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Research Article

MICROSATELLITE MARKER ASSISTED SELECTION AND GENERATION OF F1 HYBRIDS FOR RICE BIOFORTIFICATION

BRAR BASANTI[1]*, CHAUDHARY DEEPIKA[2], JAIN R. K.[1] AND JAIN SUNITA[1]

[1]Department of molecular Biology, Biotechnology and Bioinformatics, Chaudhary Charan Singh Haryana Agricultural University, Hisar, 125004, Haryana
[2]Chaudhary Devi Lal University, Sirsa, 125055, Haryana.
*Corresponding Author: Email-basantibrar@gmail.com

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Abstract- Biofortification is an approach for enhancement of the micronutrient content of staple crops. This possible because only due to the lots of genetic variation exist within the genome of staple food crops like rice. Greater than 2 billion persons of the humanity are micronutrient- iron as well as zinc deficient. Rice (*Oryza sativa L.*) is the major source of food for more than half of the world's population. As a model cereal crop, the complete genome sequences of rice has become fundamental tool for study gene functions and correlate it with the practical applications in plants. At present, rice researchers devote much effort to generating mineral rich rice genotypes to combact the micronutrient malnutrition. Molecular analysis, genetic transformation and molecular breeding combine with mineral content examination for presented rice germplasm. In the present study we report the F1 identification of rice plant after crossing the high yielding genotypes with micronutrient (iron and Zinc) rich genotypes using microsatellite markers both by agarose as well as polyacrylamide gel electrophoresis.

Keywords- Enhancement, mineral content Micronutrient deficiency, rice and zinc.

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Introduction

Rice is the staple crop that feeds higher than 50% of the global population. It is a model cereal plant species for molecular research. Till 1990, most of the molecular breeding research was focus on increasing the grain yield and to improve the resistance to environmental stresses, pests and pathogens [1,2], but there is no attention was towards the enhancement of its nutritional quality. International Rice Research Institute (IRRI) was released the first high yielding rice variety 'IR8' in 1966. In the posterity, a low number of this type of high genotypes is completely replace by the greater number of general varieties cultivated by farmer in earlier times. This resulted in the immesurable 'genetic erosion' and loss of biodiversity. Despite the loss, a lot of germplasm still exists and being maintained by International Rice Research Institute (IRRI, The Philippines) and national research institutions in different countries. Most of this germplasm is yet need to be tested for the nutritional quality and quantity traits. Many groups have reported the possibility of "Biofortification" approach for increasing their nutrient content because lots of substantial useful genetic changes presents in key staple crops, breeding programs can readily direct nutritional quality traits, which have been reported to be highly inherited in some crops and required traits are adequately stable across a broad range of growing environments as well as traits for high nutrient trait can be pooled with superior agronomic and high yield features [3]. The most destructive micronutrient deficiencies in the world are the outcome of low dietary intake of iron, zinc and vitamin A [4]. Iron deficiency marks in anaemia in human beings that decrease the immune capability, maintains homeostasis and affect the growth of brain [5,6]. A great number of abnormalities are caused by zinc deficiency such as retarded growth, dejected immune function, skeletal abnormalities and diarrhoea [7-9]. Improvement of micronutrient (e.g. Fe and Zn) rich and resourceful crops using novel molecular tools has gained attention only after Goto *et al.,* [10] and Ye *et al.,* [11] reported the engineering of soybean ferritin gene and β-carotene biosynthesis pathway, respectively, in rice. Along with the micro-nutrients, mainly of work has been completed to modify transgenic plants having high iron content as well as bioavailability [12-16]. Some progress has been made on the way to the improvement of zinc rich transgenic plants with high zinc content and bioavailability [17-19]. Molecular markers have confirmed helpful in basic and purposeful research, such as varietal recognition, variety analysis, and, DNA fingerprinting, phylogenetic examination of genes in rice [20-25]. Rice genome sequencing and comparison of sequence databases of *indica* and *japonica* rice genomes have provided an almost unlimited number of DNA markers such as SSR for high-resolution genetic analysis. The studies revealed that SSR are abundant and dispersed all through the rice genome. The present study is undertaken to initiate the crossing program between the selected mineral-rich and commercially important rice genotypes using conventional and molecular breeding techniques.

Materials and methods

Plant material

Plant material was collected from rice research station Kaul. Mineral rich (Fe and Zn) rice varieties - Palman 579, HKR95-157, Taraori Basmati, BR4-10, TNG67, Jaya; High yielding varieties- HKR47, PAU201, Pusa Basmati 1 were selected for the present study.

