Linkage mapping of QTLs for grain minerals (iron and zinc) and physio-morphological traits for development of mineral rich rice (*Oryza sativa* L.)

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In the present investigation, experiments were conducted to evaluate F_3 and F_4 populations derived from cross between PAU201 (high-yield) and Palman 579 (iron-rich) *indica* rice varieties for various physio-morphological traits and minerals (iron and zinc) content. Phenotypic correlation analysis showed no correlation between grain iron and zinc content in F_3 and F_4 population (s). A DNA fingerprint database of 33 PAU201 × Palman 579 derived F_4 plants was prepared using 61 polymorphic SSR markers distributed on the entire genome of rice. The results of NTSYS-pc UPGMA tree cluster analysis and two and three dimensional principal component analysis (PCA) scaling showed scattering of the F_4 population between the two distinct parent genotypes; but more inclined towards Palman 579. The SSR data was used to identify quantitative trait loci (QTL) for grain mineral content and physio-morphological traits. A total of 128 alleles and three new recombinant alleles were identified in F_4 plants population. Composite interval mapping (CIM) analysis by WinQTL cartographer 2.5 revealed a total of six QTLs for mineral content (five for iron and one for zinc) in rice grains on chromosome 5, 6, 7, 9 and sixteen QTLs for various physio-morphological traits on chromosomes 2, 5, 7, 8, 9, 10 and 12. Linkage mapping of QTLs of minerals (iron and zinc) can greatly enhance the efficacy of breeding programs to improve mineral density in rice. The QTLs for minerals identified can successfully employed to improve the target traits through marker assisted selection.

Key words: Rice, minerals (Fe and Zn) content, SSR markers, CIM, QTLs

Introduction

Rice (*Oryza sativa* L.), one of the most important food crops in the world, forms the staple diet of over 50% of the world population. Micronutrient (Fe and Zn) malnutrition is recognized as a massive and rapidly growing public health issue especially among poor people living on an unbalanced diet dominated by a single cereal such as rice.

Iron (Fe) and Zinc (Zn) deficiencies cause immune dysfunction and may impair growth and development of individuals. Iron deficiency is one of the most World Health Organization (WHO) report, iron deficiency anemia affects around 2 billion people in both developed and developing countries[1]. Worldwide, zinc deficiency is responsible for approximately 16% of lower respiratory tract infections, 18% of malaria and 10% of diarrheal disease. In total, 1.4% (0.8 million) of deaths worldwide are attributable to zinc deficiency: 1.4% in males and 1.5% in females[2]. Bio-fortification has the

potential to provide coverage for remote rural population, and it inherently targets the poor who consume high levels of staple foods and little else.

Molecular markers have proven useful in both basic and applied research, such as DNA fingerprinting, varietal identification and diversity analysis, phylogenetic analysis, linkage mapping of genes/quantitative trait loci (QTL), marker assisted breeding and map based cloning of genes in rice[3-7]. Among the various types of available molecular markers, microsatellites (also known as simple sequence ... ed to know about the genetic architecture of complex traits in rice, based on traditional QTL linkage[8]. Using molecular linkage maps, it is possible to estimate the number of QTLs controlling genetic variation for a target trait in a segregating population and to characterize these loci with regard to their map positions on the genome, gene action, phenotypic effects, pleiotropic effect and epistatic interaction between the QTLs[9]. In QTL mapping, genes controlling genetic variation of quantitative traits in segregating populations are resolved into individual Mendelian factors by detecting marker-trait associations[10].

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Linkage mapping, QTL analysis and marker-assisted selection (MAS) have been successfully employed to improve the efficacy of breeding programs for several complex polygenic traits such as submergence tolerance, bacterial leaf blight resistance, blast resistance, yield improvements and root traits (length, thickness, dry weight and root shoot ratio)[11-15]. But to date, there have been only a few studies to map genes/QTLs for mineral content in rice[16-20]. If micronutrients could be enhanced through breeding with the help of MAS in a staple food crop such as rice, expenditures for a micronutrient/mineral intervention program could decrease markedly.

Materials and Methods

Plant Material

Seeds harvested from selected PAU201 × Palman 579 derived F_2 plants were used to raise F_3 (177 plants) and F_4 (106 plants) population in the field at CCS Haryana Agricultural University, Rice Research Station, Kaul (Kaithal) Haryana, which falls under semi-tropical regions of North India. Seed harvested from the F_2 (selected iron-rich) plants were used to generate the F_3 and F_4 plant population (s).

Evaluation of PAU201 × Palman 579 Derived F_3 and F_4 Populations

In the present study, experiments were conducted to evaluate PAU201 × Palman 579 derived F_3 (177 plants) and F_4 (106 plants) populations and data was taken on various physio-morphological traits (plant height, effective number of tillers/plant, panicle length, grains per panicle, grain yield per plant, grain appearance and 1000 grain weight), during kharif seasons of 2011 and 2012, respectively at CCS Haryana Agricultural University, Rice Research Station, Kaul (Kaithal). The selection was done on the basis of iron and zinc content in F_3 population, so the population was decreased from 177 plants in F_3 to 106 plants in F_4 population. Frequency distribution curves for various agronomic traits of PAU201 × Palman 579 derived F_3 and F_4 population and parental rice genotypes were measured.

Mineral (Iron and Zinc) Content Analysis

Seeds harvested from PAU201 × Palman 579 derived F_3 and F_4 populations were analyzed for minerals (Fe and Zn) content (μg/g) in dehusked rice grains samples. Mineral content analysis was carried out using diacid mixture (HNO_3:$HClO_4$; 5:1 v/v) by atomic absorption spectrophotometer 2380 (Perkin

Elmer, USA) according to the method of Lindsay and Norwell[21].

Statistical Analysis

The statistical analysis of F_3 and F_4 population's data was done for determination the variability and phenotypic correlation coefficient (r) analysis using OPSTAT software of CCS Haryana Agricultural University, Hisar. Mean values were taken from the measurement of three replicates and standard error of the means was calculated. Difference between means was determined by one way ANOVA. Phenotypic correlation coefficients were calculated against standardized tabulated significant value of r with (n – 2) degree of freedom as per the procedure described by Fisher and Yates[22].

