Derived Segregating Population Revealed by SSRs

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Abstract

Rice nutritional profile defines and characterizes the dimensions of grain quality. Biofortification of rice requires intense genetic and phenotypic screening to find donor parents to improve nutritional attributes of newly developed breeding lines. To detect significant marker trait associations for utilization in future breeding programs for improvement of grain quality, a segregating rice population was developed using Super Basmati and IRBB-57 to map the genetic basis of grain nutritional attributes. A total of 213 plants of F_2 population were characterized for nutritional assays and genotyped using 94 SSR Markers. Based on linkage map of 94 markers, QTL analysis detected Twenty six main effect QTLs associated with 5 nutritional traits (protein, lipids, iron, zinc and calcium) on six linkage groups with major alleles contributed by Super Basmati with significant additive and phenotypic variance. Lipids, iron, zinc and calcium were newly examined in Basmati genome background with significant phenotypic variation (5.45– 26.25%). A main effect QTL (*qPC-8b*) flanked by RM 313 and RM 309 on chromosome 8 influenced the protein content with phenotypic variation of 13.32%. For lipid contents, 18% phenotypic variation was explained by a major QTL (*qLC-1*) on chromosome 1 linked with RM 426 and RM 428. Maximum phenotypic variation (26.25%) was explained by a main effect QTL (*qFe3*) flanked by RM 211 and RM 233 controlling iron content on chromosome 3. The findings reveal that Super Basmati may be used as potential donor parent for nutritional improvement of future rice varieties. The linked SSR makers with all the associated traits could possibly be used to transfer the target loci for nutritional improvement of future rice varieties using Marker Assisted Selection (MAS). © 2019 Friends Science Publishers

Key words: Biofortification; Marker trait association; Quantitative trait loci (QTLs); Grain quality; SSR markers

Introduction

Rice (*Oryza sativa* L.) serves half of the world population as a prominent staple food. Components of nutritional profile define and characterize the dimensions of rice grain quality (Cuevas *et al*., 2018). In a breeding program, new lines are selected based on minor and major traits associated with grain quality. All the major traits (starch, RVA properties, gel consistency and gelatinization temperature) and minor traits (protein, lipids, zinc, iron, and calcium) play leading role in defining the rice grain quality and texture (Sands *et al.*, 2009). The varieties within same amylose class could be classified based on nutritional profile dominated by different components (Champagne *et al.*, 2010). Paradigm of rice quality is based on nutritional profile, eating profile, sensory profile, cooking and physical profile (Yang *et al.*, 2014; Pang *et al.*, 2016). All of these profiles are attributed by numerous components under varying concentrations determined by physical and biochemical properties and controlled by complex inheritance under changing environments (Tran *et al*., 2011).

Rice biofortification focuses on genetic, physiological and environmental nature of targeted parameters to develop a new variety with improved nutritional traits. Textural properties of cooked rice provide more insights to study the role of nutritional attributes in defining the quality model (Li *et al.*, 2014). Many findings reveal the association of nutritional and starch attributes with genetic diversity of rice germplasm with *GBSS* and effect of changing climates globally (Tran *et al.*, 2011; Wattoo *et al.*, 2015; Hori *et al.*, 2016; Li *et al.*, 2016). Triploid nature of endosperm, epistatic interactions and cytoplasmic effects also lead to a complex genetic inheritance of rice grain components associated with its quality (Kumar and Khush, 1986; Pooni *et al.*, 1993).

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Many studies reported the role of waxy gene for starch properties and expression in different rice accessions controlled by allelic diversity at waxy locus directing the synthesis of Granule Bound Starch Synthase (GBSS) (Tan *et al.,* 1999; Wang *et al.,* 1999; Tian *et al.,* 2005). Rice grain lipids constitute a small proportion compared to starch but significantly contribute the nutritional attributes (Moazzami *et al.,* 2011). Oil of rice bran is preferred in many Asian countries with significant impact on human health and nutrition (Ghosh, 2007). Phospholipids are major component in rice grain and are composed of phosphates and lipids constituting about 10% of total lipids in rice grain. The effects of theses phospholipids are also found beneficial in many human diseases including heart inflammation and cancer (Kullenberg *et al.,* 2012). Grain lipids are grouped in to five classes (phospholipids, acylglycerols, wax easters, free fatty acids and glycolipids) based on chemical structure and linkage. Rice bran contains primarily concentrated amount of lipids (19.5–25.5%) compared with milled rice with 0.8% (Juliano *et al.*, 1992). However, the quantity of phospholipids and their distribution in different rice accessions varies based on genetic diversity and geographic distribution.