Crossing between the selected mineral-rich and commercially important rice genotypes

Mineral content reported according to Brar et al. [26,27] iron and zinc content of rice varieties were selected and thirteen crosses were made between mineral rich

(Fe and Zn) rice varieties (Palman 579, HKR95-157, Taraori Basmati, BR4-10, TNG67, Jaya) and high yielding varieties (HKR47, PAU201, Pusa Basmati 1). All the thirteen crosses were attempted using mineral rich rice genotypes as male parent and high yielding rice genotypes as female parent.

Microsatellite marker analysis of plants

Genomic DNA was isolated using CTAB method of Saghai-Maroof *et al*. [28]. RNA contamination was removed by adding 1 µl of 10 mg/ml RNase (RNase-A, Sigma chemical Co. USA, No.R-5503) in the dissolved DNA samples. Completely dissolved DNA was checked for its quality and concentration by running DNA samples on 0.8% agarose gels electrophoresis using a standard containing 100 ng/µl genomic λ DNA. Microsatellite markers were used for preparation of molecular database for fourteen rice genotypes and F_1 plants. PCR amplifications were performed using PTC – 100™ 96V thermo cycler (MJ Research, Inc., Watertown, MA, USA). The PCR reaction was conducted in a reaction volume of 20µl containing 1X PCR buffer, 100 µM dNTPs, 0.3 µM of each primer, 1.5 unit Taq DNA polymerase and 50-75 ng template DNA. Polymerase chain reaction was carried out for the amplification of products- initial denaturation at 94^{0}C at 5 minutes followed by 40 cycle of 94^{0}C at 1 minute, annealing at 54^{0}C for 1 minute, 72^{0}C for 1 min and final extension at 72^{0}C for 7 min before cooling at 4^{0}C. Amplification products were stored at –20^{0}C till further use. Amplification products were resolved on 2.5% w/v agarose gel as well as on 4% polyacrylamide gels using Aluminium Backed Sequencing System Model # 535 (Owl Scientific, Inc., Voburn, USA) as described by Chen *et al.* [29]. Gels were pre-run until an adequate temperature (50-60^{0}C) was reached. DNA bands were then visualized using the Silver Sequence DNA Sequencing System (Promega Technical Manual, Part # TM023).

Results

Crossing program

A crossing program between mineral rich (Fe and Zn) rice varieties and high yielding varieties was initiated at RRS, Kaul to transfer the desirable mineral-rich traits to high-yielding rice genotypes. A total of 13 crosses [Fig-1, 2] were made between mineral-rich (Fe and Zn) rice varieties (Palman 579, HKR95-157, Taraori Basmati, BR4-10, TNG67 and Jaya) and high-yielding rice varieties (HKR47, PAU201 and Pusa Basmati 1) with mineral-rich rice genotype as male parent and high-yielding rice genotype as the female parent [Table-1]. [Table-1] illustrates the results of attempted crosses between micronutrient rich and high yielding rice varieties. A total of 473 seeds were obtained from thirteen crosses, out of which 166 seeds germinated. From these germinated seeds, DNA was isolated from 100 plants and analysed for F_1 identification. A total of forty two plants were found to be PCR positive F_1 plants.

Table-1 *Results of attempted crosses between micronutrient rich and high yielding rice varieties*

Sr. No.	Female x Male	No. of seeds obtained	No. of seed germinated	No. of plants Analyzed by PCR	No. Of PCR positive F1 Plants
1.	Taraori Basmati x Palman 579	30	15	10 (RM152)*	2
2.	HKR47 x Taraori basmati	85	15	9(RM162, RM310)*	6
3.	Pusa Basmati 1 x Taraori basmati	35	15	10(RM162, RM310)*	5
4.	Pusa Basmati 1 x Palman 579	31	15	9(RM162, RM310)*	3
5.	PAU201 x Palman 579	55	15	11(RM162, RM310)*	7
6.	HKR47 x Palman 579	04	4	4(RM162, RM310)*	3
7.	PAU201 x HKR95-157	85	15	10(RM447)*	4
8.	HKR47 x HKR95-157	10	7	5(RM162, RM310)*	3
9.	Pusa Basmati 1 x HKR95-157	28	15	3(RM162, RM310)*	3
10.	HKR47 x Jaya	06	5	5(RM162, RM310)*	0
11.	HKR47 x BR4-10	65	15	9(RM162, RM310)*	3
12.	PAU201 x TNG67	20	15	5(RM162, RM310)*	3
13.	PAU201 x BR4-10	19	15	10(RM162, RM310)*	0

* represents the molecular markers used for F_1 identification.

