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Backcross Breeding for Enhancing Minerals (Iron and Zinc) Content in Rice (*Oryza sativa* L.)

Naveen Kumar*, Rajinder Kumar Jain, Vijay Kumar Chowdhury, Sunita Jain and Rajesh

Department of Molecular Biology and Biotechnology, CCS Haryana Agricultural University, Hisar - 125 004, (Haryana), India

**Corresponding author*

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A B S T R A C T

Micronutrient malnutrition is a serious health concern resulting due to deficiency of minerals such as iron and zinc in dietary intake. Genetic enhancement of crops with higher mineral content i.e. bio-fortification is one of the most cost effective method to solve the global mineral malnutrition problem. In the present investigation, experiments were conducted to evaluate BC_1F_2 and BC_1F_3 populations derived from cross between PAU201 (high-yielding) and Palman 579 (iron-rich) *indica* rice varieties for various physio-morphological traits and minerals (iron and zinc) content. Iron and zinc content varied from 0.9- 130.5 and 0.8-143.1 µg/g in BC_1F_2 and BC_1F_3 populations, respectively. The results of phenotypic correlation analysis showed positive correlation (r=0.281) between grain iron content and zinc content in BC_1F_2 population but not in BC_1F_3 population. The frequency distribution bar diagrams for micronutrient content and frequency distribution curves for various physio-morphological traits, iron and zinc content of PAU201 × Palman 579 derived BC_1F_2 and BC_1F_3 plants along with parental rice genotypes were accessed.

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. Billions of people in developing countries suffer from a lack of micronutrients in their daily food, a form of hunger also known as 'hidden hunger'. Micronutrient malnutrition especially Fe and Zn deficiency is recognized as a massive and rapidly growing public health issue mainly among poor people living on an unbalanced diet dominated by a single cereal such as rice. Fe and Zn deficiencies lead to immune dysfunction and may impair growth and development. Iron and zinc are essential micronutrients for most organisms, including all plants and animals. Fe deficiency is one of the most widespread micronutrient deficiencies worldwide, affecting about two billion people (Stoltzfus *et al.*, 1998) and causing 0.8 million deaths annually all over the world (WHO, 2002). Fe and Zn deficiencies weaken immune function and may impair growth and development. A major etiologic factor is the low bioavailability of Fe and Zn from diets based on staple cereals. There are several potential approaches to increase the bioavailability of Fe and Zn in plant crops, including conventional breeding and genetic engineering (Frossard *et al.*, 2000).

Biofortification is the process by which the nutritional quality of food crops is improved through agronomic practices, conventional plant breeding, or modern biotechnology. Biofortification, which refers to the breeding of plants/crops with high bioavailable micronutrient content using conventional breeding and genetic engineering approaches, is being used to improve the nutritional quality of major crops (Bouis *et al.*, 2003; Welch and Graham, 2004; White and Broadely, 2005). It has the potential to provide coverage for inaccessible rural population, and it basically targets the poor people who consume high levels of staple foods and little else. Enriching crops with high bio-available micronutrient content can complement ongoing efforts of nutritional supplementation and food fortification to combat micronutrient malnutrition. Moreover, enriching crops with micronutrient is beneficial for individuals who find it difficult to change their dietary routine because of financial, cultural, regional or religious restrictions.

Particularly rice is a suitable target for biofortification because Fe-deficiency anemia is a serious problem in developing countries where it is a major staple crop (Juliano, 1993; WHO, 2002). Brown rice is rich in micronutrient content value but mostly consumed after milling, which contains low mineral levels (Grusak and Cakmak, 2005). Biofortification can be applied to increase the Fe concentration in polished grains using the modern plant breeding and biotechnology approaches. Although rice is not considered a major mineral source in the diet, any increase in its micronutrient content could significantly help to reduce iron and zinc deficiency in humans because of the high levels of rice consumption among the poor in Asia.