Microsatellite Marker Analysis

The genomic DNA was isolated from the leaf tissues of F_4 plants using CTAB method of DNA isolation[23]. Completely dissolved genomic DNA was checked for its quality and quantity by running DNA samples on 0.8% agarose gel electrophoresis using a standard containing 100 ng/μl genomic λ DNA. Molecular marker analysis was carried out using 61 polymorphic SSR primers distributed on the entire genome of rice by ethidium bromide staining based polyacrylamide gels as described by Wang and coworkers[24]. PCR amplified products from SSR markers analysis were scored visually for presence or absence of bands; data was scored as 1 (present) and 0 (absent) for each of the SSR locus. The 0/1 matrix was used to calculate similarity genetic distance using 'SIMQUAL' sub-program of software NTSYS–PC[25]. The resultant distance matrix was employed to construct dendrograms by the unweighted pair-group method with arithmetic average (UPGMA) sub program of NTSYS-PC.

QTL Mapping for Minerals (iron and zinc) Content in F_4 Population

Windows QTL cartographer version 2.5 was used to map QTL in this study. The map positions (cM or centimorgan) of all the 61 markers chosen for study were obtained from the gramene website (http://www.gramene.org). SSR allelic data was scored according to the translation available in the manual for WinQTL cartographer. According to it, allele 'A' was assumed as diagnostic for the high (parental 1) line and the allele 'a' diagnostic for the low (parental 2) line. In this study, Palman 579 was considered as the high (parental 1) line whereas

PAU201 was taken as low (parental 2) line, because of the high mineral content of Palman 579 over the PAU201. The number 2, 0 and 1 were assigned to Palman 579, PAU201 and heterozygous alleles, respectively. However, numbers 12 and 10 were given to recombinant alleles according, to their band size closeness to the parental line Palman 579 and PAU201, respectively whereas -1 was assigned to the missing allele. QTL positions were assigned to the point of maximum LOD (log 10 of an odd ratio) score above the threshold value in the target regions. These LOD or LRS (likelihood ratio statistics) profiles were used to identify the most likely position for a QTL in relation to the linkage map, which was the position where the highest LOD value was obtained. An output excel file was created which includes all the details of the QTLs (i.e. chromosome location, map positions, additive effect, dominant effect, LRS value etc.). In addition, the proportion of observed phenotypic variance explained by each QTL and corresponding additive effect was estimated. The information in this file was used for localising the QTLs on various chromosomes.

Results

Evaluation of PAU201 × Palman 579 Derived F_3 and F_4 Populations

Data was collected on various physio-morphological and grain quality traits for PAU201 × Palman 579 derived F_3 (177 plants) and F_4 (106 plants) populations along with parental rice varieties (Table 1). Field evaluation of PAU201 × Palman 579 derived F_3 and F_4 population showed large variation for plant height (70-155; 72-130 cm), effective number of tillers per plant (2-23; 6-41), panicle length (14-35.7; 18-30 cm), grains per panicle (13-168; 19.7-225), grain yield per plant (0.8-49.7; 2.3-86.2 g) and 1000-grain weight (17-28.5; 11.1-26.5 g), respectively (Table 1).

Mineral (Iron and Zinc) Content Analysis

Seed harvested from the individual F_3 and F_4 plants was used for mineral (iron and zinc) content analysis. Mineral analysis of F_3 and F_4 population's harvest (dehusked rice samples) revealed large variation in iron and zinc contents (Table 1). Iron content of F_3 and F_4 populations differed significantly between 24.3-149.9 µg/g; 18.2-68.7 µg/g with a mean value of 45.7; 33.0 µg/g, respectively. While "PAU201" recorded an iron content of 52.3 and 55.4 µg/g, "Palman 579" consistently showed exceptionally higher iron content of 373.1 and 383.8 µg/g in two kharif seasons (2011 and 2012), respectively. The previous studies of Brar and coworkers[26] and Kumar and coworkers[27] also reported the higher iron content of Palman 579 parent. Transgressive segregation for grain iron content was noticed in one of the F_3 plant having exceptionally higher iron (746.8 µg/g) content.

Correlation Coefficient Analysis

Phenotypic correlation coefficient analysis was carried out to assess the association between various mineral (Fe and Zn) and physio-morphological traits. Phenotypic correlation coefficients among yield, yield components and mineral content in F_3 and F_4 population are given in Table 3 and Table 4, respectively. There was no correlation found between iron and zinc content in both F_3 and F_4 population.

Frequency Distribution in F_3 and F_4 Population

Frequency distribution curves for various agronomic traits of PAU201 × Palman 579 derived F_3 & F_4 population and parental rice genotypes are shown in Figure 1 and 2 respectively. In F_3

Table 1 — Mean and range for various physio-morphological traits and mineral contents in PAU201 × Palman 579 F_3 and F_4 population (s)

Traits	Average pooled data of parental rice genotypes		PAU201 × Palman 579 derived F_3 population		PAU201 × Palman 579 derived F_4 population	
	PAU201	Palman 579	Mean	Range	Mean	Range
Plant height (cm)	95.8 ± 0.40	107.6 ± 0.75	100.8	70 - 155	94.9	72 - 130
Effective number of tillers/plant	13.8 ± 0.74	15.0 ± 1.15	10.3	2 - 23	16.7	6 - 41
Panicle length (cm)	25.3 ± 0.21	26.2 ± 0.36	24.8	14 - 35.7	24.8	18 - 30
Grains/panicle	120.6 ±1.24	106.9 ±1.29	76.0	13 - 168	85.2	19.7 - 225
Grain yield/plant (g)	40.7 ± 2.24	34.0 ± 1.59	18.3	0.8 - 49.7	29.0	2.3 - 86.2
1000-grain weight (g)	24.2 ± 0.10	21.5 ± 0.16	22.6	17.0 - 28.5	20.8	11.1 - 26.5
Fe content (µg/g)	53.9 ± 0.26	378.5 ± 0.41	45.7	24.3 - 149.9	33.0	18.2 - 68.7
Zn content (µg/g)	16.3 ± 0.19	22.3 ± 0.31	27.9	0.1 - 72.6	18.5	0 - 41.8

Table 2 — Phenotypic correlation coefficients among yield, yield components and mineral content in F_3 population

	Plant height (cm)	Effective number of tillers/ plant	Grain yield/ plant (g)	Grains/ panicle	Panicle length (cm)	1000-grain weight (g)	Fe content (µg/g)	Zn content (µg/g)
Plant height (cm)	1							
Effective number of tillers/plant	0.241**	1						
Grain yield/ plant (g)	0.417**	0.705**	1					
Grains/ panicle	0.284**	0.016	0.636**	1				
Panicle length (cm)	0.514**	0.295**	0.547**	0.546**	1			
1000-grain weight (g)	0.511**	0.101	0.125	-0.129	0.153*	1		
Fe content (µg/g)	-0.055	-0.039	-0.051	-0.066	-0.052	0.022	1	
Zn content (µg/g)	-0.222**	-0.104	-0.148*	-0.197**	-0.272**	-0.004	0.127	1

