International Journal of Agriculture, Environment and Biotechnology
Citation: IJAEB: 11(1): 111-120, February 2018
DOI: 10.30954/0974-1712.2018.00178.15

GENETICS AND PLANT BREEDING

Phenotyping for Grain Mineral Contents (Iron and Zinc) in PAU201 × Palman 579 F_5 and BC_1F_4 Populations in Rice (*Oryza sativa* L.)

Amit Pippal[1]*, Rajinder K. Jain[1], Sunita Jain[1], Sandeep Kumar[2] and Nabin Bhusal[3]

[1]Department of Molecular Biology, Biotechnology and Bioinformatics, CCS Haryana Agricultural University, Hisar-125004, Haryana, India
[2]Department of Veterinary Physiology and Biochemistry, LUVAS, Hisar-125004, Haryana, India
[3]Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar-125004, Haryana, India

*Corresponding author: amitpippal@gmail.com (ORCID ID: 0000-0002-6590-1040)

Paper No. 658 Received: 03-09-2017 Accepted: 02-01-2018

ABSTRACT

Rice (*Oryza sativa* L.) occupies an enviable prime place among the food crops cultivated around the world. Biofortification refers to the development of micronutrient-dense staple crops using the best traditional breeding practices and modern biotechnology. F_5 (278) and BC_1F_4 (212) plants derived from the cross between PAU201 (high yielding) and Palman 579 (Iron rich) were phenotype during 2013-14 crop season. The results showed 17.14% plants in F_5 and 5.60 % plants in BC_1F_4 populations perform higher than Palman 579 for zinc content. Likewise, 1.07% F_5 and 31.30% BC_1F_4 populations performed higher than PAU201 for grain yield/plant and 17.14% F_5 population and 61.21% BC_1F_4 population performed higher than PAU201 for 1000-grain weight. Plants showed large variation for various grain yield related traits for iron and zinc contents. Pearson's correlation coefficients showed Iron, zinc content and grain yield/plant were positively correlated to all the studied traits in both the populations except plant height in BC_1F_4 population. 1000-grain weight showed significant positive correlation in both the population with panicle length, grain yield/plant, iron content and zinc content. Notably, one F_5 plant (plant number 48-14-3-2) had exceptionally high iron content (296.5 μg/g). The distribution curves showed normal parabolic distribution for effective number of tillers/plant and zinc content. Frequency distribution curves for iron content were skewed towards Palman 579 in F_5 and BC_1F_4 populations. This indicated that available populations is feasible to plan a breeding program to develop high-yielding, mineral rich rice genotypes and to identify genomic location for micronutrients content.

Highlights

- The mean performance of the parent PAU201 showed higher grain yield/plant and 1000-grain weight than Palman 579, while it performed lower for remaining traits. The result indicated that Palman 579 has higher iron and zinc content however PAU201 has higher grain yield. Performance of both the parents showed that they were contrasting for grain yield and micronutrients content. The contrasting behavior of these parents was also identified by the variation observed among F_5 and BC_1F_4 population. These F_5 and BC_1F_4populations could be utilized in identifications and introgression of QTL for iron and zinc content.

Keywords: Rice, Biofortification, Micronutrients, Phenotype, Iron content, Zinc content

Rice is one of the chief grains of the world including many developing countries in Asia, Africa and Latin America. More than half of the world's population use rice as a dominant staple dietary food grain. India ranks first in the world in rice cultivation in an area of 433.88 lakh tonnes and second in production with 944.8 lakh tonnes (Annual report 2017). Therefore staple crop like rice may play a major role in reducing malnutrition and hidden hunger like problems in developing countries which usually affect children and pregnant women. Nearly, half of the deaths in children under the age of five are mainly attributed to nutrient deficiency of micro elements (Fe and Zn). It causes greater risk in children and pregnant women by common infections. It may increase in frequency and severity, which ultimately leads to death of the patients (Keiss 2017).

Global studies estimated that 2 billion people suffer from iron deficiency and contribute to 0.8 million death per year worldwide. India has the highest burden of malnutrition in the world. According to hunger index 2016 India ranks 97 among 118 nations, it has been found that the key under lying this is micro nutrient deficiency. While, during 2012 NASS reported that 50 per cent malnourished children are of underweight and 70 per cent suffer from serious nutrient deficiency. In India, 52 per cent of the women were affected by certain level of anemia, while 74 per cent of their children had similar degree of anemia (IIPS 2000). Iron is essential for the formation of hemoglobin. Low level of hemoglobin causes iron deficiency (anemia IDA). IDA can affect the productivity of hemoglobin and can cause serious health consequences including impaired cognitive development in children and increased risk of morbidity (Black *et al*. 2003). It is difficult to imagine a reversal in the global incidence and impact of hidden hunger without new biofortification approaches to complement conventional nutrition interventions. “Biofortification” refers to the development of micronutrient-dense staple crops using the best traditional breeding practices and modern biotechnology (Gregorio 2002; Pfeiffer and McClafferty 2007). It is an effective and cheaper alternative to enrich the micro nutrient level of staple crops through a traditional biological method or a technique of plant breeding and genetic engineering (Khurram 2013).