Materials and Methods

In the present study, experiments were conducted to evaluate PAU201 × Palman 579 derived BC_1F_2 and BC_1F_3 populations for 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), and micronutrients (Fe and Zn) content during kharif seasons of 2011 and 2012, respectively at Rice Research Station, Kaul (Kaithal). The BC_1F_1 plants were developed by crossing F_1 plants (of cross PAU201 and Palman 579) with recurrent parent i.e. in this case PAU201. The seeds were harvested from these PAU201× Palman 579 derived BC_1F_1 rice plants. A total of seven iron-rich backcross (BC_1F_1) plants were selected on the basis of grain iron content. The BC_1F_2 population was raised from the seeds of selected plants during kharif season of 2011 and BC_1F_3 population in the kharif season of 2012 at Rice Research Station Kaul (Kaithal), which falls under semi-tropical regions of North India.

The preparation of the land, seeds and establishment of plants was as per CCS Haryana Agricultural University, Rice Research Station Kaul (Kaithal) standard practices. Seeds were grown in a single row of 2.5 m length in nursery beds at field. After thirty days the seedlings were transplanted at main field with plant to plant spacing of 15 cm and row to row spacing of 20 cm. The data on various physio-morphological traits was recorded for PAU201 × Palman 579 derived BC_1F_2 (466 plants) and BC_1F_3 (106 plants) populations, including parent genotypes. Each mature plant was harvested separately by cutting from the base of the stem. Sun drying for five days was sufficient to get the grain moisture content of around 14%. The grains were threshed manually and cleaned removing unfilled grains. These populations (BC_1F_2 and

BC_1F_3) were analyzed for micronutrients (Fe and Zn) content (µg/g) in dehusked rice grain samples. Micronutrient 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 Lindsey and Norwell 1978.

The data was subsequently analyzed using online OPSTAT software (http://hau.ernet.in/opstat.html) to determine the variability and phenotypic (r) correlation coefficient. 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. The phenotypic correlation coefficients were tested against standardized tabulated significant value of r with (n-2) degree of freedom as per the procedure given by Fisher and Yates 1963. The frequency distribution bar diagrams for micronutrient content and frequency distribution curves for various agronomic traits and micronutrients (Fe and Zn) content of PAU201 × Palman 579 derived BC_1F_2 and BC_1F_3 populations along with parental rice genotypes were also determined.

Results and Discussion

Evaluation of PAU201 × Palman 579 derived BC_1F_2 and BC_1F_3 populations

Field evaluation of various agronomic and grain quality traits for PAU201 × Palman 579 derived BC_1F_2 (466 plants) and BC_1F_3 (106 plants) populations along with parental rice varieties showed large variation (Table 1). Mineral analysis of BC_1F_2 and BC_1F_3 population's harvest (dehusked rice grain samples) revealed large variation in iron and zinc contents (Table 1). Iron content of BC_1F_2 and BC_1F_3 populations differed significantly between 0.9-101.7 µg/g; 17.1-130.5 µg/g with a mean value of 32.5; 40.7 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 µg/g and 383.8 µg/g in consecutive two kharif seasons (2011 and 2012), respectively.

Phenotypic correlation analysis

Phenotypic correlation coefficient analysis of BC_1F_2 population, showed a positive correlation (0.281, p=0.01) between Fe and Zn content (Table 2). In this population, iron content revealed a positive correlation with effective number of tillers/plant (0.226, p=0.01), grain yield/plant (0.291, p=0.01) and grains/panicle (0.150, p=0.01). Zinc content showed positive correlation with effective number of tillers/plant (0.109, p=0.05) and grain yield/plant (0.104, p=0.05). Grain yield/plant revealed a positive correlation with plant height (0.226, p=0.01), effective number of tillers/plant (0.716, p=0.01), grains/panicle (0.612, p=0.01), panicle length (0.391, p=0.01) and 1000 grain weight (0.200, p=0.01). Plant height showed significant and positive correlation with effective number of tillers/plant (0.126, p=0.01), grains/panicle (0.240, p=0.01) and panicle length (0.434, p=0.01).