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

Table 3 — Phenotypic correlation coefficients among yield, yield components and mineral content in F_4 population

	Plant height (cm)	Effective number of tillers/ plant	Grain yield/ plant (g)	Grains/ panicle	Panicle length (cm)	1000-grain weight (g)	Fe content (µg/g)	Zn content (µg/g)
Plant height (cm)	1							
Effective number of tillers/plant	0.136	1						
Grain yield/plant (g)	0.363**	0.727**	1					
Grains/panicle	0.161*	-0.145	0.463**	1				
Panicle length (cm)	0.321**	0.025	0.282**	0.397**	1			
1000-grain weight (g)	0.635**	-0.002	0.289**	0.239**	0.250**	1		
Fe content (µg/g)	-0.070	0.081	0.060	0.011	0.066	0.014	1	
Zn content (µg/g)	0.022	0.002	-0.084	-0.242**	-0.134	0.049	0.065	1

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

Table 4 — QTLs identified for grain iron and zinc contents and various physio-morphological traits in PAU201 × Palman 579 F_4 population

Trait	QTL name	Chromosome number	Position (cM)	Flanking markers	Position of flanking markers (cM)	LOD	Additive effect	R_2 %	DPE
Fe content	qFE5.1	5	41.0	RM 2488 - RM440	10.1 - 92.7	27.3	171.83	64.1	PL
	qFE5.2	5	106.6	RM440 - RM31	92.7 - 123	27.3	170.23	95.2	PL
	qFE5.3	5	118.9	RM440 - RM31	92.7 - 123	28.5	170.38	95.2	PL
	qFE7.1	7	87.4	RM432 - RM429	43.5 - 96.9	27.0	174.92	95.2	PL
	qFE9.1	9	51.7	RM566 - RM434	47.7 - 57.7	25.4	174.85	34.6	PL
Zn content	qZN6.1	6	38.9	RM585 - RM3	25.1 - 74.3	6.3	-5.86	25	P
Plant height	qPH2.1	2	21.6	RM6616 - RM53	13.1 - 32.7	4.3	-11.48	7.5	P
	qPH7.1	7	31.1	RM481 - RM418	3.2 - 42.1	3.6	2.34	21.5	PL
Tillers/ plant	qTP10.1	10	48.2	RM6868 - RM184	30.2 - 58.3	3.3	5.34	21.4	PL
	qTP10.2	10	57.2	RM6868 - RM184	30.2 - 58.3	4.2	4.43	21.0	PL
	qTP10.3	10	70.3	RM184 - RM228	58.3 - 96.3	3.5	4.44	23.1	PL
Yield/ plant	qYP8.1	8	38.0	RM152 - RM310	9.4 - 57	2.8	5.42	56.9	PL
	qYP8.2	8	50.6	RM152 - RM310	9.4 - 57	3.3	5.50	59.5	PL
	qYP12.1	12	48.7	RM19 - RM519	20.9 - 62.6	3.8	-8.27	6.4	P
Grains/ panicle	qGP2.1	2	129.4	RM263 - RM221	127.5 - 143.7	5.5	-3.43	14.3	P
	qGP5.1	5	50.0	RM2488 - RM440	10.1 - 92.7	2.9	85.99	27.5	PL
	qGP7.1	7	10.1	RM481 - RM418	3.2 - 42.1	3.2	-46.89	9.4	P
	qGP9.1	9	1.0	RM296 - RM524	0 - 13.2	5.4	0.05	5.2	PL
1000-grain weight	qTG8.1	8	42.0	RM152 - RM310	9.4 - 57	3.1	0.22	19.5	PL
	qTG8.2	8	47.6	RM152 - RM310	9.4 - 57	3.6	1.09	18.3	PL
	qTG9.1	9	13.2	RM524 - RM566	13.2 - 47.7	3.8	0.79	12.4	PL
	qTG10.1	10	29.7	RM5708 - RM6868	23.7 - 30.2	2.7	0.35	5.0	PL

DPE (direction of phenotypic effect); PL and P indicate "Palman 579" (iron rich) and "PAU201" alleles, respectively. R_2 is phenotypic variation, Additive effect is half the difference between effect of "Palman 579" allele and "PAU201" allele; its positive value indicates that the effect of genotype "Palman 579" was in the direction of increasing the trait value, LOD - log10 of an odd ratio.
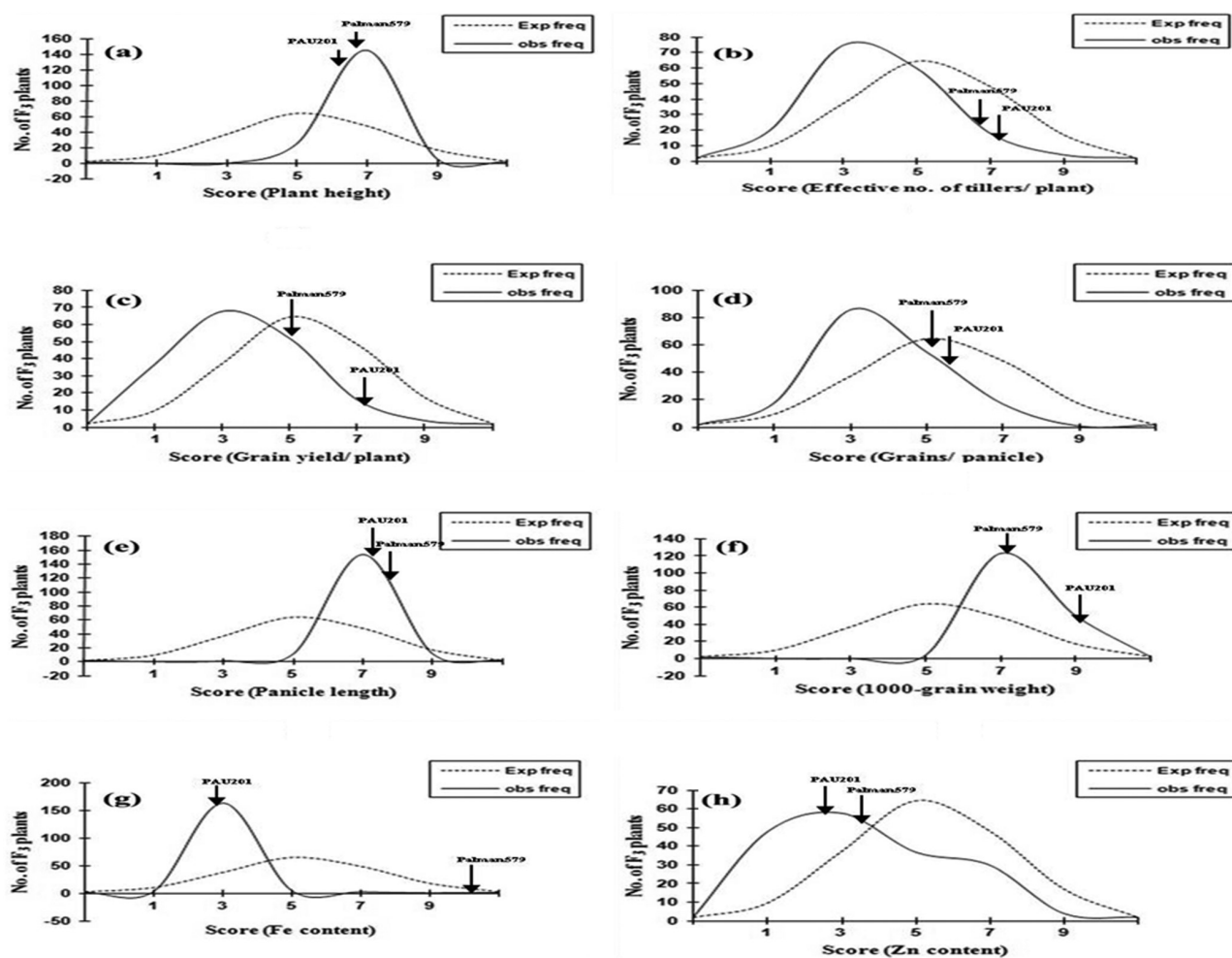
Fig. 1 — Frequency distribution curves for agronomic traits and iron and zinc contents of PAU 201 × Palman 579 F_3 population.