The genetic basis of the accumulation of micronutrients in the grain and mapping of the QTL provide the basis for preparing the strategies and improving the grain micronutrient content in rice. (Mahendra *et al*. 2016) Marker assisted selection involves selection of plant carrying genomic regions that are involved in the expression of traits of interest through molecular markers. A group of scientists from Philippines, Colombia, Indonesia, USA, Australia and Japan has successfully developed rice with increased levels of iron (Fe) and zinc (Zn) through biofortification (Trijatmiko *et al.* 2016). Biofortification of rice with Fe and Zn is a cost-effective and sustainable solution to mitigate Fe and Zn deficiency problems in the rice consuming malnourished Asian populations. There is a significant variation for mineral content in rice genotypes suggesting the existence of genetic potential to increase the concentrations of these micronutrients in rice grain through various breeding approaches. Keeping in view the above points, the present study was undertaken with the objective to find out Genetic variation for grain mineral contents (iron and zinc) in PAU201 × Palman579 derived F_5 and BC_1F_4 population in rice (*Oryza sativa* L.).

MATERIALS AND METHODS

Plant material

Seeds harvested from the F_4 and BC_1F_3 (selected Fe-Zn rich) plants were used to raise the F_5 (278) and BC_1F_4 (212) plants derived from the cross between PAU201 (high yielding) and Palman 579 (Iron rich) varieties, which were used in the present investigation. Both the parents used were developed at Punjab Agricultural University Ludhiana. The genotype PAU201 is an *indica* rice variety and Palman 579 is a cross breed *indica* rice variety derived from (IR 8 and Tadukan).

Field evaluation and data collection on various physio-morphological traits

The crop was raised during the kharif seasons of 2013-2014 at Rice Research Station, Kaul (Kaithal), CCS Haryana Agricultural University, which falls under semi-tropical regions of North India. The F_5 (278) and BC_1F_4 (212) plants were sown in a single row of 2.5 m length in nursery beds. After 30-35 days, seedlings were transplanted in the main field with plant to plant spacing of 15 cm and row to row spacing of 20 cm. Data were recorded for various and grain yield related traits Plant height (cm), Effective no. of tillers/plant, Panicle length (cm), Grain yield/plant (g), 1000-grainweight (g), Iron content (μg/g) and Zinc content (μg/g).

Fe and Zn estimation

Dehusked rice grains after hand hulling using palm dehusker were ground in pestle and mortar. Approximately, 500 mg of powdered rice grain samples were collected in 100 ml conical flask. To this, 15 ml of diacid mixture (HNO_3:$HClO_4$; 5:1 v/v) was added and kept for overnight digestion. Next day, the digested samples were kept on a hot plate at 100**°C** and after a few minutes brown fumes evolved. This indicated the starting of digestion process. Finally a white precipitate was seen by clearing the solution. The digested sample was cooled for 20 minutes then the content was filtered through Whatman No. 42 filter paper. The filtrate was diluted to 50 ml with double distilled water. The 50 ml digested samples was then transferred into a plastic bottle that was used for the determination of iron and zinc contents by Atomic Absorption Spectrophotometer 2380, Perkin Elmer (USA) according to the method of Lindsey and Norwell (1978). For the measurement of iron, $FeSO_4$ was used as the standard and for zinc the standard was $ZnSO_4$.

The data were subsequently analyzed using OPSTAT (http://hau.ernet.in/opstat.html) to determine the variability and phenotypic (r) correlation coefficient analysis. 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 tested against standardized tabulated significant value of r with (n-2) degree of freedom as per the procedure described by Fisher and Yates (1963).

RESULTS AND DISCUSSION

Mean performance and variation among the traits

Mean performance of the traits showed large variation for all the studied traits (Table 1). Palman 579 showed higher mean performance than PAU201 for all the studied traits except grain yield/plant and 1000-grain weight. It had 21.6g grain yield /plant and 20.2 g 1000-grain weight while, PAU201 had 23.3g and 22.8 g grain yield/plant and 1000-grain weight, respectively. Both the parents were contrasting for iron and zinc content. Palman 579 had 332.8 µg/g iron content and 51.4 µg/g zinc content however, PAU201 had 51.5 and 25.0 µg/g iron and zinc content, respectively. Among the F_5 and BC_1F_4 population high variation was recorded for iron and zinc content. The mean performance of F_5 population showed higher values for iron and zinc content while, the remaining traits were higher in BC_1F_4 Population. Plant height varied between 67-125 and 60-120 cm, Effective numbers of tillers/plant varied between 4-24 and 5-30, Panicle length varied between 12.0-27.9 cm and 15.5-27.4 cm, grain yield per plant varied between 0.76-41.6 g and 6.5-44.79 g, 1000-grain weight varied between 4.7-37.4 g and 11.9-34.1 g among F_5 and BC_1F_4, respectively. Likewise, Iron content ranged from 4.6-312 µg/g among the F_5plants and 3.9-86.4 µg/g in BC_1F_4 population. Zinc content in dehusked rice grains of F_5 population varied between 2.2 and 117.5 µg/g with a mean value of 31.3 µg/g while, it ranged from 1.5 to 140.6 µg/g with a mean value of 30.2 µg/g in BC_1F_4 population.