Phenotypic correlation coefficient analysis of BC_1F_3 population, showed a positive correlation (0.237, p=0.05) between Fe content and effective number of tillers/plant (Table 3). Zn content showed a positive correlation (0.207, p=0.05) between Zn content and 1000-grain weight. In this population, grain yield/plant revealed a positive correlation with plant height (0.256, p=0.01), effective number of tillers/plant (0.869, p=0.01), grains/panicle (0.456, p=0.01) and panicle length (0.444, p=0.01). Plant height showed significant and positive correlation with grains/panicle (0.240, p=0.01), panicle length (0.434, p=0.01) and 1000-grain weight (0.341, p=0.01). 1000-grain weight showed a negative correlation with effective number of tillers/plant (-0.196, p=0.05) (Fig. 1 and 2).

Frequency distribution curves for various physio-morphological traits and micronutrient content

Frequency distribution curves for various physio-morphological traits and micronutrient (Fe and Zn) content of PAU201 × Palman 579 derived BC_1F_2 population and parental rice genotypes are shown in Figure 3. For plant height, frequency distribution curve was skewed towards Palman 579. For effective number of tillers/ plant, grain yield/ plant and grains/ panicle, the frequency distribution curve was parabolic and slightly tilted towards Palman 579. Frequency distribution curve for panicle length was skewed towards Palman 579. The frequency distribution curved of 1000-grain weight, iron and zinc content showed different pattern of distribution and skewed towards PAU201.

Frequency distribution curves for various physio-morphological traits and mineral content of PAU201 × Palman 579 BC_1F_3 population and parental rice genotypes are shown in Figure 4. For plant height, grain yield/ plant, panicle length and grain zinc content, the frequency distribution curves of BC_1F_3 population were skewed towards Palman 579, but for 1000-grain weight and iron content, curves were inclined towards PAU201. The frequency distribution curved of grains/panicle and iron content showed parabolic distribution of BC_1F_3 plants and slightly skewed towards Palman 579.

Table.1 Mean and range for various agronomic traits and mineral contents in PAU201 × Palman 579 BC_1F_2 and BC_1F_3 populations

Traits	Average pooled data of parental rice genotypes		PAU201 × Palman579 derived BC_1F_2 population		PAU201 × Palman579 derived BC_1F_3 population	
	PAU201	Palman 579	Mean	Range	Mean	Range
Plant height (cm)	95.8±0.40	107.6±0.75	106.0	84-177	100.5	85-119
Effective no. of tillers/plant	13.8±0.74	15.0±1.15	10.8	3-24	12.4	4-25
Panicle length (cm)	25.3±0.21	26.2±0.36	23.5	16.3-29.3	24.0	20.5-27.5
Grains/panicle	120.6±1.24	106.9±1.29	96.9	33-180	130.1	76-206.7
Grain yield/plant (g)	40.7±2.24	34.0±1.59	25.6	3-84.8	38.7	9.5-107.2
1000-grain weight (g)	24.2±0.10	21.5±0.16	24.1	17.4-28	24.1	20.9-28.2
Fe content (µg/g)	53.9±0.26	378.5±0.41	32.5	0.9-101.7	40.7	17.1-130.5
Zn content (µg/g)	16.3±0.19	22.3±0.31	21.0	0.8-143.1	18.5	9.6-27.2

Table.2 Phenotypic correlation coefficients among yield, yield components and mineral content in BC_1F_2 population