Polyacrylamide and agarose gel electrophoresis for F_1 identification

Several microsatellite DNA markers which showed polymorphism among parental rice varieties were used for the identification of F_1 plants derived from nine parental rice genotypes using 4% PAGE [Table-2]. To reduce cost of PAGE, experiments were also conducted to identify F_1 plants using 2.5% w/v agarose gels. DNA was isolated from 100 putative F_1 plants and analyzed for allelic profile using one or more microsatellite DNA marker [Table-2]. Out of 100 plants raised from the crossed seed, 42 plants were confirmed as true F_1 hybrids [Fig-3 and 4].

Table-2 *Allelic profile of parent rice genotypes and their F_1 hybrids using at four RM loci.*

Marker	Cross combination	Allele size (bp)								
		HKR47	BR4-10	PAU-201	TNG67	Palman579	Taraori Basmati	Pusa Basmati 1	HKR95-157	Hybrid
RM310	HKR47 x BR4-10	118	87							118, 87
	PAU201 x TNG67			80	123					80, 123
	PAU201 x Palman 579			80		98				80, 98
	HKR47 x Taraori Basmati	118					98			118, 98
	Pusa Basmati 1 X Taraori Basmati						98	118		98, 118
	HKR47 X Palman 579	118				98				118, 98
	Pusa Basmati 1 X HKR95 -157							118	80	118, 80
	HKR47 X HKR 95-157	118							80	118, 80
	Pusa Basmati 1 X Palman579					98		118		98, 118
RM 162	HKR47 x BR4-10	220	217							220, 217
	PAU201 x TNG67			220	225					220, 225
	PAU201 x Palman 579			220		225				220, 225
	HKR47 x Taraori Basmati	220					245			220, 245
	Pusa Basmati 1 X Taraori Basmati						245	255		245, 255
	HKR47 X Palman 579	220				225				220, 225
	Pusa Basmati 1 X HKR95-157							255	227	255, 227
	HKR47 X HKR95-157	220							227	220, 227
	Pusa Basmati 1 X Palman 579					225		255		225, 255
RM152	Taraori Basmati X Palman 579					128	148			128,148
RM447	PAU201 x HKR95-157			170					110	170, 110