Pearson's correlation coefficients and frequency distribution

Phenotypic correlation coefficient analysis of PAU201 × Palman 579 F_5 and BC_1F_4 population showed significant correlation between mineral content (iron and zinc) and grain yield related traits (Table 2). Iron content, zinc content and grain yield/ plant showed significant positive correlation with all the studied traits in both the populations except plant height in BC_1F_4 Population. 1000-grain weight showed significant positive correlation in both the population with panicle length, grain yield/plant, iron content and zinc content.

Frequency distribution curves for various physio-morphological traits of PAU201 x Palman 579 F_5 population and parental rice genotypes are shown in Fig. 1. Frequency distribution curves for plant height, panicle length and iron content were skewed towards Palman 579. The distribution curves showed normal parabolic distribution for effective number of tillers/plant and zinc content. Frequency curves for 1000-grain weight, and grain yield/ plant were tilted towards PAU201. However, Frequency distribution in BC1F4 population was shown in Fig. 2. The traits *viz.*, plant height, panicle length and grain zinc content, frequency distribution curves were skewed towards Palman 579, but for effective number of tillers/plant, 1000-grain weight and

Table 1: Mean and Range for various traits and mineral contents in PAU201 × Palman 579 F_5 and BC_1F_4 population(s)

Traits	PAU201	Palman 579	F_5 Population		BC_1F_4 Population	
			Mean	Range	Mean	Range
PH	92±0.51	102±1.15	89.80	67-125	92.3	60-120
ENT	12.3±1.95	9.6±1.06	9.30	4-24	13.08	5-30
PL	21.9±0.24	23.2±0.53	21.70	12-27.9	21.56	15.5-27.40
GY	23.3±0.67	21.6±0.31	9.06	0.76-41.6	21.38	6.5-44.79
TGW	22.8±0.50	20.2±0.52	19.80	4.7-37.4	23.8	11.9-34.10
Fe	51.5±0.77	332.8±1.35	66.70	4.6-312	37.08	3.9-86.40
Zn	25.0±1.11	51.4±0.63	31.30	2.2-117.5	30.2	1.5-140.60

± indicates standard error; PH: Plant height (cm), ENT: Effective no. of tillers/plant, PL: Panicle length (cm), GY: Grain yield/plant (g), TGW: 1000-grain weight (g), Fe: Iron content (μg/g), Zn: Zinc content (μg/g)

Table 2: Pearson's correlation coefficients for various grain yield related traits and mineral contents in PAU201 × Palman 579 F_5 and BC_1F_4 population(s)

Traits	PH	ENT	PL	GY	TGW	Fe	Zn
F_5 Population							
PH	1						
ENT	0.098	1					
PL	0.336**	0.222**	1				
GY	0.303**	0.576**	0.400**	1			
TGW	0.394**	0.008	0.218**	0.196**	1		
Fe	0.439**	0.428**	0.414**	0.843**	0.692**	1	
Zn	0.451**	0.226**	0.338**	0.548**	0.927**	0.912**	1
BC_1F_4 Population							
PH	1						
ENT	-0.074	1					
PL	0.261**	0.077	1				
GY	-0.043	0.788**	0.241**	1			
TGW	0.066	0.070	0.217**	0.145*	1		
Fe	-0.017	0.732**	0.287**	0.947**	0.455**	1	
Zn	0.018	0.548**	0.302**	0.734**	0.778**	0.913**	1

*** Significant at (P< 0.01) per cent LSD and * significant at (P<0.05) per cent LSD; PH: Plant height (cm), ENT: Effective no. of tillers/plant, PL: Panicle length (cm), GY: Grain yield/plant (g), TGW: 1000-grain weight (g), Fe: Iron content (μg/g), Zn: Zinc content (μg/g)*

grain yield/plant the curves were inclined towards PAU201. The frequency distribution curves of iron content showed parabolic distribution of BC_1F_4 plants and were slightly skewed towards Palman 579.

Micronutrients are not only essential for plant growth and development but are also integral to human and animal health. In the last two decades, the concept of hidden hunger (deficiency of certain vitamins and micronutrient nutrients despite eating enough calories) has been well established (Nilson and Piza 1998). Rice is the most well-known cereal and staple food, which serves as a major carbohydrate source for more than half of the world population. Half of the world's population is suffering from one or more vitamin and/or mineral deficiency (World Food Program 2015). The amount of mineral nutrients in rice grain is a key determinant of its nutritive value (Anuradha *et al*. 2012a). In brown rice, concentration of Fe, Zn, Mn and Cu were estimated as 22, 14, 11 and 2.4 μg/g, respectively, which are not enough to meet all the requirements of a healthy body (Brinch -Pedersen *et al*. 2007). Over the last decade, several