Proteins affect the physiochemical properties of cooked rice. In general, protein content and cooking quality traits of rice are negatively correlated (Abacara *et al.,* 2016). Rice protein is uniquely enriched with lysine (4%) when compared with other cereals. Similarly, other grain components like zinc (Zn), iron (Fe) and calcium (Ca) are associated with grain texture and rheological properties (Cuevas *et al.,* 2018; Rehman *et al.,* 2018). Knowing the genetic basis of all the major and minor grain quality attributes would help to transfer of useful traits form one variety to other using genomic assisted selection techniques to improve the nutrient content of rice. Moreover, holistic breeding strategies aided with advanced tools are needed for rice nutritional development and product release.

Fe and Zn play key role for plants homeostasis, translocation, buffering and metabolic regulations which favours the growth and development (Trijatmiko *et al.,* 2016). Zn promotes protein coordination, gene transcription, lipid and carbohydrates metabolism (Bashir *et al.,* 2012). Similarly, Fe is a key element in electron-transfer, chlorophyll biosynthesis, respiration and photosynthesis (Yun *et al.,* 2014). Variation in Fe and Zn concentration may cause metabolic disorders leading abnormal growth and development of plants.

Simple Sequence Repeats (SSR) are valuable biological tools for genetic mapping of different attributes in rice. These markers have been successfully utilized to study genetic diversity (Zou *et al.,* 2000), QTL mapping (McCouch *et al.,* 2002), Allelic diversity (Ravi *et al.,* 2003), gene identification (Wattoo *et al.,* 2015), varietal purity testing (Coburn *et al.,* 2002), phylogenetic relationship (Joshi and Behera, 2006) and introgression of desired genes (McCouch *et al.,* 2002). SSRs are progressively used to construct genetic linkage map of rice to link genotype and phenotypic variation of different traits (Singh *et al.,* 2004). Most of genetic studies convened the Basmati genotypes in to separate cluster showing their complex patterns of evolution when compared within *indica* genotypes (Jain *et al.,* 2004).

The current study was designed to find the new genetic loci associated with grain nutritional profile using Basmati derived segregating population with SSR microsatellites. The results would further help to study the genetic basis of oil traits and nutritional profile in rice. The associated SSR markers with all the traits would be used by Marker Assisted Selection (MAS).

Materials and Methods

Plant Material

Two parent rice varieties Super Basmati and IRBB-57 were used to derive segregating mapping population for genetic mapping studies and biochemical assays of rice grain. Super Basmati rice is known as superior rice due to its aroma and quality of kernel with intermediate amylose contents, gel consistency and gelatinization temperature. The latter variety contains poor grain quality attributes with high amylose contents, gelatinization temperature and hard gel consistency. The mapping population was developed at the Agriculture experiment farm of University of Punjab, Lahore during rice growing season of 2016–2017 under standard agronomic practices. A total of 213 F_2 plants were developed and harvested at maturity for SSR genotyping and grain profile assays.

SSR Genotyping

DNA of both parents and 213 F_2 plants was extracted using CTAB method as described by Healey *et al*. (2014). A total of 154 SSR markers were surveyed on two parents Super Basmati and IRBB-75. 94 SSR markers distributed on different rice chromosomes showed polymorphism and were later used for SSR genotyping using PCR followed by Poly Acrylamide Gel Electrophoresis (PAGE) (Chen *et al.,* 1997; Temnykh *et al.,* 2000). Different SSR microsatellites were optimized using different levels annealing temperature range.

Genetic Linkage Map

Genetic linkage map was constructed using 94 polymorphic SSR markers distributed on 6 linkage groups. An integrated map was used to estimate the distance between markers (www.gramene.org).