	Plant height (cm)	Effective no. 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 no. of tillers/plant	0.126**	1						
Grain yield/ plant (g)	0.226**	0.716**	1					
Grains/ panicle	0.240**	-0.028	0.612**	1				
Panicle length (cm)	0.434**	0.179**	0.391**	0.453**	1			
1000-grain weight (g)	0.025	-0.149**	0.200**	0.314**	0.096*	1		
Fe content (µg/g)	0.029	0.226**	0.291**	0.150**	0.071	-0.036	1	
Zn content (µg/g)	-0.005	0.109*	0.104*	-0.030	-0.060	-0.077	0.281**	1

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

Table.3 Phenotypic correlation coefficients among yield, yield components and mineral content in BC_1F_3 population

	Plant height (cm)	Effective no. 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 no. of tillers/plant	0.129	1						
Grain yield/ plant (g)	0.256**	0.869**	1					
Grains/ panicle	0.246*	0.047	0.456**	1				
Panicle length (cm)	0.324**	0.253**	0.444**	0.525**	1			
1000-grain weight (g)	0.341**	-0.196*	-0.004	0.034	-0.071	1		
Fe content (µg/g)	0.052	0.237*	0.162	-0.009	-0.061	-0.013	1	
Zn content (µg/g)	0.184	-0.023	0.120	0.190	0.116	0.207*	0.021	1

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

Fig.1 Frequency distribution for grain Fe and Zn content in PAU201 × Palman 579 BC_1F_2 population

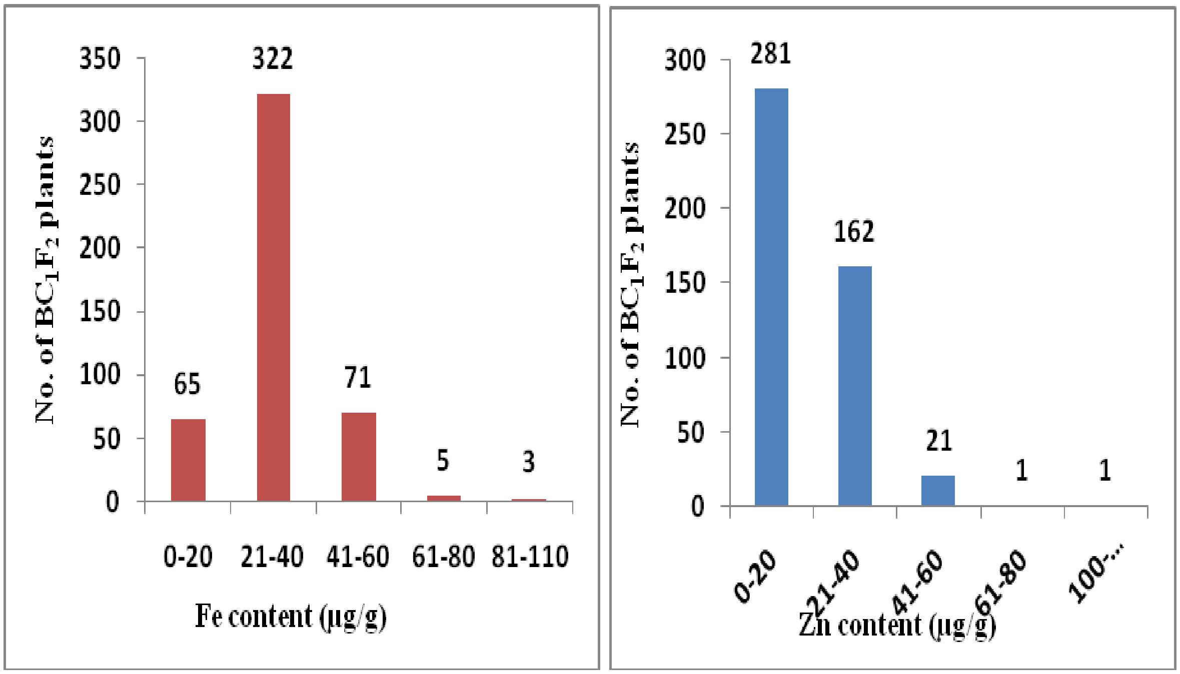


Fig.2 Frequency distribution for grain Fe and Zn content in PAU201 × Palman 579 BC_1F_3 population