Fig-1 Generation of F1 hybrids in pot house

Fig-2 Seeds obtained from cross

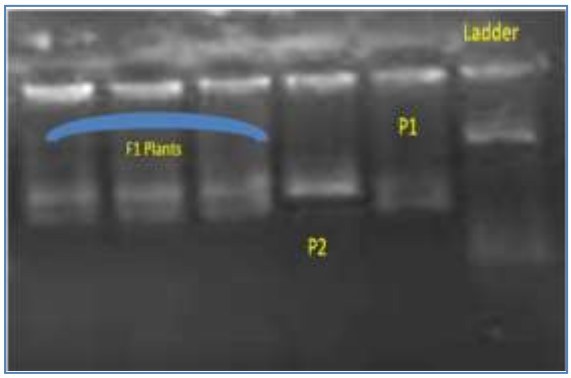

Fig-3 Agarose gel electrophoretic separation for detection of F1 hybrids

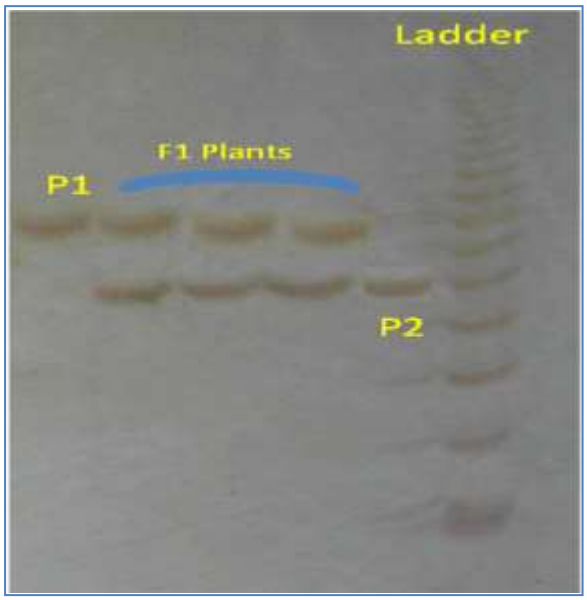

Fig-4 Polyacrylamide gel electrophoretic separations for detection of F1 hybrids

Taraori Basmati (148 bp) and Palman 579 (128 bp) amplified different size alleles at RM152. While two of the ten putative Taraori Basmati x Palman 579 F_1 plants (148 and 128 bp) had alleles from both the parents, the rest of the eight plants amplified female specific band only. Only two of the ten plants raised from the HKR47 x Taraori Basmati crossed seed had alleles from both HKR47 (220 bp, 118 bp) and Taraori Basmati (245 bp, 98 bp) at RM162 and RM310 loci, respectively, and were confirmed as true F_1 hybrids. Molecular markers, RM162 and RM310, amplified different alleles in Pusa Basmati 1 (255 bp, 118 bp) and Palman 579 (225 bp, 98 bp), respectively. Three of the ten putative Pusa Basmati 1 x Palman 579 F_1 plants had alleles from both the parents, while rest of the seven plants amplified female specific bands only. Similarly, four of the ten HKR47 x Palman 579 F_1 plants amplified alleles of both the parental lines at RM162 (220 and 225 bp) and RM310 (118 and 98 bp) and were confirmed as true F_1 hybrids. PAU201 (170 bp) and HKR95-157 (110 bp) amplified different alleles at RM447. Four of the ten PAU 201 x HKR95-157 F_1 plants (170 and 110 bp) showed alleles from both the parents and rest of the six plants amplified female specific band only. Similarly polymorphism at RM162 and RM310 loci, HKR47 (220 and 118 bp) x HKR95-157 (227 and 80 bp), amplified different alleles. Polymorphism at these markers was used to identify true F_1 hybrids; three of the five F_1 plants tested, three plants were found to be true hybrids. Similarly polymorphism at RM162 and RM310 loci, was used to identify true F_1 hybrids from the cross between HKR47 (220 and 118 bp) and BR4-10 (217 and 87 bp). A total of three plants were found to hybrid out of four putative plants obtained from cross (PAU201 x TNG67). PAU201 (220 and 80 bp) and TNG67 (225 and 123 bp) also amplified different alleles at these RM loci. All the three Pusa Basmati 1 (255 and 118 bp) x HKR95-157 (227 and 80 bp) F_1 plants were confirmed as hybrids by analyzing the allelic profile at these two loci. A total of seven plants were found to hybrid out of eleven putative plants obtained from cross between PAU201 and Palman 579. PAU201 (220 and 80 bp) and Palman 579 (225 and 98 bp) amplified different alleles at RM162 and RM310, respectively. The rest four plants gave female specific bands. Pusa Basmati 1 and Taraori Basmati showed polymorphism at RM162 (245, 255) and RM310 (98, 118), respectively. Out of the 10 putative F_1 plants, only two plants had alleles from both the parents and rest eight plants give female specific bands.

Discussion

Microsatellite markers have also been used earlier for fingerprinting of hybrids, assessing variations within parental lines and testing the purity of hybrid seed lot in rice [30]. Along with parental bands non-parental has been observed that may be due to different amplified fragments of heteroduplex molecules were a suggestive characteristic for hybrid individuals identification [30]. Four sets of markers (RM206, RM 216, RM 258 and RM 263) has been reported for differentiating all the hybrids from other, which can be used as referral markers for unambiguous identification and protection of these hybrids [30]. DNA profiling of 14 rice genotypes has been reported using 48 microsattelite markers [25, 27, 32]. In the present study, after screening 220 rice genotypes [26], a number of mineral (Fe and Zn) -rich rice genotypes were selected for the crossing program. A total of 13 crosses were made between mineral-rich (Palman 579, HKR95-157, Taraori Basmati, BR4-10, TNG67, Jaya) and high yielding rice varieties (HKR47, PAU201, Pusa Basmati 1). The crossed seeds were recovered from 13 crosses. Maximum seeds were obtained from HKR47 x Taraori Basmati, PAU201 x HKR95-157 and PAU201 x HKR95-157 (85) followed by HKR47 x BR4-10 (65), PAU201 x Palman 579 (55), Pusa Basmati 1 x Taraori Basmati (35), Pusa Basmati 1 x Palman 579 (31), Taraori Basmati x Palman 579 (30), Pusa Basmati 1 x HKR95-157 (28), PAU201 x TNG67 (20), HKR47 x HKR95-157 (10), HKR47 x Jaya (6) and HKR47 x Palman 579 (4). Out of 13 crosses, eleven crosses gave putative F_1 plants. DNA was isolated from 100 F_1 plants and analyzed for F_1 identification using the molecular markers (RM152, RM 162, RM 310 and RM447). Out of these 42 plants were found to be true F_1 hybrid. Breeding experiments have also been conducted earlier to improve the micronutrient (Fe and Zn) content in rice. Large variation for both Fe and Zn in rice, has been observed [33-35]. The results showed that a high-iron and zinc trait can be combined with high yielding traits. This was demonstrated in the serendipitous discovery of an aromatic variety already in the