QTL Analysis

QTL mapping was performed with a linkage map of 94 SSR markers evenly distributed on six linkage groups. QTLs for grain protein content, lipids, iron, zinc and calcium were

identified using both regression and non-regression mapping approaches. QTL cartographer version 2.5 and Map marker version 3.0 (Lincoln *et al.*, 1992) were used to detect marker and phenotypic association using Simple Interval Mapping (SIM) and Composite Interval Mapping (CIM) approaches. A threshold P value ($P < 0.05$) was used with permutation rate 1000 for QTL detection. Phenotypic variation (R^2) was determined using likelihood ratio test to set hypothesis to calculate Logarithm of the Odds (LOD) values with dominance and additive effects (H3: H0). A LOD score ≥ 5.0 was set to detect the significance of a QTL.

Grain Assays

Protein estimation: For protein estimation, 2.5 g of grinded rice powder was added to a sample tube followed by digestion with strong acid (sulfuric acid, 25 mL) with 2 tablets following classical Kjeldahl Method. The samples were allowed to steam distillate and titrated back with sodium hydroxide. The nitrogen values were multiplied with a constant factor (5.95) to determine the protein content. Each sample was performed thrice and protein content was averaged over replications.

Lipids estimation: Mature grain samples of 213 plants of F_2 population were milled and grinded into fine powder. 50 mg powder was weighed to determine the lipid contents following the method described by Browse *et al*. (1986). All the samples were assayed twice and replicated for average in percentage.

Mineral Analysis

For mineral assay of different grain components, 0.3 g of grinded powder of white rice was taken in Polytetrafluoroethylene (PTFE) tube for digestion with nitric acid and hydrogen peroxide (2 mL and 0.5 mL) following shifting of digestion solution to volumetric flask (25 mL) and filling the volume with distilled water. Iron, zinc and calcium concentrations were determined using ICP-MS, Agilent, CA, USA following method described by Wu *et al.* (2010) expressed in μg/g.

Statistical Analysis

All the phenotypic data were statistically analyzed using SPSS 19.0. Linear regression model and Pearson correlation was performed to check the correlation among different nutritional attributes.

Results

Protein Contents

The protein contents of all the 213 samples of segregating populations varied significantly. The protein contents of both parents (Super Basmati and IRBB-57) were recorded as 7.9 and 5.2%, respectively. The range of protein in segregating samples was 4.3 to 10% (Fig. 1).

Lipids Contents

Lipids contents in brown rice samples of mapping population varied significantly (0.5–3.2%). Super Basmati and IRBB-57 showed lipid contents 0.8 and 2.5% respectively. The statistical analysis revealed three distinct groups based on lipids variation. One group showed similarity range with IRBB-57 with lipids contents range of 2.5–3.1%. Other group showed similarity range with Super Basmati (0.5–1.0%). Remaining samples showed diverse range (0.5–3.2%) (Fig. 1).

Mineral Components

A significant degree of variation in Zn was recorded in mapping population. Zn contents of Super Basmati and IRBB-57 were recorded 9.6 μg/g and 12.8 μg/g respectively. The range varied from 7.5–15.8 μg/g in mapping population. Some of samples were recorded with values significantly different from both parents showing recombinant groups (Fig. 1).

Similarly, Fe contents showed a statistically significant range in mapping population. Super Basmati was detected with 10 μg/g of Fe while IRBB-57 with 7.5 μg/g. The range in mapping population was observed between 5.5–12.6 μg/g (Fig. 1) showing three distinct groups with parental range and recombinants. Ca contents of mapping population also revealed a significant range. Super Basmati was detected with 250 μg/g of Calcium while IRBB-57 with 280 μg/g. The range of Ca contents comprised three groups within the quantitative scale of both parents and recombinants (Fig. 1).

Correlation Analysis

Statistical analysis revealed significant correlation among all the traits (Table 1). Protein content showed positive correlation with calcium (0.63), zinc (0.85) and iron (0.60) while negatively correlated with lipids (-0.45) (Table 1). Similarly, lipids contents showed positive correlation with iron (0.75), zinc (0.65) and calcium (0.59). Iron revealed positive correlation with lipids (0.75). Calcium was also positively correlated with protein content (0.63), lipids (0.59), iron (0.75) and zinc (0.45) (Table 1).