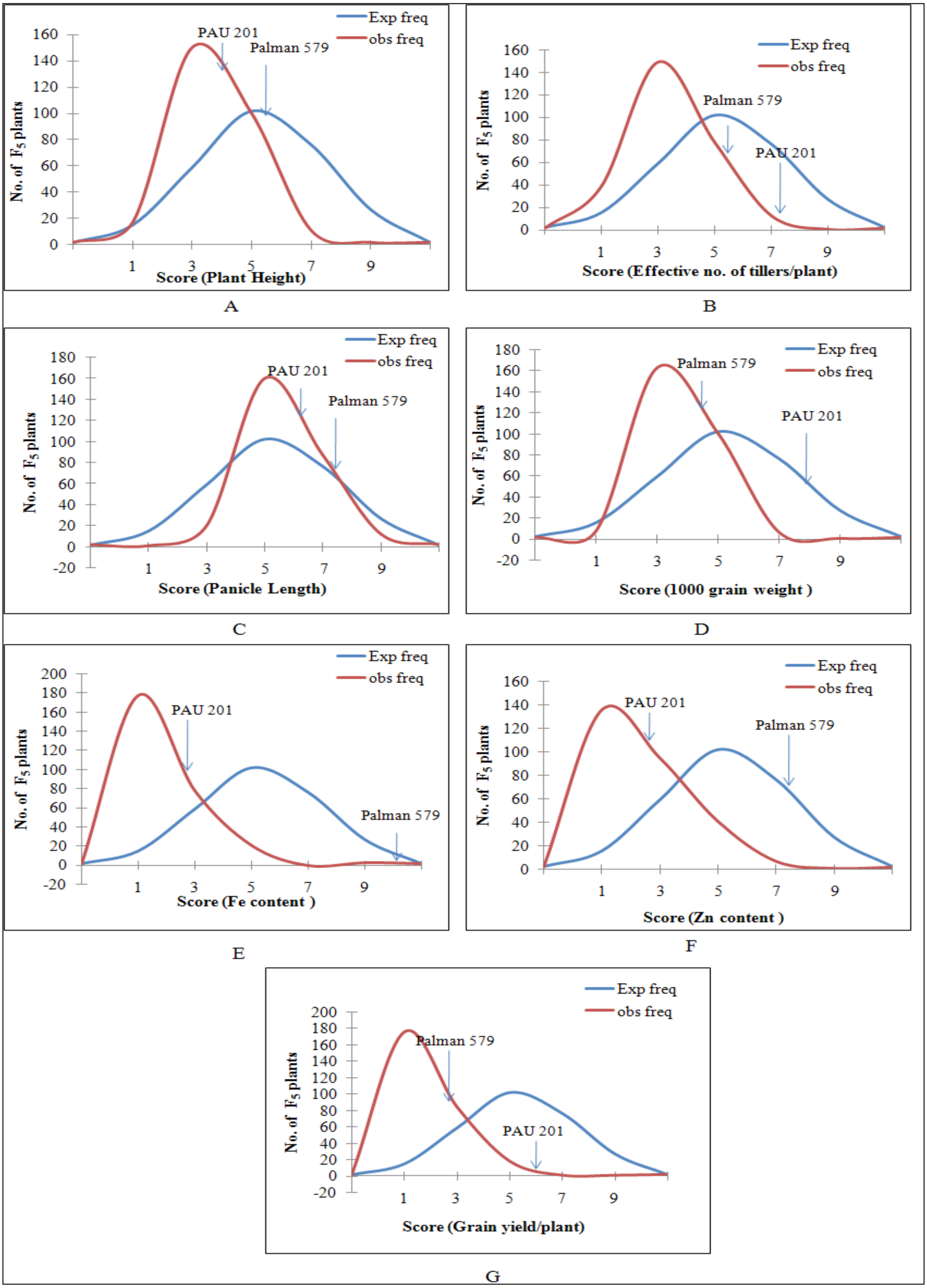

Fig. 1: Frequency distribution curves for Physio-morphological traits and iron and zinc content of PAU 201 × Palman 579 F_5 Population