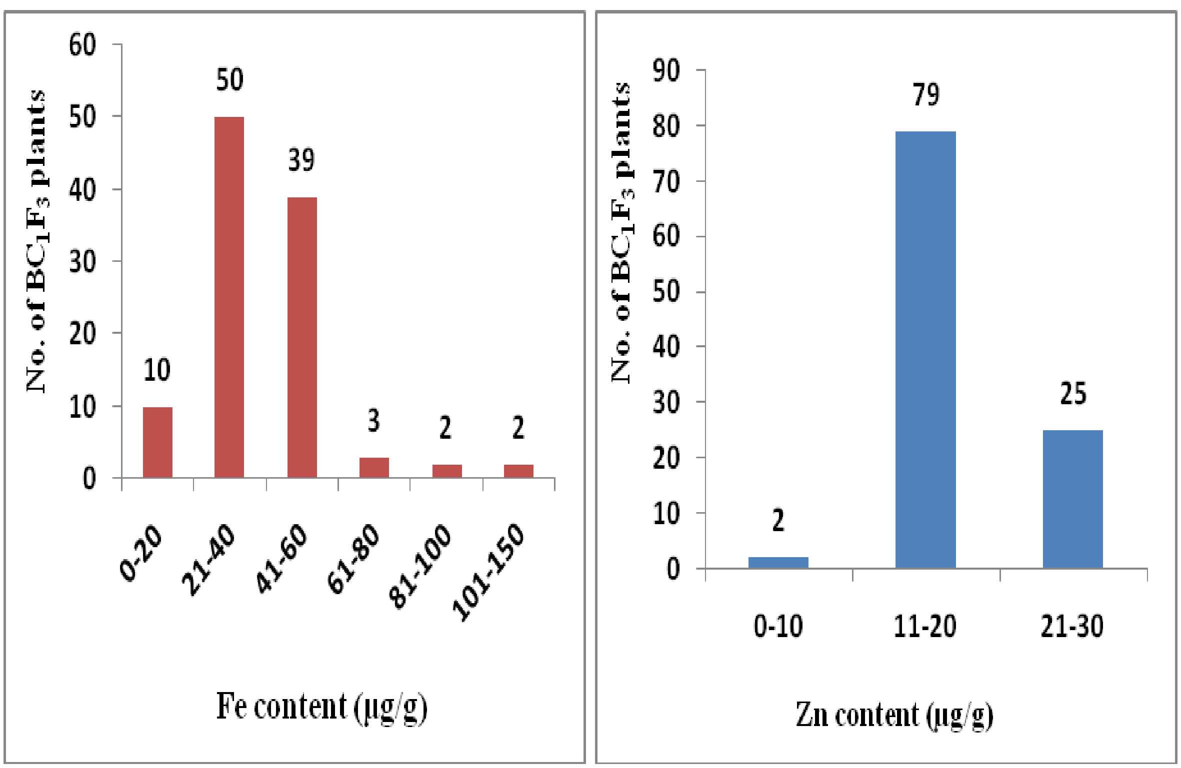


Fig.3 Frequency distribution curves for physio-morphological traits and iron and zinc contents of PAU201 × Palman 579 BC_1F_2 population