QTL Analysis

Protein contents: QTL analysis detected 26 main effect QTLs for all the five nutritional attributes (Protein contents, Lipid contents, Zinc, Iron and Calcium). For Protein content, five QTLs were detected on chromosomes, 1, 4, 7 and 8 (Table 2; Fig. 2). On chromosome 1, one QTL was detected as *qPC-1*which showed LOD score of 5.31 with phenotypic variation 7.26. One QTL *qPC-4* was detected on chromosome 4 with LOD score of 9.26 and phenotypic variation of 9.45% (Table 2; Fig. 2). The other QTL *qPC-7* was detected on chromosome 7 showing LOD score 5.62 and 6.23% phenotypic variation.

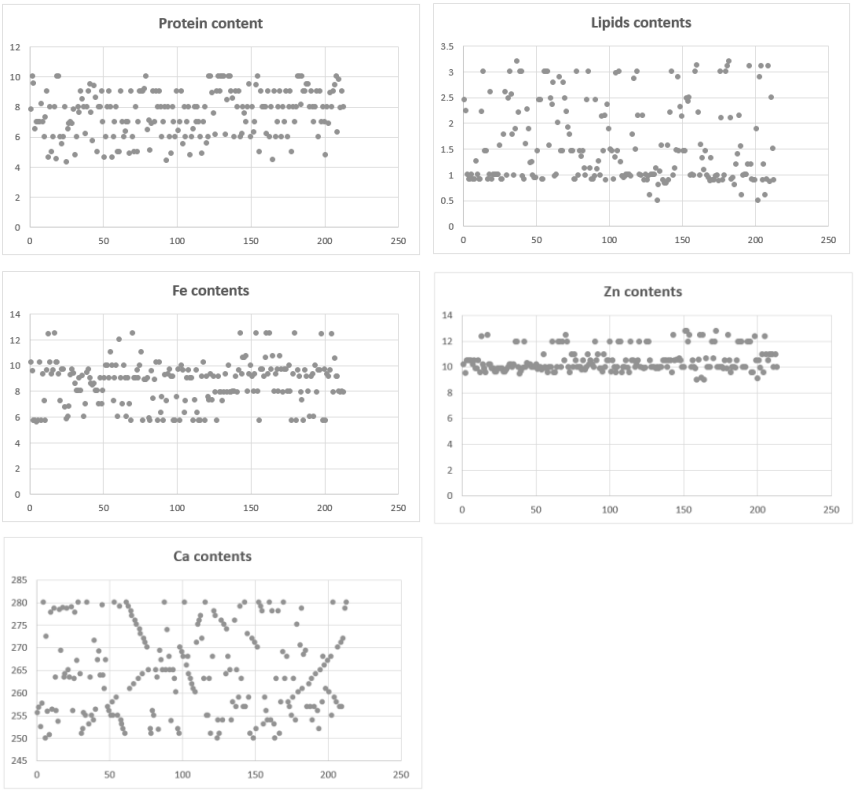

Fig. 1: Statistical distribution showing range of nutritional attributes in mapping population. Proteins and lipids contents are expressed in % age while Fe, Zn and Ca contents are expressed in μg/g while taking plants on x-axis and phenotypic range on y-axis