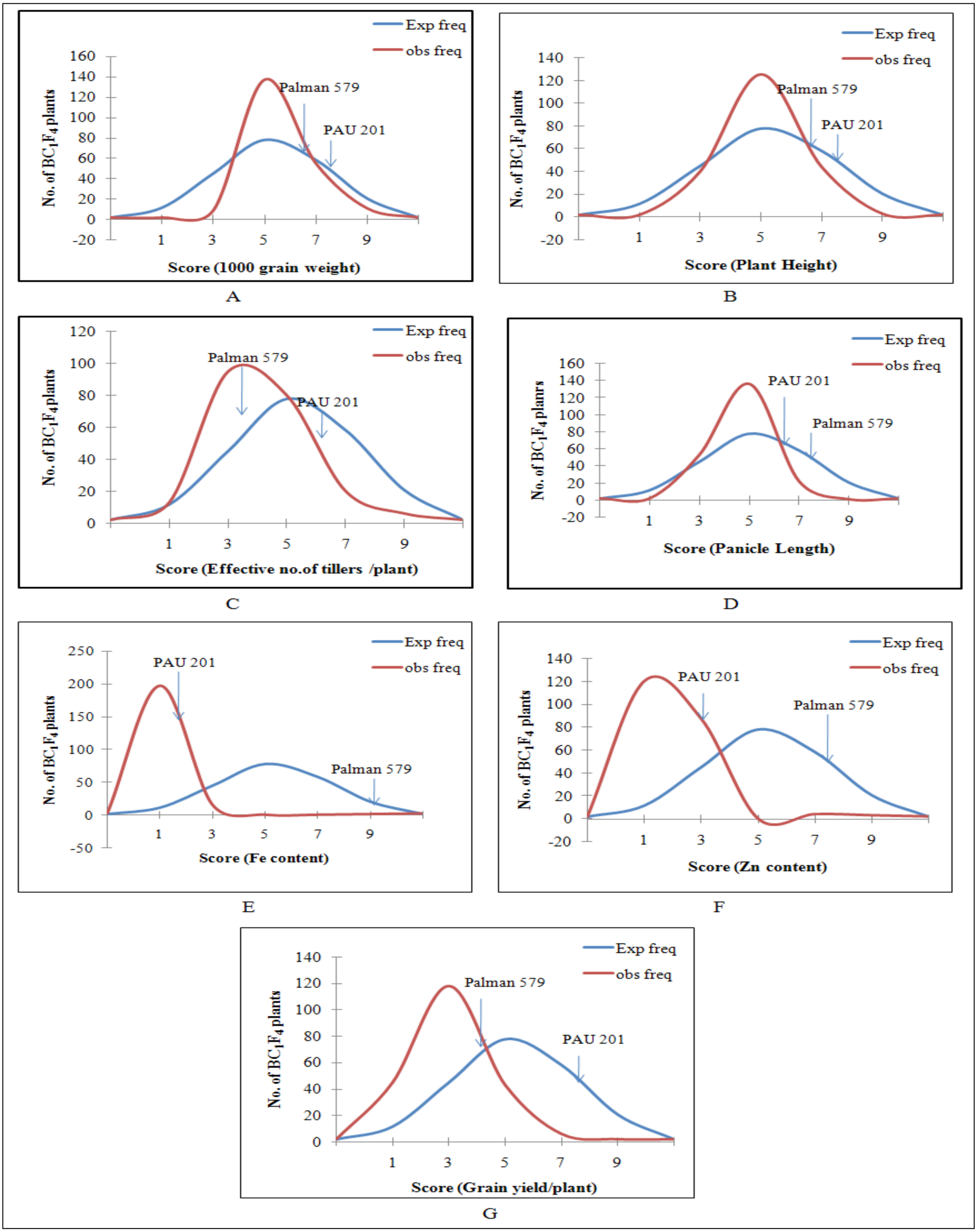

Fig. 2: Frequency distribution curves for Physio-morphological traits and iron and zinc content of PAU201 × Palman 579 BC_1F_4 Population

