Identification of Cytoplasmic Influence on Grain Quality and Micro-nutrient Traits Using Reciprocal Crosses and Mitochondrial Marker Based Polymorphism in Rice (*Oryza sativa* L.)

YALLAPPA HARIJAN AND SHAILAJA HITTALMANI

Department of Genetics and Plant Breeding, College of Agriculture, UAS, GKVK, Bengaluru-560 065

E-mail: shailajah_maslab@rediffmail.com

ABSTRACT

The data on plant, grain quality and nutrient traits were recorded to study genetic variability, heritability, genetic advance, correlation and path coefficient in both direct and reciprocal F_2 crosses of rice involving BPT-5204 and HPR-14 parents. The objective was to observe the effect of cytoplasmic influence on these traits. On the basis of mean performance, protein, iron, carbohydrates, phytic acid contents and grain yield per plant was higher in direct cross compared to its reciprocal cross. The percentage of transgressive segregants obtained in reciprocal cross for iron, zinc, amylose contents and grain yield per plant was higher than the direct cross. Both the crosses showed high heritability with low PCV, GCV and genetic advance for iron, zinc, carbohydrate and phytic acid contents. For grain yield per plant, moderate heritability with high PCV, GCV was observed.Zinc content in grain exhibited maximum positive direct effect on grain yield compared to iron content. Phytic acid had negative correlation with protein, zinc and amylose content in both crosses. In the parental polymorphism effort using 20 mitochondrial markers,13 markers indicated similarity for both parents and mostly showed monomorphism.

Keywords: cytoplasmic-inheritance, reciprocal-crosses, protein, zinc and iron, phytic acid and mitochondrial DNA markers

RICE (*Oryza sativa* L.) is India and South Asia's staple food. India is the second largest producer of rice and most of it is consumed in the country. It is rich in energy sources like carbohydrate but deficient in nutrients of protein, zinc and Fe hence, most rice eating population sufferes from miconutrient deficiency and leading to diseases in children and elderly persons from the lower strata of the society. It is estimated that more than 3.5 billion people in the world are deficient in vitamin A, iodine, iron and zinc (Virk *et al.,* 2007). Also World Health Organization (2011) reported approximately 1.62 billion people suffer from Iron deficiency (Anemia) and more than half the world suffering from Zinc deficiency. Zinc is an essential trace element for microorganisms, plants and animals. For humans, sufficient zinc is needed to maintain health and plays a role in many major metabolic pathways (Hanamareddy Biradar *et al.,* 2007).

Rice is a crop rich in genetic diversity, and known to have more than 1,27,000 germplasm in the world (IRRI, 2017). From among these, some donor genotypes with higher nutritional value could contribute to development of rice varieties that are high in nutrients and with higher yield.

Cytoplasmic effects can contribute substantially to the phenotype of an individual, despite evidence that maternal effects can have a large influence on offspring phenotype. Variation in an individual's phenotype may be determined not only by the genotype and environment of that individual but also by maternal effects, *i.e.,* the contribution of the maternal parent to the phenotype of its progenies beyond the equal chromosomal contribution expected from each parent. Maternal effects will increase the amount of environmental noise and thus slow the response to selection. Cytoplasmic or nuclear genetic maternal effects will inflate the amount of genetic variance but may slow the response to selection if the trait is completely under maternal control. Molecular and quantitative genetic studies have shown that cytoplasmic factors contribute to heritable variation in both qualitative and quantitative traits in plants. (Alexander and Wulff, 1985).

DNA markers are useful tools in identification of desired trait in plants or organisms. They help to find out if any association exists for selection (Hittalmani *et al.,* 1995 and 1997) and help in pyramiding of the traits. Mitochondrial markers may help to find out there is any cytoplasmic inheritance for the traits under study. Further, they could also be useful for selection for traits. The use of chloroplast (cp) DNA and / or mitochondrial (mt) DNA polymorphisms to investigate evolutionary phenomena requires a knowledge of the mode of organellar inheritance. For example, the frequent occurrence of uniparental inheritance of organellar genomes results in a characteristic decrease in the expected level of standing organellar variation in a population (as compared to the biparental case; Birky *et al.*, 1989). In addition, to the extent that they are uniparentally inherited. Organellar polymorphisms can provide insights into phenomena such as sex-biased gene flow (McCauley, 1994) and the directionality of introgression in populations of hybrid origin (Edwards-Burke *et al.,* 1997).