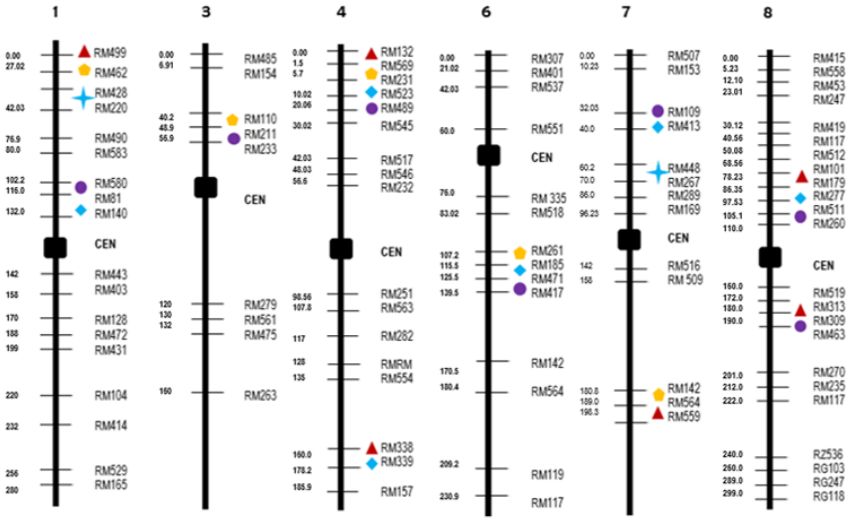

Fig. 2: A linkage map of polymorphic SSR markers between two parents (S.B & IRBB-57) showing distribution of 26 main effect QTLs associated with all the 5 nutritional traits on 6 linkage groups. The nutritional traits are represented with different symbols like ▲ Protein contents, ⬟ Lipids contents, ◆ Zinc contents, ⬬ Iron contents and ✦ Calcium contents

Similarly, two QTLs were identified on linkage group 8 as *qPC-8a* and *qPC-8b* with LOD score 5.31 and 6.32, respectively. The phenotypic variation of *qPC-8a* was detected 10.21 while *qPC-8b* was detected with 13.32% phenotypic variation (Table 2; Fig. 2).

Lipids contents: Five QTLs were identified for lipids contents on linkage groups 1, 3, 4, 6 and 7 (Table 2; Fig. 2). One major QTL *qLC-1* on chromosome 1 was detected with LOD score 10.2 and phenotypic variation 18.12%. Other QTL *qLC-3* was detected on linkage group 3 with LOD

Table 1: Correlation among different traits of grain nutritional profile. PC (protein contents), LC (lipid contents), Fe (iron), Zn (zinc) and Ca (calcium)

Variables	PC	LC	Fe	Zn	Ca
PC	1				
LC	-0.45*	1			
Fe	0.60*	0.75**	1		
Zn	0.85**	0.65*	0.69*	1	
Ca	0.63	0.59	0.75	0.45	1

****** $P < 0.01$; * $P < 0.05$

Table 2: Summary of different QTLs associated with all the nutritional attributes

Traits	QTLs	Marker Interval	LOD Score	Additive	Phenotypic variance (%)
Protein Contents	*qPC-1*	RM499-RM462	5.31	1.6	7.26
	qPC-4	RM132-RM569	9.26	4.89	9.45
	qPC-7	RM564-RM559	5.62	3.45	6.23
	qPC-8a	RM101-RM179	5.31	2.16	10.21
	qPC-8b	RM313-RM309	6.32	3.26	13.32
Lipids contents	*qLC-1*	RM462-RM428	10.2	3.16	18.12
	qLC-3	RM110- RM211	5.13	0.45	10.56
	qLC-4	RM569- RM231	6.32	5.2	14.12
	qLC-6	RM261- RM185	5.16	2.9	8.26
	qLC-7	RM142- RM564	6.56	3.6	7.32
Iron (Fe)	*qFe-1*	RM580- RM81	8.26	2.15	9.65
	qFe-3	RM211- RM233	9.12	1.69	26.25
	qFe-4	RM489- RM545	6.23	0.65	11.26
	qFe-6	RM471- RM417	5.32	4.36	5.45
	qFe-7	RM109- RM413	9.23	5.29	7.46
	qFe-8a	RM511- RM260	6.23	0.60	10.45
	qFe-8b	RM309- RM463	5.12	2.30	13.45
Zinc (Zn)	*qZn-1*	RM81- RM140	10.2	2.9	11.12
	qZn-4a	RM523- RM489	6.23	3.20	7.35
	qZn-4b	RM338- RM339	5.92	0.90	6.12
	qZn-6	RM185- RM471	5.12	0.43	7.65
	qZn-7	RM413- RM448	6.24	0.53	10.24
	qZn-8	RM179- RM277	6.89	4.06	9.23
Calcium (Ca)	*qCa-1*	RM428- RM220	6.12	-0.90	10.10
	qCa-7	RM448- RM267	9.32	-0.74	12.46

score 5.13 and phenotypic variation 10.56% (Table 2; Fig. 2). Similarly, a major QTL, *qLC-4* was identified on chromosome 4 with LOD score 6.32 and phenotypic variation 14.12%. Linkage groups 6 and 7 were detected with *qLC-6* and *qLC-7* with LOD 5.16, 6.56% and 8.26, 7.25% phenotypic variation respectively (Table 2; Fig. 2).