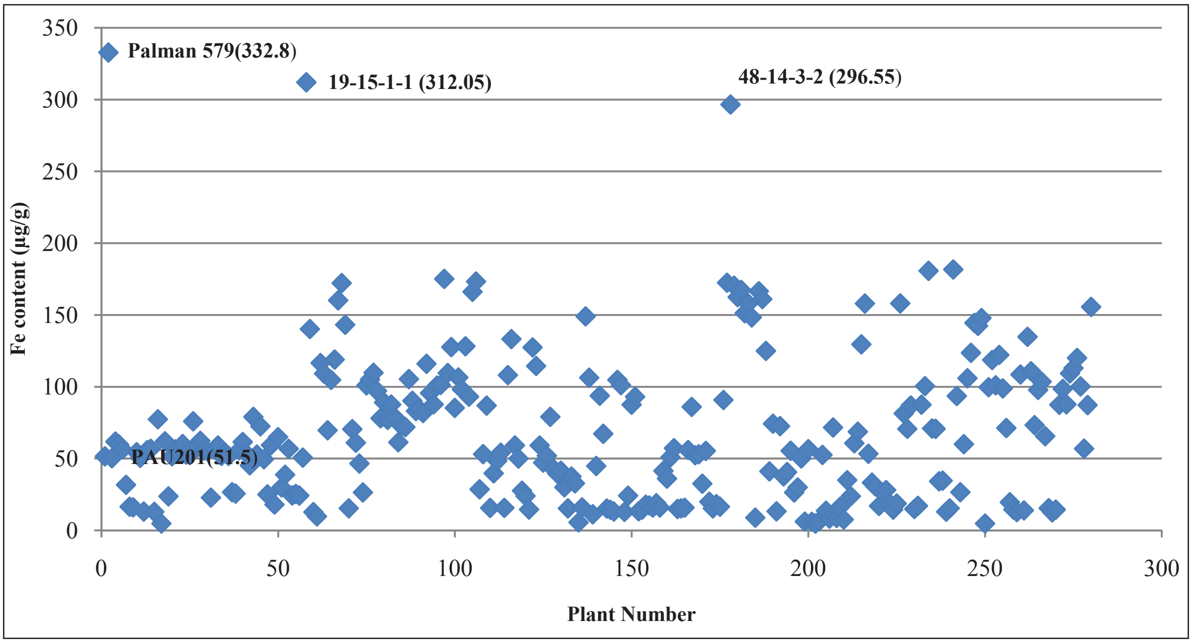

Fig. 3: Variation for grain Fe content in PAU201 ×Palman 579 F_5 population

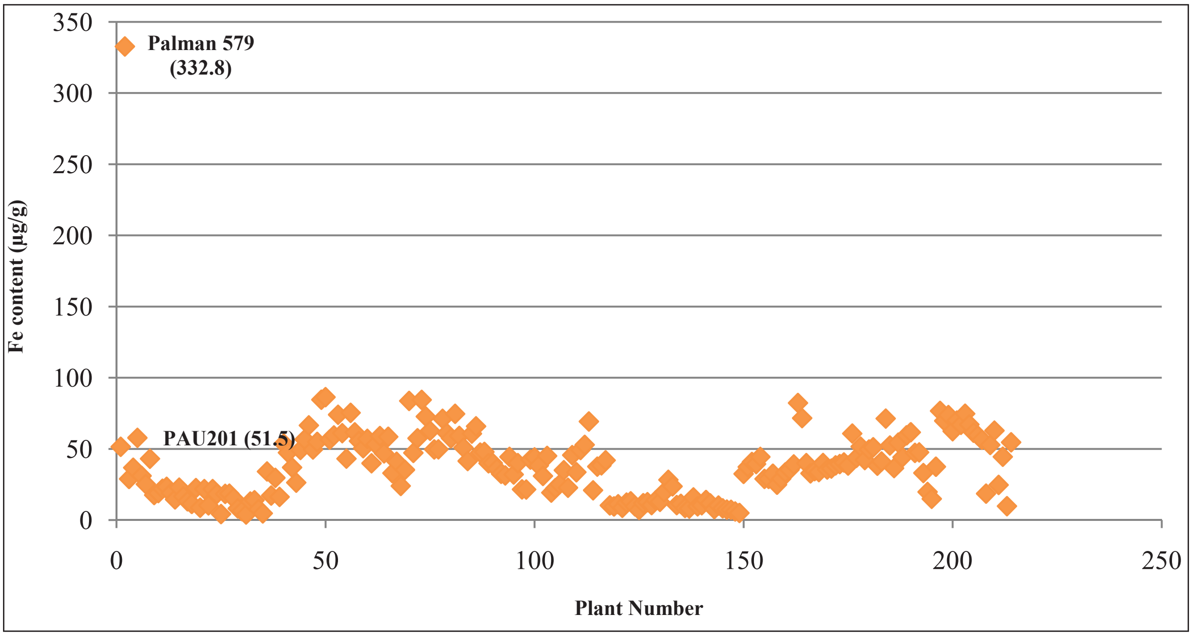

Fig. 4: Variation for grain Fe content in PAU201 ×Palman 579 BC_1F_4 population

efforts have been made to biofortify food crops with micronutrients, which lead to a significant understanding of the physiological, genetic and molecular basis of high iron and zinc accumulation in grains and also the influence of agronomic management and environmental factors on iron and Zinc uptake, translocation and loading into grains (Impa *et al*. 2012).

However, phenotyping for the mineral nutrients is one of the major factors for crop improvement and previous studies suggested that there is significant genetic diversity in rice genome to increase iron and zinc concentration in rice grain by combining the high iron traits with high yielding traits (Graham *et al*. 1999; Gregorio *et al*. 1999; Brar *et al*. 2011; Nachimuthu *et al*. 2014). At the same time identification of micronutrient-rich genotypes opens up the possibilities for the linkage mapping of genomic regions or QTLs responsible for mineral uptake and translocation, which can be subsequently used as a donor for developing nutrient enriched varieties.

In the present investigation we evaluated the F_5 and BC_1F_4 populations derived from PAU201 and Palman 579 for various traits along with their parents. The mean performance of the parent PAU201 showed higher grain yield/plant and 1000-grain weight than Palman 579 while, it performed lower for remaining traits. The result indicated that Palman 579 has higher iron and zinc content however, PAU201 has higher grain yield. Performance of both the parents showed that they were contrasting for grain yield and micronutrients content. The contrasting behavior of these parents was also identified by the variation observed among the F_5 and BC_1F_4 population derived from these parents. The iron and zinc contents analysis of populations revealed large variation in grain harvests. Iron content differed significantly among the F_5 and BC_1F_4 population ranging between 4.6-312.0 µg/g and 3.9-86.4 µg/g, respectively (Fig. 3 and 4).