Using reciprocal crossing, maternal effects have been reported for several traits in rice, including grain weight and filled-grain ratio (Toa *et al.*, 2011), grain quality trait (Asfaliza *et al.*, 2012), protein content (Shi *et al.*, 1996) and milling quality traits (Shi and Zhu, 1995). The reciprocal effect for a trait is based on the assumption that if the difference exists between F_2 and its reciprocal F_2 population, they would be expected to be due to maternal effects and this contributes consequently for the design and interpretation of genetic studies (Mosjidis and Yermanos, 1984). Phytic acid (PA; myo-inositol hexaphosphate) is a ubiquitous biomolecule present abundantly in plants wherein PA phosphorus constitutes the major portion of total phosphorus in several seeds and grains (Harland and Overleas, 1987). It accounts for 50–80 per cent of the total phosphorus in different cereals (Raboy, 2003; Stangoulis *et al.,* 2007). However, the presence of certain forms of a particular nutrient can hinder the uptake of other nutrients especially micronutrients.

The present study was conducted to find out if there exists any variation for observed traits in the progenies of reciprocal crosses due to cytoplasmic inheritance involving a superior grain quality popular variety BPT 5204 and a local donor for micronutrients and protein HPR 14 and Further, explore the possibility of selecting desired combination by testing them using genetic-statistical parameters.

MATERIAL AND METHODS

The experiment was carried out during *Kharif*, under aerobic condition representing the eastern dry zone which is located at the latitude of 12^0 58' North; longitude 77^0 35' East and altitude of 930 meters above mean sea level. The reciprocal crosses previously developed by Mahadevaiah and Shailaja Hittalmani (2015) were obtained from Marker Asssited Selection laboratory, UAS, GKVK, Bengaluru for the present study. It composed of 479 direct cross (BPT 5204 x HPR-14) and 487 individuals plants of reciprocal cross (HPR-14 x BPT 5204) of F_2 population. The BPT 5204 is a highly popular variety grown in irrigated conditions in South India is known to petch high price for its excellent cooking and milling quality. The HPR 14 is low yielder, short duration for growth, bold grain type, low tillering ability and having high protein content of 13-14 per cent.

The Near Infrared Reflectance spectroscopy (NIR system, FOSS, Denmark) system was used for the estimation of total protein, iron, zinc, amylose, carbohydrates and moisture present in the grain. The phytic acid content present in the parents and two crosses of F_2 population were estimated by following the standard protocol of Gao *et al.,* 2007 and results were expressed as a percentage of dry weight (mg /100 g). Using the above procedure 400 samples across two crosses were estimated and developed the standard spectra for phytic acid content in Near Infrared Reflectance spectroscopy (NIR system, FOSS, Denmark) system and remaining samples were analyzed for phytic acid content using NIR. Correlation and path analysis were done for grain quality and micronutrients in both the crosses using software, INDOSTAT version 8.5. The SPSS version 16.0 software was used to obtain frequency distribution curves for grain nutrients traits.

A total of 20 different universal mitochondrial (mt) DNA specific primers primarily used by Khera *et al.,* 2012 (Table I) synthesized by Eurofins

TABLE I

List of mitochondrial DNA specific primers

Primer Name	Primer Sequence 5' to 3'	Annealing Temp. (°C)
Nad1 F	GCATTACGATCTGCAGCTCA	57.3
Nad1 R	GGAGCTCGATTAGTTTCTGC	57.3
Nad4ex1 F	CAGTGGGTTGGTCTGGTATG	59.4
Nad4ex1 R	TCATATGGGCTACTGAGGAG	57.3
Nad4ex2 F	TGTTTCCCGAAGCGACACTT	57.3
Nad4ex2 R	AACCAGTCCATGACTTAACA	53.2
rpS14 F	CACGGGTCGCCCTCGTTCCG	67.6
rpS14 R	GTGTGGAGGATATAGGTTGT	55.3
coxII F	AATCCAATCCCGCAAAGGATT	55.9
coxII R	AGAAGATGATCCAGAATTGGG	55.9
Rrna F	GTGTTGCTGAGACATGCGCC	61.4
Rrna R	ATATGGCGCAAGACGATTCC	57.3
alp F	TTTGCCAGCGGTGTTAAAGG	57.3
alp R	CTTCGCCATATTGTGCCAATTC	58.4
Atp6 F	GGAGGTGGAAATTCAGTTCCAA	58.4
Atp6 R	TAGCATCATTCAAGTAAATACA	50.9
Cob F	AGTTATTGGTGGGGGTTCGG	59.4
Cob R	CCCCAAAAGCTCATCTGACCCC	64.0
coxIA F	GGTGCCATTGCTGGAGTGATGG	64.0
coxIA R	TGGAAGTTCTTAAAAGTATG	49.1
coxIB F	GGCTTTTCTCCACTAACCACAA	58.4
coxIB R	GGAGGGCTTTGTACCATCCATTC	62.4
cox2 F	GATGCTGCTGAACCTTGGCA	59.4
cox2 R	TCCGATACCATTGATGTCC	54.5
cox3 F	GTAGATCCAAGTCCATGGCCT	59.8
cox3 R	GCAGCTGCTTCAAAGCC	55.2
Nad1A F	CTAGCTGAACGTAAAGTAATGGC	58.9
Nad1A R	CCAACCTGCTATAATTATTCC	54.0
Nad3 F	AATTGTCGGCCTACGAATCTG	57.9
Nad3 R	TTCATAGAGAAATCCAATCGT	52.0
Nad3A F	AATTGTCGGCCTAGGAATGTG	57.9
Nad3A R	GCTCGTGTACGGTCTGTGGG	63.5
Nad4A F	ATACGATTGATTCGGTCTGTG	55.9
Nad4A R	TGAACTGGTACCATAGGCACTTT	58.9
Nad5A F	GAAATCTTTGATGCTTCTTGGG	56.5
Nad5A R	ACCAACATTGGCATAAAAAAAGT	53.5
Nad5D F	ATAAGTCAACTTCAAAGTGGA	52.0
Nad5D R	CATTGCAAAGGCATAATGAT	51.2
Rps14 F	ATACGAGATCACAAACGTAGA	54.0
Rps14 R	CCAAGACGATTTTTTTATGCC	54.0

