Oryza Vol. 49. No. 2, 2012 (97-101)

Molecular mapping of the chromosomal regions associated with high iron and zinc content using SSR markers

G. Usharani*[1], C.N. Neeraja[2], V. Ravindra Babu[2], P. Nagesh[1], M.V. Brahmeswara Rao[1] and S. Sumathi[1]

*[1]*College of Agriculture, Rajendranagar, Acharya N. G. Ranga Agricultural University, Hyderabad*

[2]*Directorate of Rice Research, Rajendranagar, Hyderabad*

ABSTRACT

The present study was undertaken with the prime objective of mapping the chromosomal regions associated with high iron and zinc content involving the F2 populations derived from the cross of Samba Mahsuri with Ranbir Basmati using SSR markers derived from the genomic regions associated with iron and zinc metabolism. Out of the 35 microsatellite markers used for the parental polymorphism studies in Samba Mahsuri and Ranbir Basmati, 13 markers were polymorphic, 19 markers were monomorphic and 3 were not amplified. Most of the markers studied in the mapping experiment have shown a clear association with the trait.

Key words: *mapping, biofortification, iron, zinc, Samba Mahsuri, Ranbir Basmati*

Nutrient deficiencies pertaining iron and zinc is becoming a serious public health problem concerning about 124 million children worldwide. The poor masses in India, who consume rice as the staple food have limited resources to invest on non staple food like fruits, vegetables, meat and milk products etc. and their changing habit of shift from millet based staple to rice based staple food have resulted in widespread micronutrient deficiency termed as the 'hidden hunger'.

Even though the levels of carbohydrates are adequate in rice, parallel analysis of the levels and bioavailability of the other micronutrients in rice revealed that the levels are very low and consumption of rice alone cannot meet the recommended daily allowance for a range of vitamins, minerals and proteins. Biofortification is a genetic approach which aims at biological and genetic enrichment of food stuffs with vital nutrients such that the farmer can grow the variety indefinitely without any additional input to produce nutrient packed rice grains in a sustainable way (Bouis, 2002).

Rice has been at the forefront of plant genomics because of its small genome size, genetic relatedness to other major cereals, relatively low amount of repetitive DNA, its diploid nature and its ease of manipulation in tissue culture. For effective utilization of molecular markers in commercial breeding programs, tight linkage of the molecular markers with the trait of interest is an absolute necessity (Biradar *et al*., 2004). With expanding population and pressure on food crops, now breeders are focusing on breeding for nutritional enhancement. The range of iron and zinc concentrations in brown rice is 6.3-24.4 μg g^{-1} and 13.5-28.4 μg g^{-1}, respectively. There was approximately a fourfold difference in iron and zinc concentrations, suggesting some genetic potential to increase the concentration of these micronutrients in rice grains (Gregario, 2002). Potential exists for developing improved rice varieties with high iron and zinc content in the grain. Therefore, The present study was undertaken with the prime objective of mapping the chromosomal regions associated with high iron and zinc content

MATERIALS AND METHODS

Parental lines Samba Mahsuri (A popular variety in Andhra Pradesh with fine grain quality having high consumer acceptance but low zinc content) and Ranbir Basmati (A high zinc containing genotype) along with the mapping population consisting of 90 F_2 plants derived