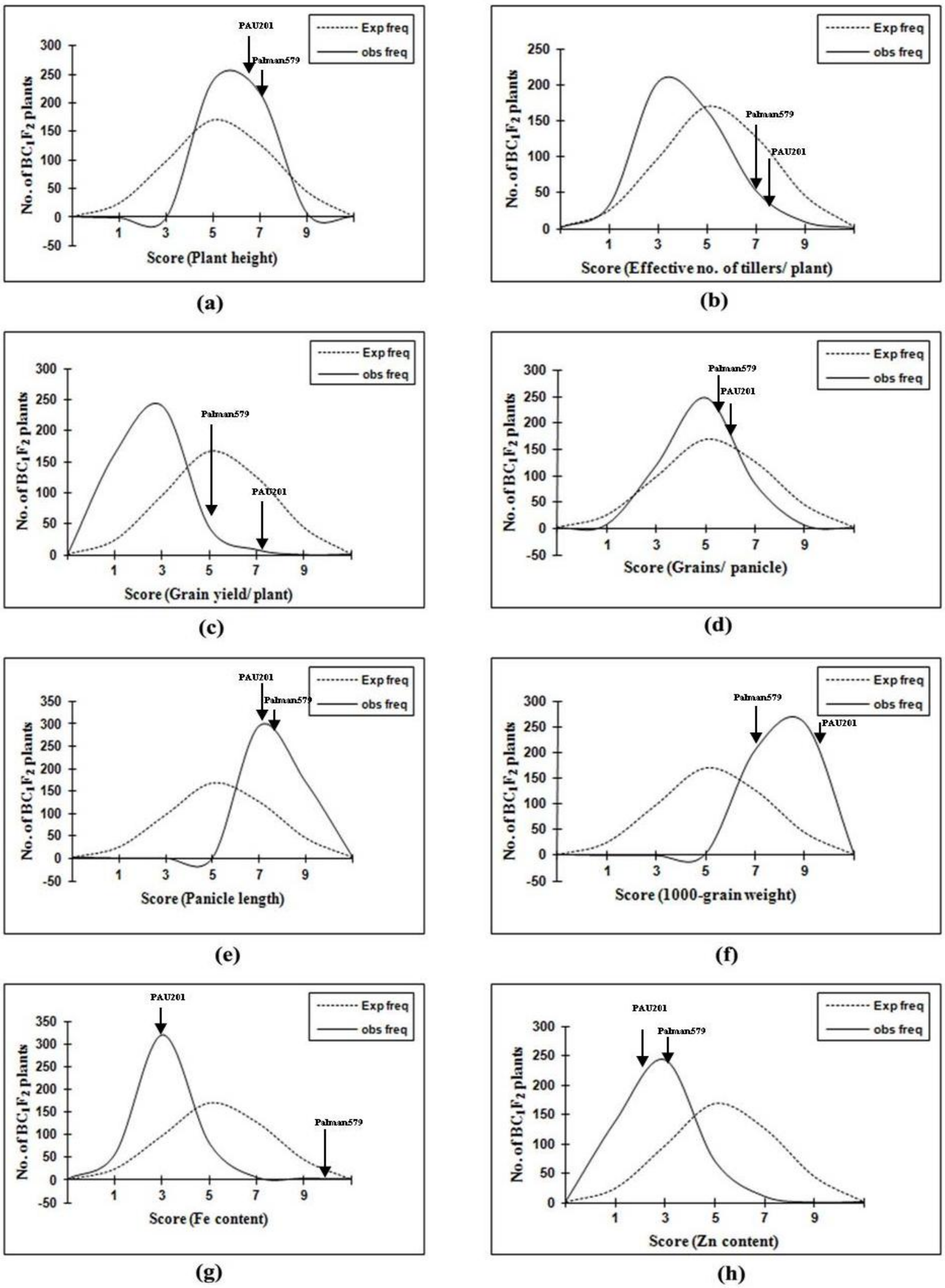