Genomics India Pvt. Ltd., Bengaluru were used in this study. These universal mt DNA primers were designed based on consensus mt DNA (Petit *et al.,* 1998; Wu *et al.,* 1998) derived from the various mitochondrial genes. The parental polymorphism on 3 per cent agarose gel electrophosis and 6 per cent polyacrylamide gel electrophosis was carried out using 20 mitochondrial specific simple sequence repeats (SSRs) following the methods described by (Sambrook *et al.,* 1989).

RESULTS AND DISCUSSION

The mean performance of grain nutrient traits like protein (9.70 %), iron (11.86 ppm), carbohydrates (83.05 %), phytic acid (733.44 mg / 100 g) and grain yield per plant (15.10 g) was higher in direct cross compare to its reciprocal cross (Table II). Frequency distribution was normal for grain nutrient traits and grain yield per plant both in direct and reciprocal crosses (Fig.1). The present investigation lead to the identification of superior promising individual plants for the traits under observation in F_2 segregating generations in the two crosses. The percentage of transgressive segregants obtained in reciprocal cross for iron were 76.39 per cent, zinc 69.61 per cent, amylose 98.15 per cent and phytic acid 28.54 per cent in reciprocal cross. For grain yield per plant 95.49 per cent of transgressive segregants were observed in reciprocal cross and which was higher than direct cross (94.15 %). The transgressive segregnts were lesser for protein 19.30 per cent and moisture content, 45.38 per cent in reciprocal cross compared to direct cross. The *per cent* of transgressive segregants in respect of carbohydrates was almost same in both the crosses *i.e..* 62 per cent (Table III).

The percentage of phenotypic and genotypic coefficients of variation for iron, zinc and carbohydrate were low in both the crosses. High heritability coupled with low genetic advance were observed for iron (97.65 %, 1.54%), zinc (61.10%, 1.75%) and carbohydrate content (73.92%, 8.46%) in direct cross, respectively. Similar results of high heritability and low genetic advance were obtained in reciprocal cross as well. *i.e.,* iron (98.05 %, 1.59%), zinc (66.71 %, 2.09 %) and carbohydrate contents (76.04 %, 8.27 %), respectively. For amylose content, the percentage of

Comparison of various grain nutrient traits in direct and reciprocal crosses in F_2 generation of rice under aerobic condition

| Grain nutrient traits	Parameters	BPT 5204 X HPR - 14	HPR - 14 X BPT 5204	Parents	
		Direct cross	Reciprocal cross	BPT 5204	HPR 14
Protein (%)	Mean	9.70	9.56	8.12	12.35
	Range	5.16-14.75	5.65-15.82		
Iron (ppm)	Mean	11.86	11.29	11.66	12.68
	Range	9.47-14.87	8.53-14.62		
Zinc (ppm)	Mean	28.92	30.37	28.14	30.23
	Range	25.10-31.85	26.37-32.98		
Amylose (%)	Mean	28.82	34.34	27.14	28.01
	Range	10.92-59.13	15.03-57.03		
Carbohydrate (%)	Mean	83.05	80.39	79.6	84.57
	Range	69.96-86.91	68.87-87.05		
Moisture (%)	Mean	12.12	12.13	12.48	11.97
	Range	10.69-13.15	11.06-13.04		
Phytic Acid (mg/100g)	Mean	733.44	720.80	848.06	636.59
	Range	492.21-1016.66	389.97-1072.43		
Grain yield per plant (g)	Mean	15.10	14.41	14.63	13.52