from the above cross were selected for the mapping purpose. The DNA was extracted from freshly germinated young seedlings of parental lines and F_2 population using the method of Zheng *et al*. (1991). Thirty well spot test plate available from Thomas Scientific, USA was used for DNA isolations. The purity and concentration of the isolated genomic DNA samples were estimated by UV- absorption spectrophotometer (Beckman DU 650 model) as per the procedure described by Sambrook *et al*. (2001). Agarose gel electrophoresis (0.8%) was carried out for confirming the quality and quantity of the isolated DNA using a known concentration of ë DNA. The genomic DNA was subjected to PCR amplification as per the procedure described by Chen *et al*. (1997). DNA samples were amplified in 10µl reaction volumes containing 1X PCR buffer [10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (v/v) gelatin] (Bangalore Genei, India), 0.2 mM of each dNTPs (Bangalore Genei, India), 10 pmol of each primer and 1 U of *Taq polymerase* (Bangalore Genei, India). PCR was carried out in a Thermal cycler (Perkin–Elmer-Gene Amp PCR System 9700, USA). A PCR profile consisting of 5 min of initial denaturation at 94°C, 35 cycles of 1 min of denaturation at 94°C, 1 min of annealing at 55°C, 2 min of extension at 72°C and 7 min of final extension at 72°C was followed. The amplified products were resolved on 3% agarose gels, stained with ethidium bromide and visualized under UV in a gel documentation system (Alpha Innotech, USA). Microsatellite markers located in the vicinity of the putative candidate genes have been selected for mapping the chromosomal regions associated with iron and zinc rich regions in rice grains. Parental polymorphism was surveyed between Samba Mahsuri and Ranbir Basmati using 35 SSR markers derived from the genomic regions associated with iron and zinc metabolism (Table 1). Iron and zinc content of grain samples were estimated by Atomic Absorption Spectrophotometer as suggested by Lindsay and Novell (1978). The strategy of selective genotyping was carried out with the F_2 plants showing extreme phenotypes exhibiting high and low zinc content in grains individually with all polymorphic markers as suggested by Nandi *et al*. (1997). The markers which were found to be associated in the selective genotyping were used for analysis of all the 90 individuals. Each gel was scored for maternal, paternal and heterozygous banding pattern and scored accordingly. The maternal band was designated as 'A', paternal band 'B' and heterozygous band 'H'. Homozygotes were given a value of 0 or 1 based on their phenotype group. Heterozygotes were given a value of 0.5. Recombination frequency in percentage in relation to the total sample was calculated manually.

Recombination Frequency =

$$\frac{\text{Number of Recombinant Progeny}}{\text{Total Number of Progeny}} \times 100\%$$

Table 1. Details of SSR markers used and their chromosomal location

S.No.	SSR s	Nature of thetargeted gene	Chr.
1	SC 103	*ZT*	3
2	SC 116	*ZT*	8
3	SC 120	*Ysl*	4
4	SC123	*Ysl*	4
5	SC 126	*Ysl*	8
6	SC 129	*ZIP*	3
7	SC 131	*ZIP*	6
8	SC 135	*ZIP*	5
9	SC 141	*NRAMP*	12
10	SC 408	*ZIP*	3
11	SC 409	*ZIP*	3
12	SC 410	*ZIP*	3
13	SC 413	*ZIP*	5
14	SC414	*ZIP*	5
15	SC 418	*NRAMP*	12
16	SC 419	*NRAMP*	12
17	SC 420	*NRAMP*	12
18	SC 423	*ZT*	3
19	SC 424	*ZT*	3
20	SC 425	*ZT*	3
21	SC 428	*ZIP*	6
22	SC 429	*ZIP*	6
23	SC 430	*ZIP*	6
24	SC 433	*Ysl*	4
25	SC 434	*Ysl*	4
26	SC 435	*Ysl*	4
27	SC 438	*Ysl*	4
28	SC 439	*Ysl*	4
29	SC 440	*Ysl*	4
30	SC 443	*ZT*	8
31	SC 444	*ZT*	8
32	SC 445	*ZT*	8
33	SC 448	*Ysl*	8
34	SC 449	*Ysl*	8
35	SC 450	*Ysl*	8

SC indicates the microsatellite markers developed at Directorate of Rice Research; *ZT: Zinc Transport* gene, *YSl : Yellow Stripe like* gene, *ZIP : Zrt/Irt related protein* gene, *Zrt: Zinc regulated transporter* gene, *Irt: Iron regulated transporter* gene, *NRAMP: Natural Resistance-Associated Macrophage Protein* gene