population, for plant height, frequency distribution curve was skewed towards Palman 579. For effective number of tillers/ plant, grain yield/ plant and grains/ panicle, frequency distribution curves were parabolic and slightly tilted towards Palman 579 while for panicle length it was skewed towards Palman 579 in F_3 population. For plant height, grain yield/ plant, grains/ panicle and panicle length, the frequency distribution curve of F_4 population was skewed towards Palman 579 in F_4 population. For effective number of tillers/plant, the frequency distribution curve showed normal parabolic distribution of F_4 population. The frequency distribution curved of 1000-grain weight, iron and zinc content showed different pattern of distribution of F_3 and F_4 plants and inclined towards PAU201.

Microsatellite Marker Analysis

A total of 33 selected F_4 plants covering entire range of mineral content were used for construction of DNA fingerprint database. Ethidium bromide stained polyacrylamide/ agarose gels showing allelic polymorphism for some of the markers have been shown in Figure 3 and 4. A total of 128 alleles were identified in the selected 33 F_4 plants of PAU201 × Palman 579 and parental rice genotypes. Three (RM53, RM209 and RM481) of 61 SSR markers amplified recombinant (new) alleles which were different to those present in two parental rice varieties. Eleven out of thirty three selected F_4 plants showed recombinant alleles at one or more loci. On an average, 49.6 and 50.4% alleles were from PAU201 and Palman 579, respectively, in all 33 F_4 plants. Genetic similarities between the genotypes were measured by the similarity coefficient based on the proportion of shared electromorphs using 'SIMQUAL' sub-program of NTSYS-PC (Version 2.02 Exeter Software, Setauket, NY, USA) package[25]. The resultant distance matrix data was used to construct dendrogram (Fig. 5) and for two-dimensional scaling
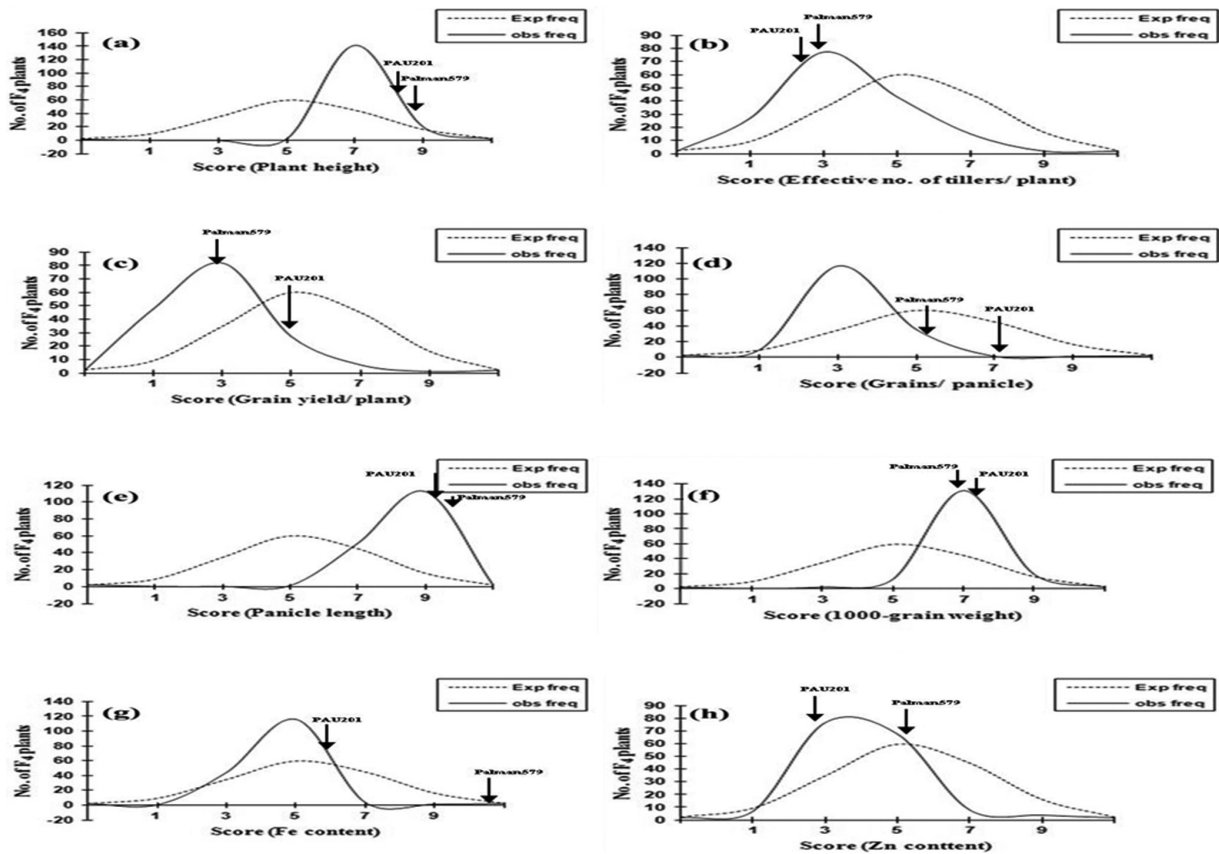
Fig. 2 — Frequency distribution curves for agronomic traits and iron and zinc contents of PAU 201 × Palman 579 F_4 population.
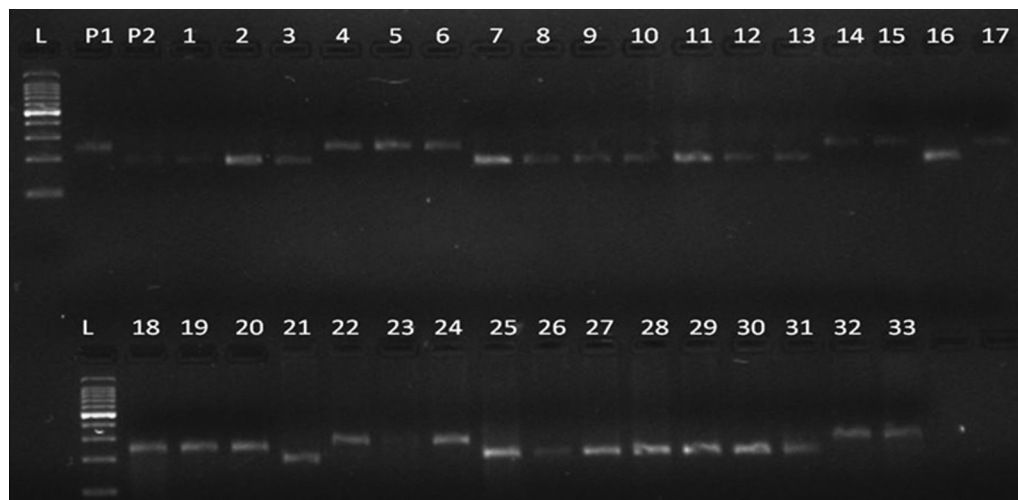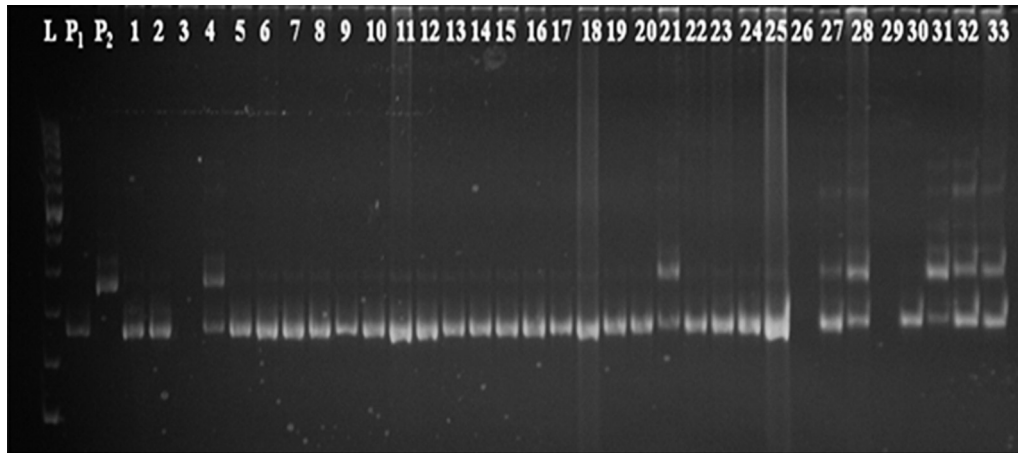