Iron contents: Seven QTLs were identified for iron contents on linkage groups 1, 3, 4, 6, 7 and 8 (Table 2; Fig. 2). Chromosome 1 was identified with one QTL *qFe-1* with LOD score 8.26 and phenotypic variation of 9.65%. Similarly, one major QTL *qFe-3* with phenotypic variation 26.25% was identified on chromosome 3 showing LOD score 9.12 (Table 2; Fig. 2). Other QTL *qFe-4* was detected on linkage group 4 with LOD score 6.23 and phenotypic variation 11.26%. Chromosomes 6 and 7 were detected with *qFe-6* and *qFe-7* with LOD score 5.32 and 9.23 and phenotypic variation 5.45 and 7.46%, respectively. Chromosome 8 was detected with two QTLs *qFe-8a*&*qFe-8b* with LOD score 6.23, 5.12 and phenotypic variation of 10.45 and 13.45% respectively (Table 2; Fig. 2).

Zinc contents: Six QTLs were identified for Zinc contents on linkage groups 1, 4, 6, 7 and 8 (Table 2; Fig. 2). Chromosome 1 was detected with one QTL *qZn-1* with significant phenotypic variation of 11.12% and LOD score 10.2% (Table 2; Fig. 2). Similarly, two QTLs *qZn-4a* & *qZn-4b* were detected on linkage group 4 with LOD score 6.23, 5.92 and phenotypic variation 7.35 and 6.12% respectively. Linkage groups 6 and 7 were identified with two QTLs *qZn-6* and *qZn-7* with LOD score 5.12, 6.24 and phenotypic variation 7.65 and 10.24%, respectively. One QTL *qZn-8* was detected on chromosome 8 with LOD score 6.89 and phenotypic variation 9.23% (Table 2; Fig. 2).

Calcium contents: Two QTLs were detected for calcium contents on chromosomes 1 and 7 with significant phenotypic variation (Table 2; Fig. 2). The QTL on chromosome 1 *qCa-1* was detected with LOD score 6.12 and phenotypic variation 10.10%. Similarly, chromosome 7 was detected with one QTL *qCa-7* with LOD score 9.32 and phenotypic variation 12.46% (Table 2; Fig. 2).

Discussion

Deficiency of essential minerals, vitamins and micronutrients in rice is serious concern in the regions where it is consumed as staple diet (Farooq *et al*., 2018). Fe and Zn deficiencies are reported as main concern in resource poor regions around the globe (Sands *et al.,* 2009). In current study, many QTLs were identified to be linked with various quality attributes. Super Basmati is being used as potential donor parent to restore best alleles for grain quality improvement. IRBB-57 is used as donor of blight resistant genes in promising cultivars following by restoration of target genome using Marker Assisted Backcrossing (MAB). Both parents have significant variation in all the studied grain quality traits.

For protein contents, five QTLs were identified on 4 linkage groups (1, 4, 7 and 8). One major QTL was detected on chromosome 4 between marker interval RM132-RM569 with LOD score 9.26 and additive variance 4.89% and phenotypic variance 9.45% (Fig. 2; Table 2). Chromosomes 1 and 7 were also previously detected to have QTLs for protein contents (Qin *et al.,* 2009; Zhong *et al.,* 2011; Bruno *et al.,* 2017). Chromosomes 4 and 8 have been detected first time to have QTLs for quantitative variation of protein contents. Super Basmati favored the contribution of positive alleles for protein content. Similarly, on linkage groups 7 and 8, Super Basmati showed additive effect of 3.45 and 3.26 respectively (Fig. 2; Table 2). Rice protein is an important nutritional component and is reported by many researchers as an important factor involved in the variability of eating and cooking quality of rice accessions within same amylose class (Zhong *et al*., 2011). Our results clearly indicated that Super Basmati can be used as donor parent for introgression of protein associated alleles in newly breeding cultivars.