RESULTS AND DISCUSSION

The parental polymorphism was studied using 35 markers in which 13 markers (37 %) were polymorphic, 19 markers were monomorphic and 3 were not amplified (Table 2). The polymorphic markers were then used for selective genotyping for rapid identification of regions associated with iron and zinc content (Table 3). To identify associated regions of the chromosomes with zinc metabolism, 90 F_2 plants were assayed with seven polymorphic markers which include SC129, SC135, SC428 and SC 430 markers based on *Zrt/Irt related Protein* (*ZIP*) gene, SC425 based on *Zinc Transport* (*ZT*) gene, SC434 marker based on *Yellow Stripe –Like transporter* (*YSL*) gene and SC418 marker based on *Natural Resistance –Associated Macrophage Protein* (*NRAMP)* gene (Table 4). The results are presented in Table 4. To identify associated regions of the chromosomes with iron metabolism, 90 F_2 individual plants were assayed individually with seven polymorphic markers which include SC129, SC135, SC428 and SC 430 markers based on *ZIP* gene, SC425 based on *ZT* gene, SC434 marker based on *YSL* gene and SC418 marker based on *NRAMP* gene (Table 5). The tentative SSR based linkage maps for regions associated with enhanced iron accumulation in F_2 lines from the cross of Samba Mahsuri and Ranbir Basmati is exhibited in Fig 1. The tentative SSR based linkage maps for regions associated with enhanced zinc accumulation in F_2 lines from the cross of Samba Mahsuri and Ranbir Basmati is exhibited in Fig 2. The segregation pattern of SC 430 in the F_2 lines derived from the cross Samba Mahsuri / Ranbir Basmati is shown in Fig 3. The SC 129 marker is located on chromosome 3 and the putative candidate gene sequence was found between '16921118 and 17018159 bp'. The studies made earlier had shown that, *OsZIP 1* was found to be on chromosome 3 at a distance of 83.3 cM (Gross *et al*., 2003). The SC 425 marker is

Table 2. Parental Polymorphism Survey between Samba Mahsuri and Ranbir Basmati

Polymorphism	No. of markers	SSR Markers
Polymorphic	13	SC-129, SC-103, SC-141, SC-120, SC-126, SC-123, SC-418, SC-448, SC-435, SC-425, SC-428,SC-430, SC-434
Monomorphic	19	SC-135, SC-131, SC-116, SC-408, SC-409, SC-410, SC-413, SC-414, SC-419, ,SC-429,SC-433,,SC-438, SC-439,SC-440,SC-443,SC-444, SC-445,SC-449,SC-450
Not amplified	3	SC-420, SC-423, SC-424

Table 3. Details of selective genotyping values of the F_2 mapping population

Quantity	F_2 population of the cross derived from parents	Fe/Zn content	mg/100g	No. of indivi-duals	Cut-off values
HIGH	Samba Mahsuri/ Ranbir Basmati	Zn	3.07-10.33	24	> 3
LOW	Samba Mahsuri/ Ranbir Basmati	Zn	0.23-2.15	22	< 2

Table 4. Recombination frequency of markers between the gene of interest and designed marker in F_2 population of Samba Mahsuri and Ranbir Basmati for zinc content

Marker	Gene	Chr. No.	Recombination frequency(cM)
SC129	ZIP	3	9.8
SC135	ZIP	5	10.5
SC428	ZIP	6	15.9
SC430	ZIP	6	6.4
SC425	ZT	3	9.8
SC434	YSl	4	8.5
SC418	NRAMP	12	14.5

Table 5. Recombination frequency of markers between the gene of interest and designed marker in F_2 population of Samba Mahsuri and Ranbir Basmati for iron content

Marker	Gene	Chr. No.	Recombination frequency(cM)
SC129	ZIP	3	12.5
SC135	ZIP	5	13.4
SC428	ZIP	6	8.8
SC430	ZIP	6	10.5
SC425	ZT	3	16.5
SC434	YSl	4	13.9
SC418	NRAMP	12	21.6