However, zinc content ranged from 2.2-117.5 and 1.5-140.60 µg/g for F_5 and BC_1F_4 population, respectively (Fig. 5 and 6). Among the F_5 and BC_1F_4 populations there was no plant available which performed higher than Palman 579 for iron content while, 17.14 % plants in F_5 population and 5.6 % plants in BC_1F_4 population performed well than Palman 579 for zinc content. Likewise, 1.07% F_5 population and 31.30% BC_1F_4population performed higher than PAU201 for grain yield/plant and 17.14% F_5 population and 61.21% BC_1F_4population performed higher than PAU201 for 1000-grain weight. The results indicated that both the populations have high genetic variation for the grain yield component traits as well as high iron and zinc content. Previously, large variation for iron and zinc content has been reported by several workers in different sets of rice germplasm and populations. Brar *et al*. (2011) reported large variation in iron and zinc contents of dehusked grains in a collection of 220 rice genotypes for Fe (5.1- 441.5 µg/g) and Zn (2.12 - 39.4 µg/g). Anuradha *et al*. (2012) described variation for iron and zinc contents in brown rice samples of 126 rice accessions. Garcia-Oliveria *et al*. (2009) reported variation for iron (4.9-20 µg/g, Tequing-7.5 µg/g) and zinc (13.3-60.1 µg/g, Tequing-16.6 µg/g) in a set of 85 introgression lines (ILs) derived from a cross between an elite *indica* cultivar Teqing and the wild rice (*Oryza rufipogon*). Stangoulis *et al*. (2007) communicated variation in the levels of phytate, inorganic P and micronutrients, especially Fe (10-53 mg/kg) and Zn (30-90 mg/kg) in the double haploid population derived from a cross between the irrigated *Indica* variety IR64 and the upland *Japonica* variety Azucena. Notably, in the present investigation one F_5 plant (plant number 48-