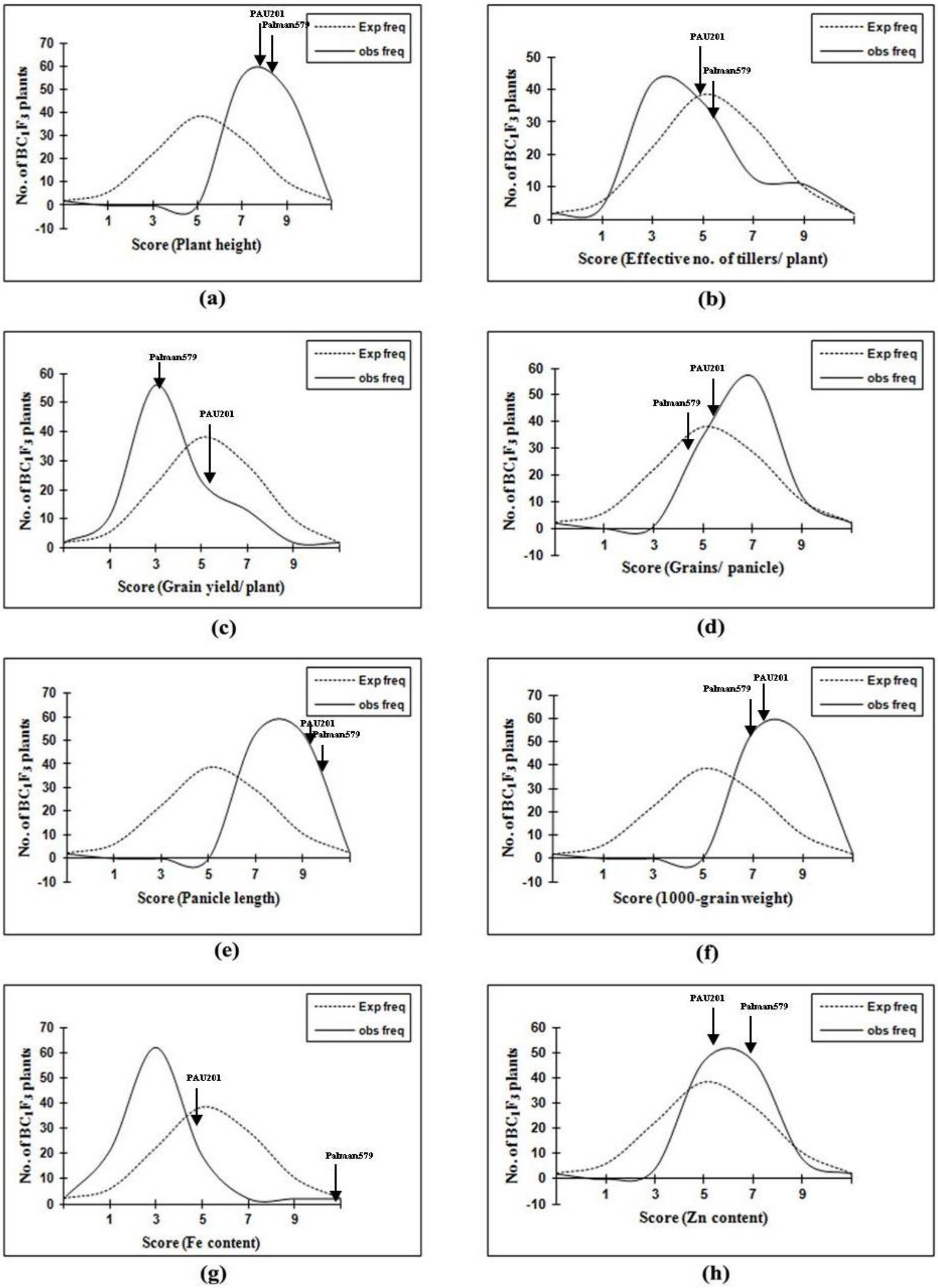