Fig. 3 — Agarose gel showing allelic polymorphism among parental rice varities and 33 selected F_4 plants at RM 585 locus. Lane L- 100 bp ladder, P_1- PAU201, P_2- Palman 579, 1-33: 33 selected F_4 plants.

of rice genotypes by two dimensional principal component analysis (Fig. 6).

QTL Mapping for Minerals (Iron and Zinc) Content in F_4 Population

Sixty one polymorphic SSR markers distributed on 12 rice chromosomes were used to map the QTLs linked with mineral content (iron and zinc) in rice grains in 33 PAU201 × Palman 579 F_4 plants. Composite interval mapping (CIM) analysis by WinQTL cartographer 2.5 revealed a total of six QTLs for mineral (iron and zinc) content in rice grains (Table 4 and Fig. 7).

Fig. 4 — Ethidium bromide stained PAGE showing allelic polymorphism among parental rice varities and 33 selected F_4 plants at RM 224 locus. Lane L- 20 bp ladder, P_1- PAU201, P_2- Palman 579, 1-33: 33 selected F_4 plants.
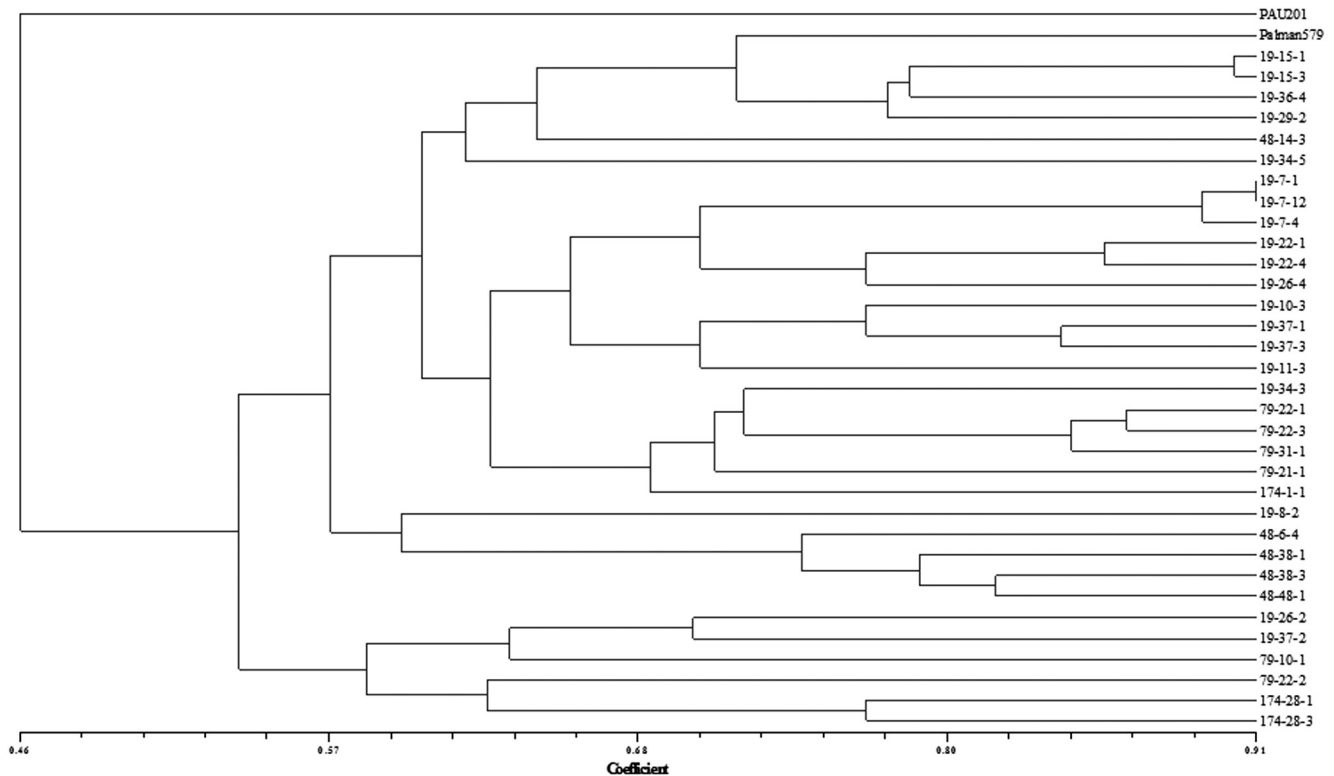