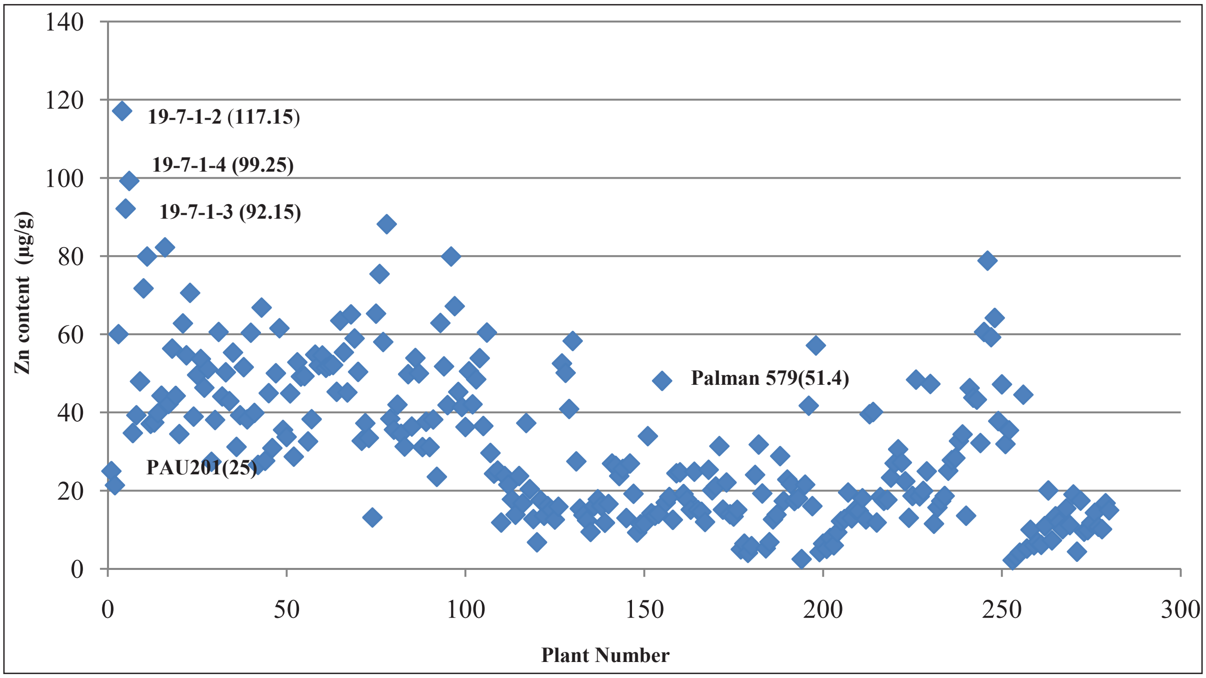

Fig. 5: Variation for grain Zn content in PAU201 × Palman 579 F_5 population

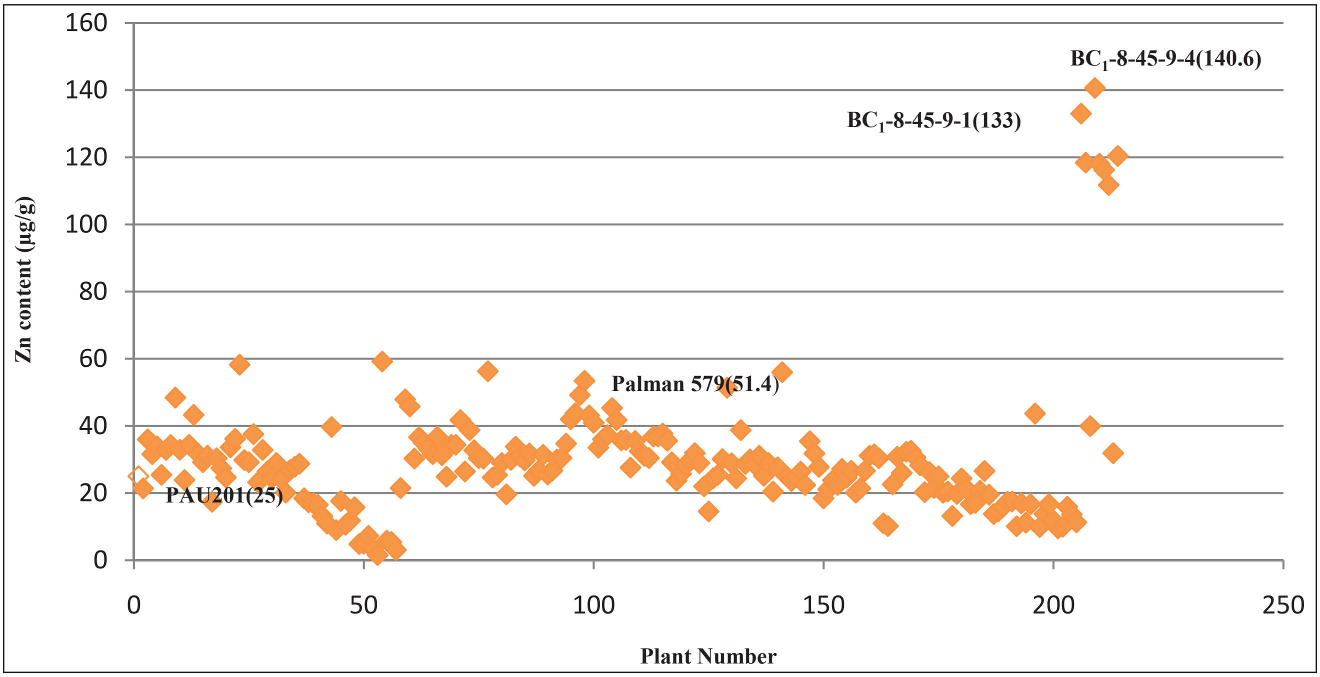

Fig. 6: Variation for grain Zn content in PAU201 × Palman 579 BC_1F_4 population

14-3-2) had exceptionally high iron content (296.5 µg/g) than others plants. It should be recognized that while Palman 579 had exceptionally higher iron content, PAU201 also had relatively higher iron content compared to the other cultivated *indica* rice varieties (Brar *et al*. 2011; Gowda *et al*. 2012).

In the present investigation we found significant positive correlation among the Iron content, zinc content and grain yield/plant showed significant positive correlation with all the studied traits in both the populations except plant height. The result indicated that increase in one of these could help to improve other associated traits as well. Previously, significant positive correlation between grain iron and zinc content was observed in 85 introgression lines (ILs) derived from a cross between an elite *indica* cultivar Teqing and the wild rice (*Oryza rufipigon*) (Garcia-Oliveria *et al*. 2009). A significant positive correlation between Fe and Zn contents in grains of PAU201 × Palman 579 derived F_2 population was also observed by Kumar *et al*. (2014).

Thus, it may be tenable to improve Fe and Zn levels simultaneously in rice grain through plant breeding with the aid of marker assisted selection. The transgressive segregation in the RIL population is an indication of the presence of different sets of genes in the parental lines for the target traits (Tiwari *et al*. 2009 Bhusal *et al*. 2016). These transgressive segregants might have resulted due to the accumulation of favorable genes controlling grain iron content or the development of new combinations of genes controlling grain traits derived from the parents. In the present study, we also identified transgressive segregants for zinc content, grain yield/plant and 1000-grain weight. Kiranmayi *et al*. (2014) also reported transgressive segregation for both grain Fe and Zn concentration in the population.

CONCLUSION

The significant variation for mineral content in F_5 and BC_1F_4 population suggests the existence of genetic potential to increase the concentrations of these micronutrients in rice grain. Pearson's correlation coefficients showed Iron content, zinc content and grain yield/plant were positively correlated to all the studied traits. The distribution curves showed normal parabolic distribution for effective number of tillers/plant and zinc content. This also indicated that available populations is feasible to plan a breeding program to develop high-yielding, mineral rich rice genotypes and identify genomic location for micronutrients content. However, more research is required to understand molecular and biochemical mechanisms for mineral uptake and transport using such novel diverse populations.

ACKNOWLEDGEMENTS

The authors are highly grateful to Dr. Sandeep Kumar and his team at Department of Veterinary Physiology and Biochemistry, LUVAS, for providing facilities for mineral content analysis.

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