IRRI testing program-a cross of a high yielding variety (IR72) and a tall, traditional variety (Zawa Bonday) from India, from which IRRI identified an improved line (IR68144-3B-2-2-3) with high a concentration of grain iron, ~21 ppm in brown rice. This elite line has tolerance to rice tungro virus and excellent grain qualities. Yields are ~10% below IR72, but, in partial compensation, maturity is earlier. This variety has good tolerance to mineral deficient soils, such as P, Zn and Fe. Several dozen crosses has been attempted using these 3 genotypes (IR69428-6-1-1-3-3, IR75862-206-2-8-3-B-B-B and Joryeongbyeo) as donor parents and IR64, a popular *indica* variety as recipient parent aimed at developing elite rice breeding lines with high grain zinc [36]. Three promising genotypes had grain zinc content significantly higher than IR64, a popular *indica* variety.

Conclusion

In the present study, a total of 13 crosses were made between mineral-rich (Palman 579, HKR95-157, Taraori Basmati, BR4-10, TNG67, Jaya) and high yielding rice varieties (HKR47, PAU201, Pusa Basmati 1) for mineral enhancement in rice. Out of which 11 crosses gave putative f1 seeds. The 100 plants were grown from the putative seeds and used for DNA extraction. The F1 identification using the molecular markers (RM152, RM 162, RM 310 and RM447), out of 100 plants, 42 plants were found to be true F1 hybrid.

Application of research: Molecular breeding helps in biofortification of rice. It helps to combat the problem of micronutrient malnutrition and helps in increasing grain yield.

Research Category: Enhancement, mineral content, Micronutrient deficiency, rice and zinc

Abbreviations:

PCV: Phenotypic coefficients of variation
GCV: Genetic coefficient of variation
Fe:Iron, Zn:Zinc, IRRI: International Rice Research Institute

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***Research Guide: Dr R K Jain**

University: Chaudhary Charan Singh Haryana Agricultural University, Hisar, 125004, Haryana
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Author Contributions: All author equally contributed

Author statement: All authors read, reviewed, agree and approved the final manuscript