Fig. 5 — Dendrogram (NTSYS pc, UPGMA) of PAU201 × Palman 579 F_4 plants and parental rice genotypes based on SSR diversity data at 61 loci.

Five QTLs associated with iron content in dehusked rice grains were mapped on chromosome 5 (*qFE5.1*, *qFE5.2* and *qFE5.3* at map positions 41.0, 106.6 and 118.9 cM respectively), chromosome 7 (*qFE7.1* at map position 87.4 cM) and chromosome 9 (*qFE9.1* at map position 51.7) (Fig. 7). All the five QTLs identified for grain iron content were from the donor parent, Palman 579. The QTL identified for grain zinc content had negative values for additive effect (-5.86) indicated that the favouring alleles for grain zinc concentration was from PAU201 parent (Table 4).

QTL mapping for Physio-morphological Traits in F_4 population

Composite interval mapping (CIM) analysis by WinQTL cartographer 2.5 revealed a total of sixteen
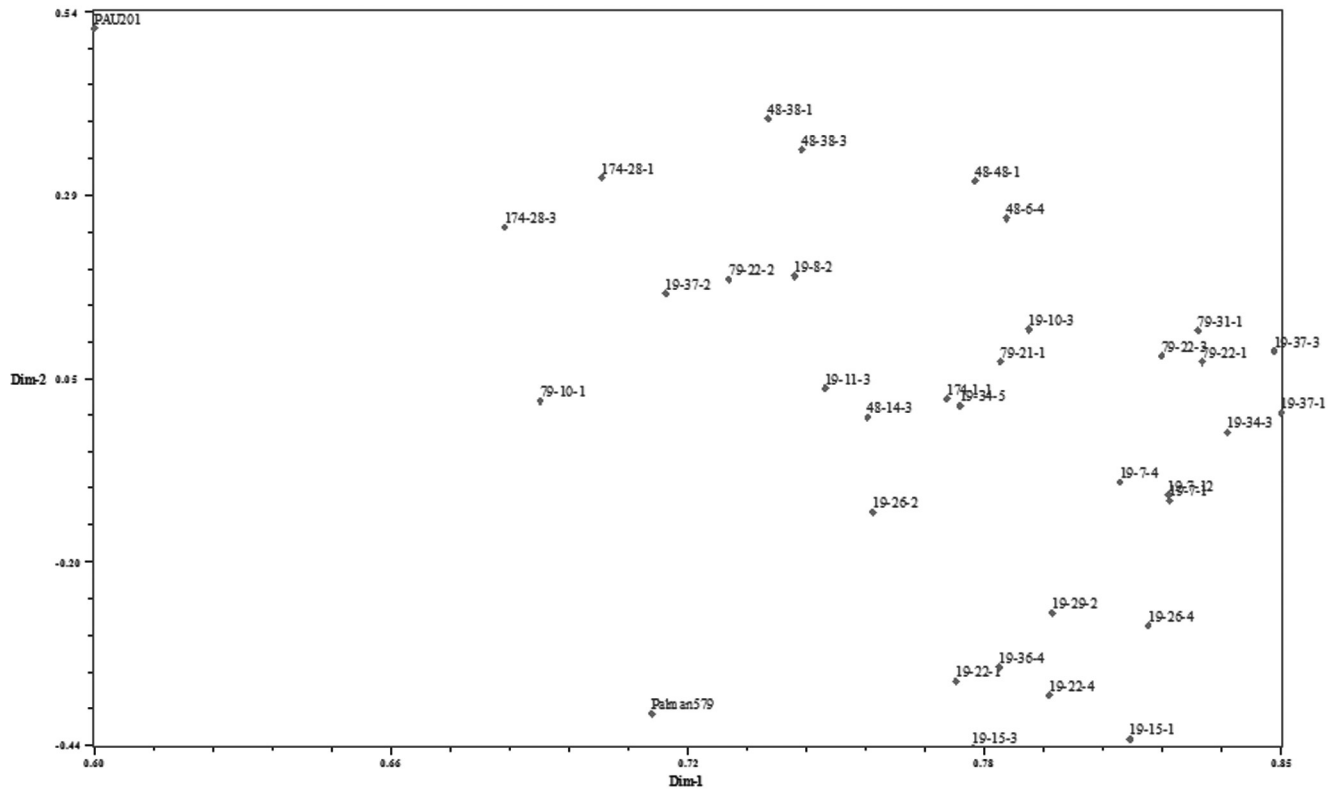
Fig. 6 — Two dimensional PCA scaling of PAU201 × Palman 579 F4 plants and parental rice genotypes based on SSR diversity data at 61 loci.
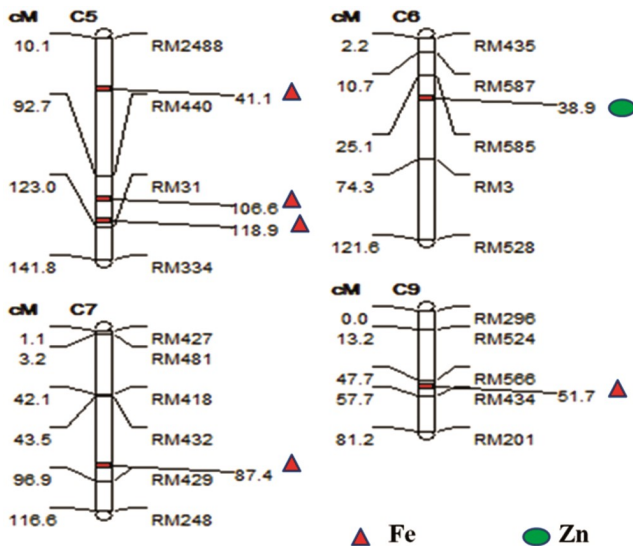