high PCV, GCV, heritability and genetic advance were noticed in both crosses. For phytic acid high heritability (99.67 %, 99.75 %) with high genetic advance as *per cent* of mean (23.71 %, 27.58 %) were recorded in both crosses, respectively. For grain yield per plant, the highest PCV (89.80 %, 89.92 %) and GCV (66.03 %, 58.36 %) were recorded in direct and reciprocal crosses, respectively (Table IV). Indicating the presence of ample variation for these traits in the present material. This implies that the trait was controlled by additive gene action, which offers scope for further improvement through selection which were in agreement with Sala., 2012; Verma *et al.,* 2000; Ashwini Samak*et al.,* 2011 and Shashidhar *et al.,* 2013.

Correlation studies for grain yield per plant and grain nutrient traits in F_2 population of direct and reciprocal crosses revealed that, grain yield had positive non-significant correlation with the zinc (0.2436), iron (0.0449), moisture contents (0.1347) while protein (-0.0865), amylose (-0.0536), carbohydrate (-0.0502) and phytic acid (-0.1103) had negative correlation in direct cross. In reciprocal cross, grain yield per plant had non-significant negative correlation with iron and positive non-significant correlation with amylose content (0.0717) (Table V & VI). These results were in accordance with the Nagesh *et al.* (2012) and Sala and Geetha (2015).
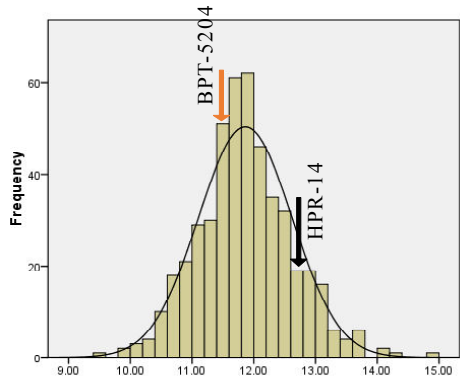
Phytic acid had significant negative correlation with protein (-0.0907) and significant positive correlation with the carbohydrate (0.01339) in direct cross whereas, protein (0.1319), iron (0.1278), carbohydrate (0.1654) had significant positive correlation and zinc (-0.3388), amylose (-0.1664) had
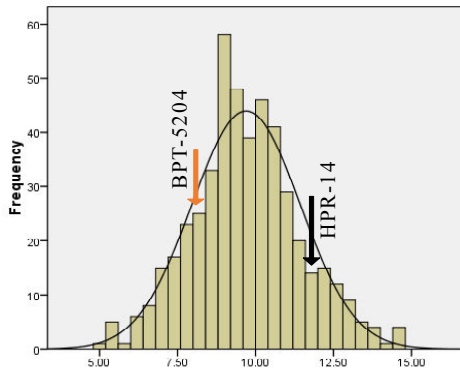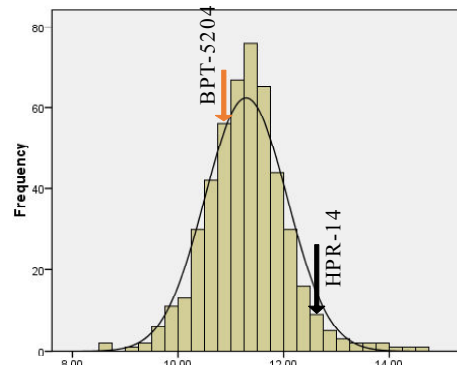
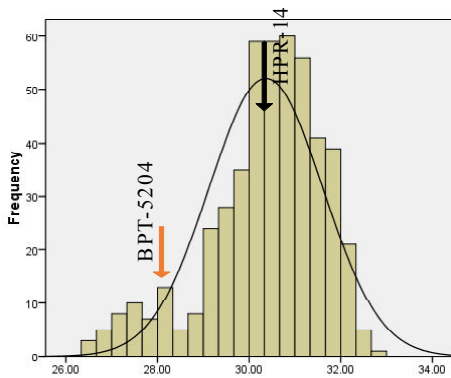1(a). Protein content (%) Direct cross
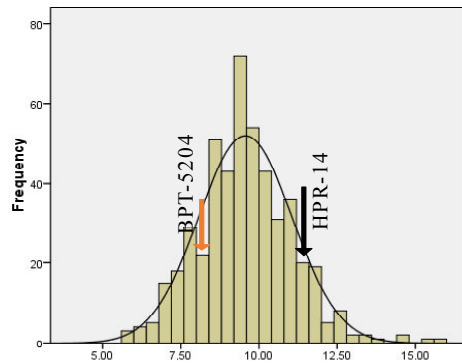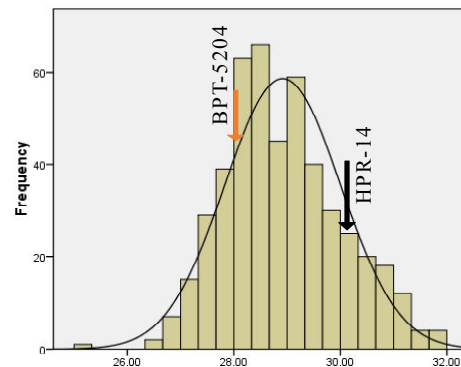
Protein content (%) Reciprocal cross
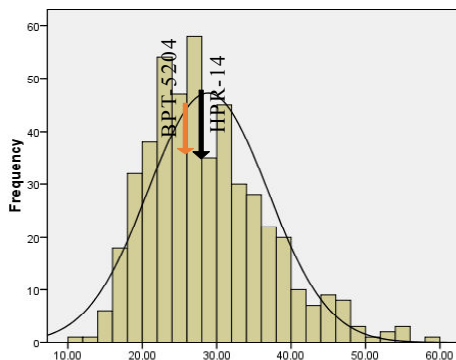
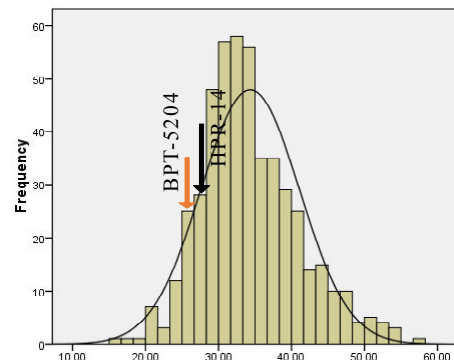1(b). Iron (ppm) Direct cross