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

References

[1] Borlaug N.E. (1998) *Food security, plant pathology and quarantine. Public discussion forum on global food Security. International Congress of Plant Pathol., Edinburgh*.
[2] Borlaug N.E. (2000) *Plant Physiol.,* 124, 487-490.
[3] Welch R.M., Graham R.D. (2004) *J. Exp. Bot.*, 55, 353-364.
[4] Zimmermann M.B. and Hurrell R.F. (2002) *Current Opinion in Biotechnol.,* 13,142–145.
[5] DeMaeyer E. and Adiels-Tegman M. (1985) *World Health Stat. Quart.*, 38, 302-316.
[6] Scrimshaw N.S. (1984) *J. Nutr. Sci. Vitamino.*, 30, 47-63.
[7] Parsad A.S. (1993) *Clinical spectrum of human zinc deficiency In: Parsad AS Biochemistry of Zinc, Plenum Press, New York*, pp 219-258.
[8] Prasad A.S. (1996) *J. Am. College of Nutr.*, 15, 113-120.
[9] Rivera J.A., Ruel M.T., Santizo M.C., Lo Ènnerdal B. and Brown K.H. (1998) *J. Nutr.*, 128,556-562
[10] Goto F., Yoshihara T., Shigemoto N., Toki S. and Takaiwa F. (1999) *Nat. Biotechnol.*, 17, 282-286.
[11] Ye X., Al-Babili S., Kloeti A., Zhang J., Lucca P., Beyer P. and Potrykus I. (2000) *Science*, 287, 303–305.
[12] Vasconcelos M., Datta K., Oliva N., Khalekuzzaman, Torrizo L., Krishnan S., Oliveira M., Goto F. and Datta S.K. (2002) *Plant Sci.*, 164, 371-378.
[13] Jain R.K., Saini N., Jain S. and Singh R. (2003) *Ind. J. Biotechnol*., 2, 121-137.
[14] Guerinot M.L. (2007) Proc. Natl. Acad. Sci. USA, 104, 7311-7312.
[15] Zhu C., Naqvi S., Gomez-Galera S., Pelacho A.M., Capell T. and Christou P. (2007) *Trends Plant Sci.*, 12, 548-555.
[16] Zheng L., Cheng Z., Ai C., Jiang X. and Bei X. (2010) Nicotianamine, a novel enhancer of rice iron bioavailability to humans. in press- available online.
[17] Ishimaru Y., Kim S., Tsukamoto T., Oki H., Kobayashi T., Watanabe S., Matsuhashi S., Takahashi M., Nakanishi H., Mori S. and Nishizawa N.K. (2007) *Proc. Natl. Acad. Sci. USA*, 104, 7373-7378.
[18] Masuda H., Suzuki M., Morikawa K.C., Kobayashi T., Nakanishi H., Takahashi M., Saigusa M., Mori S. and Nishizawa N.K. (2008) *Rice*, 1, 100–108.
[19] Lee S., Jeong H.J., Kim S.A., Lee J., Guerinot M.L. and An G. (2010) *Biomed. Life Sci.Plant Mol. Biol.*, 73, 507-517.
[20] Wu K.S. and Tanksley S.D. (1993) *Mol. Gen. Genet.*, 241, 225-235.
[21] McCouch S.R. (2001) *Plant Physiol*., 125, 152-155.
[22] Li Z. (2001) QTL mapping in rice, a few critical considerations. In, Rice genetics IV (EdsKhush, GS, Brar, DS and Hardy, B), IRRI, Los Baños, Manila, Philippines, pp 153-171.
[23] Xu Y. (2002) Global view of QTL: rice as a model In: quantitative genetics, genomics and plant breeding (ed Kang, MS), Wallingford (UK): CAB International, pp 109-134.
[24] Shen Y.J., Jiang H., Jin J.P., Zhang Z.B., Xi B., He Y.Y., Wang G., Wang C., Qian L., Li X., Yu Q.B., Liu H.J., Chen D.H., Gao J.H., Huang H., Shi T.L. and Yang Z.N. (2004) *Plant Physiol.*, 135 , 1198-1205
[25] Brar B., Jain R.K. and Jain S. (2015) *Int J CurrSci*., 2, 15, E 42-50.
[26] Brar B., Jain S., Singh R. and Jain R.K. (2011) *Indian J. Genet*., 71(1), 67-73.
[27] Brar B., Jain S. and Jain R.K. (2014) *Indian J. Genet*., 74(1), 81-85.
[28] Saghai- Maroof M.A., Soliman K.M., Jorgensen R.A. and Allerd R.W. (1984) *Proc. Natl. Acad. Sci. USA*, 81, 8014-8019.
[29] Chan M.T., Chang H.H., Ho S.L., Tong W.F. and Yu S.M. (1993) *Plant Mol. Biol.*, 22, 491-506.
[30] Nandakumar N., Singh A.K., Sharma R.K., Mohapatra .T, Prabhu K.V., Zaman F.U. (2004) *Euphytica*, 136, 257-264.
[31] Hashemi S.H., Mirmohammadi-Maibody S.A.M., Nematzadeh G.A. and Arzani A. (2009) *Afri. J. Biotechnol.,* 8(10), 2094-2101.
[32] Brar B., Jain R.K. and Jain S. (2015) *Rice Genomics and Genetics*, 6(9), 1-9.
[33] Graham R.D., Ascher J.S. and Hynes S.C. (1992) *Plant Soil.,* 146, 241-250.
[34] Graham R.D., Senadhira D., Beebe S., Iglesias C. and Monasterio I. (1999) *Field Crops Res.,* 60, 57–80.
[35] Gregorio G.B., Senadhira D., Htut H. and Graham R.D. (2000) *Food Nutr. Bull.*, 21, 382-386.
[36] Virk P. and Barry G. (2009) *Biofortified rice-towards the combating micronutrient malnutrion deficiencies. DAPO Box 7777, Metro Manila, Philippines: International Rice Research Institute.*