Fig. 7 — Chromosomal locations of quantitative trait loci (QTLs) for the mineral element (Fe and Zn) concentration identified in F_4 population derived from a cross between PAU201 × Palman 579.

at map position 31.1 cM) (Table 4, Fig. 8). Three QTLs associated with effective number of tillers/ plant were mapped on chromosome 10 (*qTP10.1*, *qTP10.2* and *qTP10.3* at map positions 48.2, 57.2 and 70.3 cM, respectively) (Table 4, Fig. 8). *qTP10.2* had maximum LOD score of 4.2 followed by *qTP10.3* (3.5 cM) and *qTP10.1* (3.3 cM). The QTLs, *qTP10.1*, *qTP10.2* and *qTP10.3* accounted for 21.4, 21.0 and 23.1% phenotypic variation, respectively. All the three QTLs identified for effective number of tillers/plant were from the donor parent, Palman 579.

Composite interval mapping revealed three QTLs associated grain yield/ plant in rice on chromosome 8 (*qYP8.1* and *qYP8.2* at map positions 38.0 and 50.6 cM, respectively) and chromosome 12 (*qYP12.1* at map position 48.7 cM) (Table 4, Fig. 8). The *qYP12.1* had the maximum LOD score of 3.8 and accounted for 6.4% phenotypic variation. The QTL, *qYP12.1* identified for grain yield/ plant had negative values for additive effect (-8.27) indicated that the favouring alleles for grain yield/ plant was from PAU201 parent (Table 4).

Four QTLs associated with grains/ panicle were mapped on chromosome 2 (*qGP2.1* at map positions
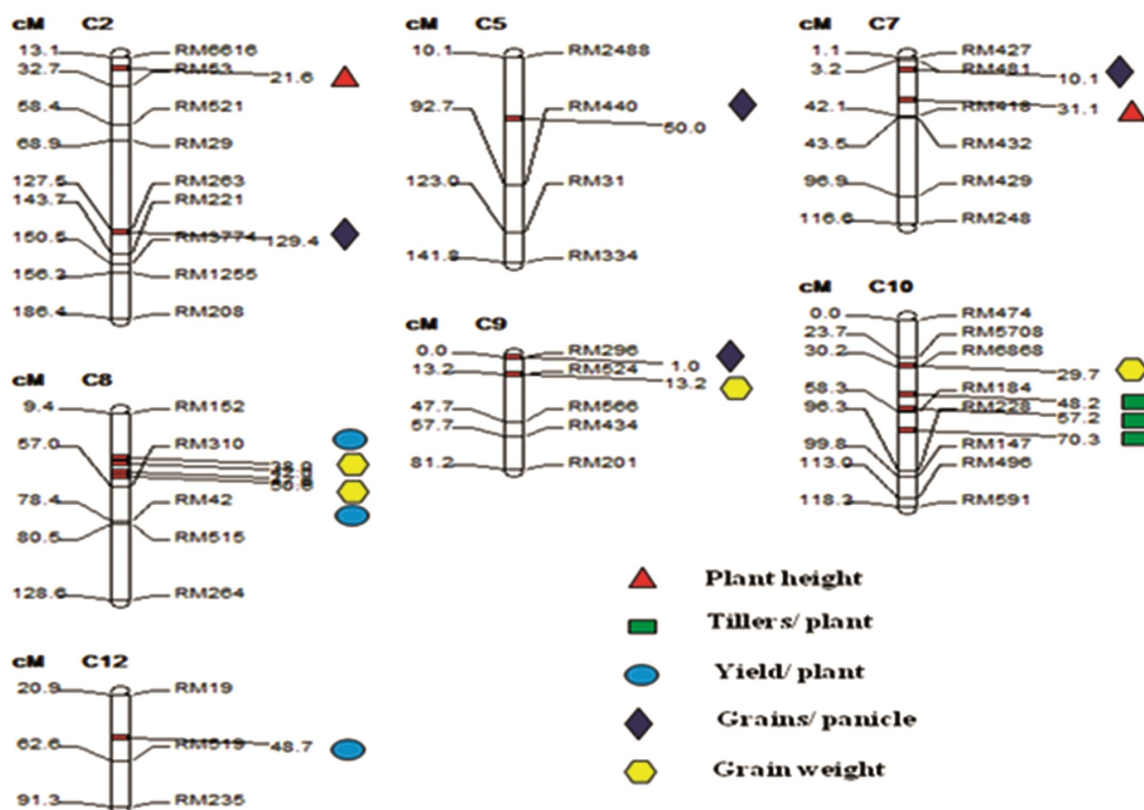
QTLs for various physio-morphological traits in rice (Table 4 and Fig. 8). Two QTLs associated with plant height were mapped on chromosome 2 (*qPH2.1* at map position of 21.6 cM) and chromosome 7 (*qPH7.1*

Fig. 8 — Chromosomal locations of quantitative trait loci (QTLs) for various physio-morphological traits identified in F_4 population derived from a cross between PAU201 × Palman 579.

129.4 cM), chromosome 5 (*qGP5.1* at map position 50.0 cM), chromosome 7 (*qGP7.1* at map position 10.1 cM) and chromosome 9 (*qGP9.1* at map position 1.0 cM) (Table 4, Fig. 6). Of the four QTLs identified for grains/ panicle, two QTLs (*qGP2.1* present on chromosome 2 and *qGP7.1* present on chromosome 7 with an additive effect of –3.43 and -46.89, respectively) were from PAU201; the remaining two QTLs were from the donor parent, Palman 579 (Table 4).

Four QTLs associated with 1000-grain weight were mapped on chromosome 8 (*qTG8.1* and *qTG8.2* at map positions 42.0 and 47.6 cM, respectively), chromosome 9 (*qTG9.1* at map position 13.2 cM) and chromosome 10 (*qTG10.1* at map positions 29.7 cM) (Fig. 8). The QTLs, *qTG8.1*, *qTG8.2*, *qTG9.1* and *qTG10.1* accounted for 19.5, 18.3, 12.4 and 5.0% phenotypic variation, respectively (Table 4). All the four QTLs identified for 1000-grain weight were from the donor parent, Palman 579.