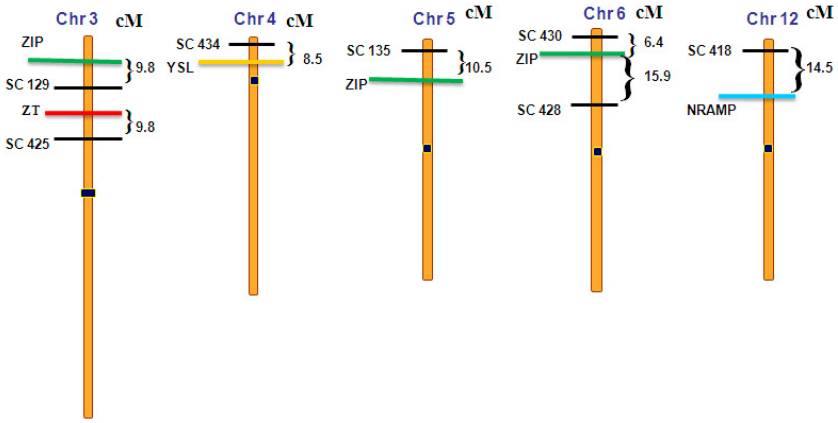

Fig. 1. Tentative SSR based linkage maps for regions associated with enhanced zinc accumulation in F_2 lines from Samba Mahsuri / Ranbir Basmati

located on the chromosome 3 and the putative candidate gene sequence was found between '26114338 – 26248438 bp'. The SC 135 marker is located on the chromosome 5 and the putative candidate gene sequence was found between '23120382-23146687 bp'. The SC 428 and SC 430 markers are located on the chromosome 6 and the putative candidate gene sequence was found between '21733234-21853800 bp'. The SC 434 marker is located on the chromosome 4 and the putative candidate gene sequence was found between '19035594-19181019 bp'. The SC 418 marker is located on the chromosome 12 and the putative candidate gene sequence was found between '24050217–24150869 bp'. It was reported earlier that *OsNRAMP7* was found on chromosome 12 at a distance of 94.6 cM (Gross *et al*., 2003).

Thus, the findings from the present study indicated that much more regions associated with iron and zinc content in the grains can be identified with the screening of more microsatellite markers located in the candidate genes associated with iron and zinc metabolism. In addition to the F_2 lines studied under selective genotyping, analysis of more F_2 lines would increase the stringency of the loci identified for the iron content in the grain. The knowledge of QTL analysis and the information of DNA in identified genes on mineral accumulation are helpful in the identification

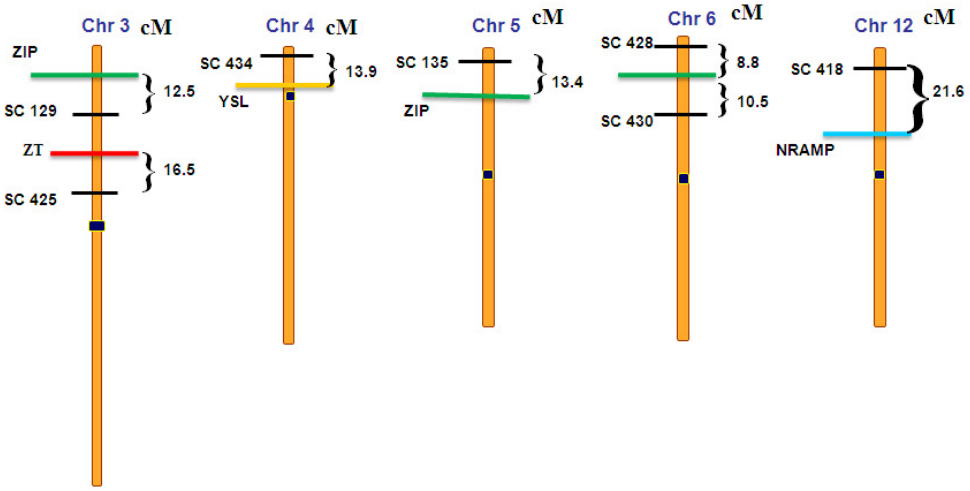

Fig. 2. Tentative SSR based linkage maps for regions associated with enhanced iron accumulation in F_2 lines from Samba Mahsuri / Ranbir Basmati