Iron (ppm) Reciprocal cross
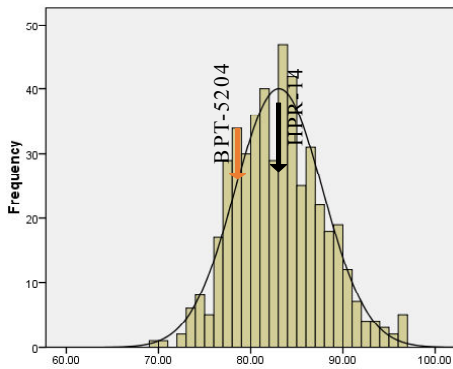
1(c). Zinc (ppm) Direct cross
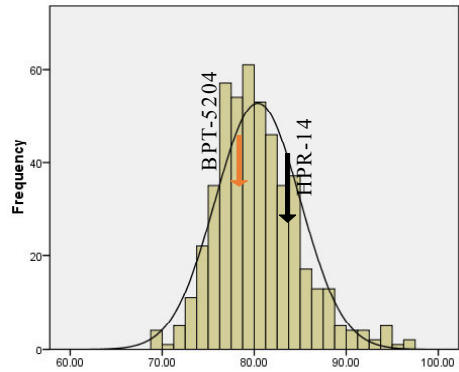
Zinc (ppm) Reciprocal cross
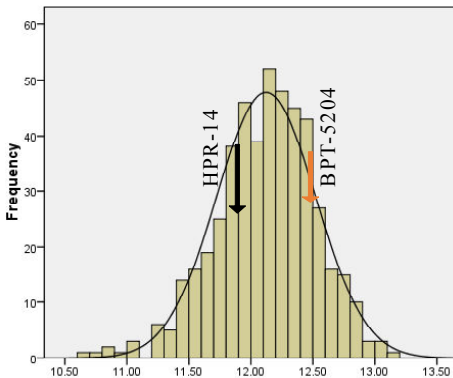
1(d). Amylose (%) Direct cross