Discussion

The present data show large variation in iron content in grain harvests of segregating F_3 and F_4 population, which could be due to different combinations of alleles from the two contrasting parents for iron content. While "Palman 579" had exceptionally higher iron content, "PAU201" also had relatively higher iron content compared to the other cultivated *indica* rice varieties[26-27]. Accumulation of minerals (iron and zinc) in grains is a complex phenomenon. Although, substantial genetic diversity for mineral content has been observed in rice, but linkage mapping of genes/QTLs for iron and zinc contents has gained momentum recently[16,18-20,28].

In the present study, a DNA fingerprint database of thirty three PAU201 × Palman 579 F_4 plants representing the entire variation for Fe and Zn contents was prepared using 61 polymorphic SSR markers. The NTSYS-pc UPGMA tree cluster analysis and two dimensional PCA scaling showed that the parent genotypes were quite distinct and diverse whereas 33 F_4 plants interspersed between the parent rice genotypes. A total of 128 alleles were identified in F_4 plants and three new recombinant alleles (different that those present in parental rice genotypes) were identified. Blair and coworkers[29] reported that such new alleles may have resulted from

unequal crossing over/replication slippage, translocation and other types of mutation or residual heterozygosity. Microsatellite marker analysis data showed that the F_4 population is skewed towards Palman 579.

The number of QTLs detected in each study depends on the genetic diversity among parental genotypes, population size and the number of molecular markers tested[11]. In this study, six QTLs for mineral (iron and zinc) content in rice grain were identified using CIM programme of WinQTL cartographer 2.5. A total of thirty three PAU201 × Palman 579 F_4 plants were used for microsatellite marker analysis and QTL identification by using the 61 polymorphic microsatellite markers. Notably, all the five putative QTLs for iron content identified in this study were from iron rich, donor parent, "Palman 579" present on three chromosomes (chromosome 5, 7 and 9). At the same time, the zinc improving allele (mapped on chromosome 6) was from the high-yielding, recipient parent, "PAU201".

Garcia-Oliveria and coworkers[18] detected a QTL for grain iron content in rice near the SSR marker RM296 on chromosome 9. Norton and coworkers[19] reported four QTLs for grain iron content on chromosome 1, 3, 4 and 7, respectively; QTL detected on chromosome 7 was at a different position. Gregorio and coworkers[30] reported three loci explaining 19-30% variation for iron content on chromosome 7, 8 and 9 in rice. In the present study, we did not detect any QTLs for iron content on chromosome 8 but QTLs were observed on chromosome 7 and 9, indicating a consensus region for iron content on these chromosomes in rice.

Anuradha and coworkers[28] reported one QTL on chromosome 5 and two QTLs on chromosome 7 for iron content in unpolished rice of Madhukar × Swarna RIL population, which accounted 69.2 and 138% phenotypic variation, respectively. Zhang and coworkers reported[20] two QTLs for grain iron content in rice on chromosome 5 and 7 using TeQing × Lemont backcross introgression lines. While the location of QTLs identified on chromosome 7 in the present study (87.4 cM) was comparable to that reported by Zhang and coworkers[20] with the position (70-120 cM); the location of QTL mapped on chromosome 5 was quite different.

In the present study, one QTL for zinc content was detected on chromosome 6 at map positions of 38.9 cM. Norton and coworkers[19] reported four QTLs

for zinc content in rice grain on chromosome 6, 7 and 10; one of these QTL was on chromosome 6 but at different map positions of 0.0 cM. Zhang and coworkers reported[20] one QTL for zinc content in rice grain on chromosome 6 using unflooded TeQing × Lemont backcross introgression lines. The location of QTL identified on chromosome 6 in the present study (38.9 cM) was comparable to that reported by Zhang and coworkers[20] at position (0-40 cM) on chromosome 6.

A notable aspect of this study was that out of six QTLs for mineral (iron and zinc) content; three QTLs for iron content were present on chromosome 5. The QTLs on chromosome 5 (*qFE5.1*, *qFE5.2*, *qFE5.3*), chromosome 6 (*qZN6.1*), chromosome 7 (*qFE7.1*) and chromosome 9 (*qFE9.1*) accounted for 254.5, 25.0, 95.2 and 34.6% phenotypic variation, respectively; QTLs for mineral content have also been reported by other researchers on these chromosomes[18-20,28]. It must be noted that these studies differ drastically with respect to the parental rice genotypes, mapping populations, environmental conditions (glass house[16]; open field[18-19]; flooded and unflooded field[20]) and locations across the continent. Though, this study has been conducted using a small, mortal F_4 population in a single environment, the results led to the identification of as many as six potential QTLs for Fe and Zn contents.

In this study, sixteen QTLs for physio-morphological traits in rice were also identified using CIM programme of WinQTL cartographer 2.5. The study also highlights the mapping of two QTLs (*qPH2.1* and *qPH7.1*) for plant height; three QTLs each for effective number of tillers per plant (*qTP10.1*, *qTP10.2* and *qTP10.3*); and grain yield per plant (*qYP8.1*, *qYP8.2* and *qYP12.1*); and four QTLs each for grains per panicle (*qGP2.1*, *qGP5.1*, *qGP7.1* and *qGP9.1*) and thousand grain weight (*qTG8.1*, *qTG8.2*, *qTG9.1* and *qTG10.1*). Ishimaru and coworkers reported three QTLs each for grains per panicle on chromosome 2, 3 and 8; panicle number per plant on chromosome 4, 7 and 8; and yield per plant on chromosome 3 and 5; and nine QTLs for plant height on chromosome 1, 2, 3, 7 and 8 in rice using backcross inbred lines of *japonica* Nipponbare × *indica* Kasalath[31]. The QTLs for grains per panicle on chromosome 2 reported by Ishimaru and coworkers was near the marker locus C1408[31]. Marathi and coworkers[32] identified QTLs for plant height, panicles per plant, filled grains per panicle,

thousand grain weight per plant and yield per plant using recombinant inbred population generated by crossing Pusa1266, a new plant type rice genotype derived from *indica × japonica* cross with a highly adapted high yielding *indica* rice variety Jaya[32]. The positions of the QTLs reported in the present study were quite different from reported by Marathi and coworkers[32].

Molecular and biochemical mechanisms for mineral uptake, translocation and accumulation, have been a subject of thorough investigation in many plant species[33-35]. With the increasing knowledge on the physiological and molecular processes underlying mineral accumulation, interesting alleles of relevant genes will be detected by QTL analysis and the DNA information of identified genes will be converted into molecular markers. With the increasing availability of more accurate genetic markers, advanced breeding tools can be used for selecting specific alleles of the genes, for example, mineral uptake or translocation to the grain.

Acknowledgement

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