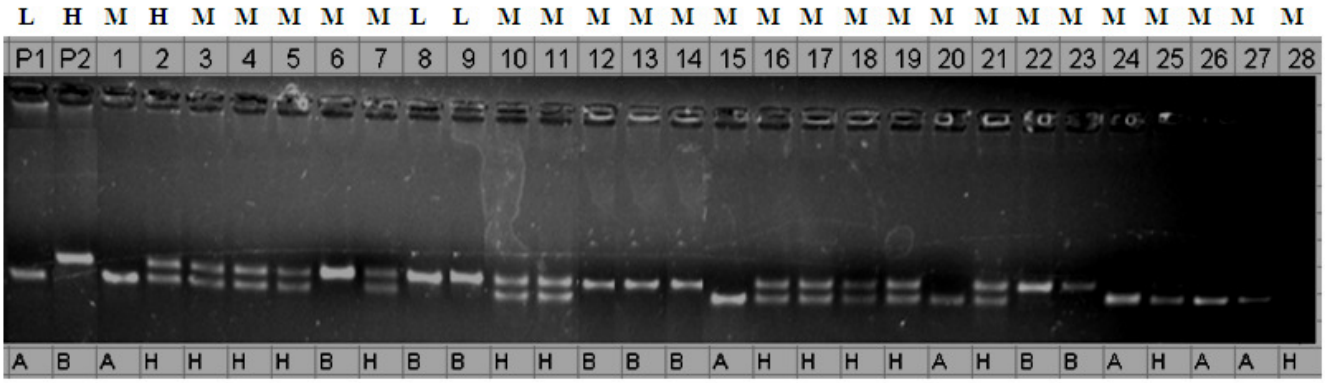

Fig. 3. Segregation pattern of SC 430 in the F2 lines derived from the cross of Samba Mahsuri / Ranbir Basmati

H- indicates the F2 lines with high iron rich regions; M- indicates the F2 lines with medium iron rich regions; L- indicates the F2 lines with low iron rich regions.

of interesting alleles of relevant genes. Most of the markers studied in the mapping experiment have shown a clear association with the trait despite the less number of F_2 samples analyzed. Thus the mapping results in the present study suggest the strategy of identification of microsatellite markers in the vicinity of candidate genes involved in the cation metabolism and their use in mapping to be very appropriate.

REFERENCES

Biradar SK, Sundaram RM, Thirumurgan T, Bentur JS, Amudhan S, Shenoy VV, Mishra B, Bennet J and Sharma NP 2004. Identification of flanking SSR markers for a major rice gall midge resistance gene Gm1 and their validation. Theoretical and applied genetics. 109 (7):1468-73.

Bouis HE 2002. Plant breeding: A new tool for fighting micronutrient malnutrition. Journal of Nutrition. 132:491-494.

Chen X, Temnykh S, Xu Y, Cho Y and Mccouch SR 1997. Development of microsatellite framework map providing genome wide coverage in rice (*Oryza sativa* L.). Theoretical and applied genetics 95: 553-567.

Gregario GB 2002. Progress in Breeding for Trace Minerals in Staple Crops. Journal of Nutrition. 132:500-502.

Gross J, Stein JS, Fett-Neto AG and Fett JP 2003. Iron homeostasis related genes in rice. Genetics and Molecular Biology 26 (4): 477-497.

Lindsay WL and Novell WA 1978. Development of a DTPA soil test for zinc, manganese and copper. Soil Sci Soci Am J 42: 421-428.

Nandi S, Subudh PK, Senadhira D, Manigbas NL, Sen-Mandi S and Huang N 1997. Mapping QTLs for submergence tolerance in rice by AFLP analysis and selective genotyping. Mol. Gen. Genet 255: 1-8.

Sambrook J and Russell DW 2001. Molecular Cloning-A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Zheng KL, Shen B and Qian HR 1991. DNA polymorphism generated by arbitrary primed PCR in rice. Rice Genetics Newsletter 8 : 134-136.