Amylose (%) Reciprocal cross

1(e). Carbohydrates (%) Direct cross

Carbohydrates (%) Reciprocal cross

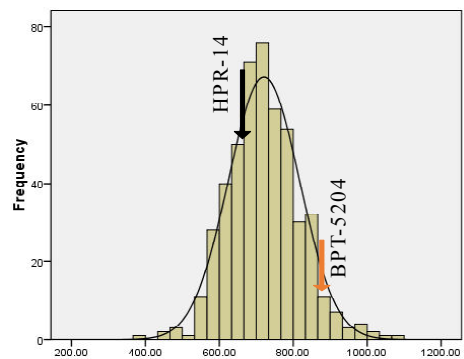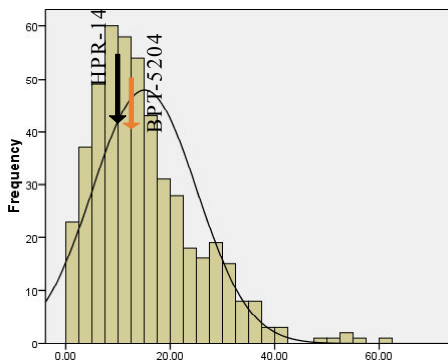1(f). Moisture (%) Direct cross
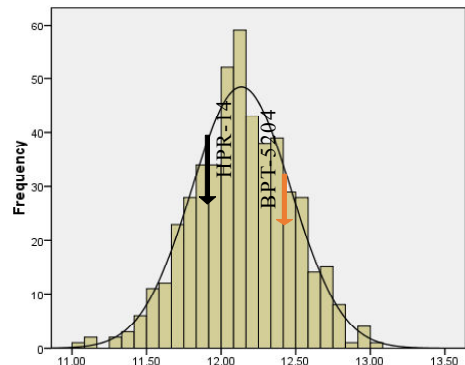
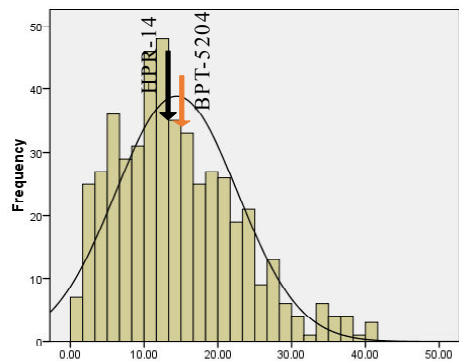Moisture (%) Reciprocal cross
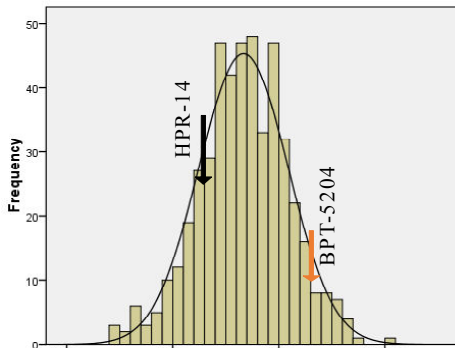
1(g). Phytic acid (mg/100g) Direct cross

Phytic acid (mg/100g) Reciprocal cross

1(h). Grain yield per plant (g) Direct cross

Grain yield per plant (g) Reciprocal cross

Fig. 1: Comparison of frequency distribution curve for grain quality parameters in direct and reciprocal crosses of F_2 generation in rice under aerobic condition.

TABLE III

Estimates of observed probability and transgressive segregants of F_2 population of direct and reciprocal crosses for grain nutrient traits in rice under aerobic condition.

Characters	Parental criteria	Observed probability (%)		Transgressive segregants (%)	
		Direct Cross	Reciprocal Cross	Direct Cross	Reciprocal Cross
Protein (%)	<BPT 5204	17.33	15.81	25.26	19.30
	>HPR 14	7.93	3.49		
	Intermediate	74.74	80.69		
Iron (ppm)	<BPT 5204	37.79	72.28	52.40	76.39
	>HPR 14	14.61	4.11		
	Intermediate	47.59	23.61		
Zinc (ppm)	<BPT 5204	24.22	7.39	37.16	69.61
	>HPR 14	12.94	62.22		
	Intermediate	62.84	30.39		
Amylose (%)	<BPT 5204	49.06	12.32	95.61	98.15
	>HPR 14	46.55	85.83		
	Intermediate	4.38	1.85		
Carbohydrates (%)	<BPT 5204	25.68	46.82	62.00	62.63
	>HPR 14	36.32	15.81		
	Intermediate	37.99	37.37		
Moisture (%)	>BPT 5204	17.12	16.02	51.15	45.38
	<HPR 14	34.03	29.36		
	Intermediate	48.85	54.62		
Phytic Acid (mg/100g)	>BPT 5204	7.72	10.06	19.61	28.54
	<HPR 14	11.89	18.48		
	Intermediate	80.37	71.46		
Grain yield per plant (g)	>BPT 5204	42.17	43.33	94.15	95.49
	<HPR 14	51.98	52.16		
	Intermediate	5.84	4.51		

<BPT 5204   =   The genotypes having value less than the parent BPT 5204 and expressed in percentage

<HPR 14   =   The genotypes having value more than the parent HPR 14 and expressed in percentage

Intermediate   =   The value between the two parents BPT 5204 & HPR 14 and expressed in percentage

TABLE IV

Estimates of PCV, GCV, heritability (broad sense) genetic advance and genetic advance per cent mean in direct and reciprocal rice crosses of F_2 generation under aerobic condition.