Fig.4 Frequency distribution curves for physio-morphological traits and iron and zinc contents of PAU201 × Palman 579 BC_1F_3 population



Micronutrient deficiency is widespread worldwide; especially among the poor people whose daily caloric intake is mainly depends on staple cereals. About 49 nutrients are essential for human metabolic need and deficiencies of these essential minerals and vitamins together are often called as 'Hidden Hunger', and it is considered as one of the most serious challenges faced globally by mankind (Hirschi, 2009; White and Broadley, 2009). Even though micronutrients are needed in very little quantities (micrograms to milligrams per day), they have remarkable impact on human health. Inadequate dietary intakes of these micronutrients can cause impairment of the immune and reproductive systems, brain function and also the energy metabolism. These deficiencies results disabilities in learning, reduced work capacity, serious illnesses and even the death of the person. Thus, micronutrient malnutrition is a serious global problem that limits the work capacity of people and seriously hinders economic development (Anonymous 1994). Efforts are being made to develop the high iron and zinc rich rice to combat the deleterious effect of hidden hunger. Rice is particular target for this biofortification because it is the staple food for more than half of the world population.

In this study, mineral analysis of PAU201 × Palman 579 derived BC_1F_2 (466 plants) and BC_1F_3 (106 plants) populations revealed large variation for iron and zinc contents in grain harvests, which could be due to different combinations of alleles from the two contrasting parents for iron and zinc content. Iron content differed significantly among the BC_1F_2 populations ranging between 0.9-101.7 µg/g; (PAU201, 52.3 µg/g; Palman 579, 373.1 µg/g). Iron content also varied among the BC_1F_3 populations ranging between 17.1-130.5 µg/g (PAU201, 54.4; Palman 579, 383.8 µg/g). 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). The backcross population developed from these parents i.e. PAU201 and Palman 579 showed large variation for various physio-morphological traits and micronutrient (Fe and Zn) content. Micronutrients (iron and zinc) accumulation in grain is a complex phenomenon. Quantitative genetic studies of plant hybrids mainly the primary cause of transgression is complementary genes action; however overdominance and epistasis also contribute to the quantitative traits. Complementary genes appear to be common for most traits, with the possible exception of those with a history of disruptive selection. These results provide credibility that hybridization is responsible for creating raw material for rapid adaptation and provide a simple explanation for niche digression and phenotypic variability often associated with hybrid lineages.

Phenotypic correlation coefficient analysis of PAU201 × Palman 579 derived BC_1F_2 and BC_1F_3 populations was carried out to assess the association between various mineral (Fe and Zn) and physio-morphological traits. Correlated characters are of interest due to connection with the genetic causes of correlation through the pleiotropic gene actions, to determine how one character selected will cause a simultaneous alter other characters, and to find out the relationship between character and fitness. In this study, grain iron content showed significant positive correlation (r=0.281) with grain zinc content only in BC_1F_2 population, while no significant correlation was observed between grain iron and zinc contents in BC_1F_3 populations. Both, grain iron and zinc content showed positive correlation with grain yield per plant (r= 0.291 and 0.104) in BC_1F_2 population, while no significant correlation was observed between grain mineral (iron and zinc) content and grain yield per plant in BC_1F_3 population. In BC_1F_2 and BC_1F_3 populations grain yield per plant showed significant positive correlation with plant height (r= 0.226 and 0.256, respectively), and effective number of tillers per plant (r= 0.716 and 0.869, respectively). Garcia-Oliveria *et al.*, 2009, also observed significant positive correlation between grain iron and zinc content in 85 introgression lines (ILs) derived from a cross between an elite *indica* cultivar Teqing and the wild rice (*Oryza rufipigon*). Monasterio and Graham 2000 reported large variation for both Fe (28.8–56.5 μg/g) and Zn (25.2–53.3 μg/g) in grains of a wide range of wheat germplasm and revealed a high correlation between grain-Fe and grain-Zn concentrations in wheat grain. The present study also showed a significant positive correlation (r=0.281) between grain Fe and Zn contents in BC_1F_2 population. Thus, it should be plausible to improve Fe and Zn levels simultaneously in rice grain through plant breeding. However, no correlation was apparently visible between grain iron and zinc content in BC_1F_3 population, which is matter of concern and being investigated in advanced generations.

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