Five QTLs were identified for lipid contents on linkage groups 1, 3, 4, 6 and 7. Maximum phenotypic variation (18.12%) was recorded on chromosome 1 for *qLC-1* with LOD score 10.2 and additive effect 3.16. Similarly, two QTLs on chromosomes *4 (qLC-4) and 7 (qLC-7)* showed significant additive values 5.2, 3.6 with phenotypic variation 14.2 and 7.32% respectively (Fig. 2; Table 2). On all the three linkage groups (1, 4 and 7), Super Basmati increased positive alleles for lipids contents. Chromosome 6 has been reported by many researchers to have waxy locus with major impact on starch biosynthesis and physiochemical properties (Bao *et al.,* 2006; Wang and Shu, 2007; Li *et al.,* 2014; Xu and Bai, 2015). We also reported the same linkage group for genetic control of lipids contents and our results provided evidence that Super Basmati may be used as potential donor parent to increase the frequency of alleles associated with increase lipids contents using linked SSR markers.

Seven QTLs were identified for iron (Fe) contents on six linkage groups (1, 3, 4, 6, 7 and 8). One QTL *qFe-3* was identified with maximum phenotypic variation 26.25% on chromosome 3 with LOD score 9.12 and additive effect 1.69% (Fig. 2; Table 2). Chromosome 1 was previously identified to have QTLs for iron content (Kaiyang *et al.,* 2008; Anuradha *et al.,* 2012). Similarly, chromosomes 7 and 8 were reported by James *et al.* (2007) and Anuradha *et al.* (2012) to have QTLs for iron content. Other chromosomes were detected first time. Two QTLs on chromosome 6 *(qFe-6)* and 8 *(qFe-8)* were detected with negative additive variances -4.36 and -0.60%, respectively (Fig. 2; Table 2). IRBB-57 decreased favorable alleles associated with iron.

For Zinc (Zn) contents, six QTLs were detected on five linkage groups (1, 4, 6, 7 and 8). Two QTLs with significant additive and phenotypic variance were identified on linkage groups 1 & 4 (Fig. 2; Table 2). Chromosomes 6,7 and 8 were previously identified by many researchers (Kaiyang *et al.,* 2008; Anuradha *et al.,* 2012) to have alleles for quantitative variation of zinc contents in rice. Other chromosomes were identified first time. Positive alleles for zinc contents were shared by Super Basmati on all the linkage groups (Table 2; Fig. 2).

Two QTLs *(qCa-1 & qCa-7)* for calcium content were identified for calcium contents on chromosomes 1 and 7 with LOD score 6.12, 9.32, Additive variance -0.90–0.74% and Phenotypic variance 10.10 and 12.46% respectively (Table 2). QTLs for calcium contents on chromosome 1 were also reported by Garcia *et al.* (2008). Super Basmati decreased the calcium contents on both linkage groups. Many QTLs were detected sharing common position on various linkage groups showing their complex haplotypes variation for phenotypic traits.

Rice biofortification with crucial minerals and micronutrients is challenging due to complex metabolic and genetic networks underlying the phenotypic expression of all the nutritional traits (James *et al.,* 2007; Bashir *et al.,* 2012). Moreover, germplasm diversity and variation in nutrients concentration may significantly affect the mobilization scheme of these nutrients under changing climatic conditions and agronomic practices leading a big challenge for breeders to maintain the required concentration of a particular micronutrient (Paul *et al*., 2012).

Conclusion

In conclusion, 26 QTLs were identified to be associated with 5 nutritional traits on six linkage groups using segregating rice population derived from Super Basmati and IRBB-57. Our results have clear implications for improvement of rice nutritional profile using Super Basmati as one of potential donor parent for sustainable and cost effective varietal development. The linked SSR makers with all the associated traits could possibly be used to transfer the target alleles for nutritional improvement of future rice varieties using Marker Assisted Selection (MAS).

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