Characters	PCV (%)		GCV (%)		h² (broad sense)		Genetic advance (%)		Genetic advance over mean (%)	
	Direct Cross	Reciprocal Cross	Direct Cross	Reciprocal Cross	Direct Cross	Reciprocal Cross	Direct Cross	Reciprocal Cross	Direct Cross	Reciprocal Cross
Protein (%)	27.34	26.14	17.77	15.64	42.25	35.80	2.31	1.84	23.80	19.28
Iron (ppm)	6.47	6.97	6.39	6.91	97.65	98.05	1.54	1.59	13.02	14.09
Zinc (ppm)	4.80	5.01	3.75	4.09	61.10	66.71	1.75	2.09	6.04	6.89
Amylose (%)	28.28	19.88	28.10	19.66	98.72	97.82	16.58	13.75	57.52	40.05
Carbohydrates (%)	6.69	5.72	5.75	5.72	73.92	76.04	8.46	8.27	10.18	10.28
Moisture (%)	17.04	16.74	3.29	2.76	3.74	2.72	0.16	0.11	1.31	0.94
Phytic Acid (mg/100g)	11.51	13.39	11.47	13.35	99.67	99.75	173.91	198.78	23.71	27.58
Grain yield per plant (g)	89.80	89.92	66.03	58.36	54.07	42.13	15.10	11.20	100.02	78.03

PCV (%) = Phenotypic coefficient of variation (%)

GCV (%) = Genotypic coefficient of variation (%)

h²= Heritability

TABLE V

Phenotypic correlations among grain nutrient traits in direct cross (BPT 5204 X HPR-14) of F_2 generations under aerobic condition.

	X1	X2	X3	X4	X5	X6	X7
X1	**1**						
X2	-0.245 ***	**1**					
X3	-0.1148 *	-0.2891 ***	**1**				
X4	-0.0549	-0.4138 ***	0.3914 ***	**1**			
X5	-0.507 ***	0.3953 ***	-0.2528 ***	-0.0098	**1**		
X6	0.0049	0.0247	0.1781 ***	0.0528	-0.0805	**1**	
X7	-0.0907 *	0.0051	-0.0877	-0.0084	0.1339 **	0.0010	**1**
X8	-0.0865	0.0449	0.2436	-0.0536	-0.0502	0.1347	-0.1103

X1= Protein (%), X2= Iron (ppm), X3= Zinc (ppm), X4= Amylose (%), X5= Carbohydrates (%), X6= Moisture (%), X7= Phytic Acid (mg/100g) and X8= Grain yield per plant (g)

* Significant at 5 per cent, ** Significant at 1 per cent, *** Significant at 0.5 per cent

TABLE VI

Phenotypic correlations among traits in reciprocal cross (HPR-14 X BPT 5204) of F_2 generations under aerobic condition.

	X1	X2	X3	X4	X5	X6	X7
X1	1						
X2	-0.0765	1					
X3	0.0276	-0.5010 ***	1				
X4	-0.0084	-0.5803 ***	0.3480 ***	1			
X5	-0.7120 ***	0.2960 ***	-0.5265 ***	-0.1333 **	1		
X6	0.0379	0.0499	0.1088 *	-0.1712 ***	-0.1130 *	1	
X7	0.1319 **	0.1278 **	-0.3388 ***	-0.1664 ***	0.1654 ***	-0.0623	1
X8	-0.0640	-0.0548	0.1062	0.0714	-0.0738	0.0066	-0.2293

X1= Protein (%), X2= Iron (ppm), X3= Zinc (ppm), X4= Amylose (%), X5= Carbohydrates (%), X6= Moisture (%), X7= Phytic Acid (mg/100g) and X8= Grain yield per plant (g)

* Significant at 5 per cent, ** Significant at 1 per cent, *** Significant at 0.5 per cent

significant negative correlation with the phytic acid content in reciprocal cross. Grain zinc content had significant negative correlation with iron in both direct and reciprocal crosses. Among the traits studied, zinc had highest direct effect on grain yield per plant followed by moisture and iron. The amylose content (-0.1448) had highest negative effect in direct cross and this amylose had highest direct effect (0.0356)

followed by iron (0.0012) on grain yield per plant and carbohydrate had highest negative direct effect in reciprocal cross (Table VII & VIII). Since, there was no significant correlation between grain nutrients under study and grain yield per plant suggests that simultaneous selection or breeding can be taken up to enhance iron, zinc contents and grain yield per plant.

TABLE VII

Genotypic direct (diagonal) and indirect effects of different grain nutrient traits in direct cross of rice F_2 generation under aerobic condition.

	X1	X2	X3	X4	X5	X6	X7
X1	-0.1121	0.0308	0.0129	0.0062	0.0841	-0.0006	0.0102
X2	-0.0156	0.0570	-0.0165	-0.0236	0.0225	0.0014	0.0003
X3	-0.0301	-0.0757	0.2617	0.1024	-0.0662	0.0466	-0.0230
X4	0.0080	0.0599	-0.0567	-0.1448	0.0014	-0.0076	0.0012
X5	0.0548	-0.0289	0.0185	0.0007	-0.0730	0.0059	-0.0098
X6	0.0004	0.0022	0.0159	0.0047	-0.0072	0.0891	0.0001
X7	0.0081	-0.0005	0.0078	0.0007	-0.0120	-0.0001	-0.0893
r	0.0097	0.0026	0.0638	0.0078	0.0037	0.0120	0.0098

Residual effect= 0.944

R square = 0.109

X1= Protein (%), X2= Iron (ppm), X3= Zinc (ppm), X4= Amylose (%), X5= Carbohydrates (%), X6= Moisture (%), X7= Phytic Acid (mg/100g), X8= Grain yield per plant (g) and r= partial R^2

TABLE VIII

Genotypic direct (diagonal) and indirect effects of different grain nutrient traits in reciprocal cross of rice F_2 generation under aerobic condition

	X1	X2	X3	X4	X5	X6	X7
X1	-0.1868	0.0143	-0.0052	0.0016	0.1330	-0.0071	-0.0246
X2	-0.0001	0.0012	-0.0006	-0.0007	0.0003	0.0001	0.0001
X3	-0.0021	0.0377	-0.0753	-0.0262	0.0396	-0.0082	0.0255
X4	-0.0003	-0.0207	0.0124	0.0356	-0.0047	-0.0061	-0.0059
X5	0.1506	-0.0626	0.1114	0.0282	-0.2115	0.0239	-0.0350
X6	-0.0003	-0.0004	-0.0008	0.0013	0.0009	-0.0078	0.0005
X7	-0.0250	-0.0243	0.0643	0.0316	-0.0314	0.0118	-0.1899
r	0.0120	-0.0001	-0.0080	0.0025	0.0156	-0.0001	0.0435

Residual effect= 0.9667

R square = 0.0655

X1= Protein (%), X2= Iron (ppm), X3= Zinc (ppm), X4= Amylose (%), X5= Carbohydrates (%), X6= Moisture (%), X7= Phytic Acid (mg/100g), X8= Grain yield per plant (g) and r= partial R^2

DNA marker validation: Molecular markers are often used to select suitable genotype in plant breeding. Twenty mitochondrial specific markers have been already mapped by Khera *et al.,* 2012. These 20 mt specific SSR markers were screened on BPT 5204 and HPR-14 genotypes. The amplified products were resolved on 3 per cent agarose and 6 per cent PAGE gel. About 65 per cent of mitochondrial SSRs or 13 SSR markers indicated similarity for both parents and showed monomorphism on 3 per cent agarose. Out of 20 markers, none of markers showed polymorphic on 3 per cent agarose (Fig. 2) and 3 markers were polymorphic on 6 per cent PAGE (Fig. 3). Majority of
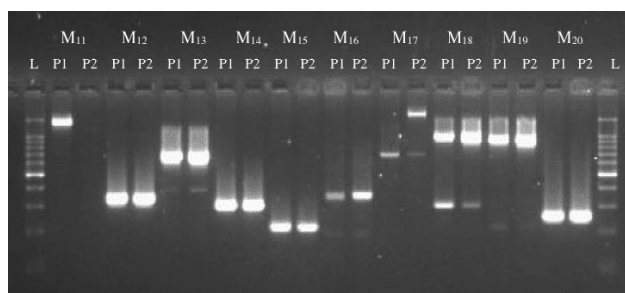

Fig. 2: Parental polymorphism on BPT 5204 (P$_1$) and HPR-14 (P$_2$) genotypes using mitochondrial specific SSRs. L =100bp Ladder ; M=Mitochonrial specific SSR markers

Fig. 3 : Parental polymorphism on BPT 5204 (P$_1$) and HPR-14 (P$_2$) genotypes using mitochondrial specific SSRs. L = 100bp Ladder ; M= Mitochonrial specific SSR markers
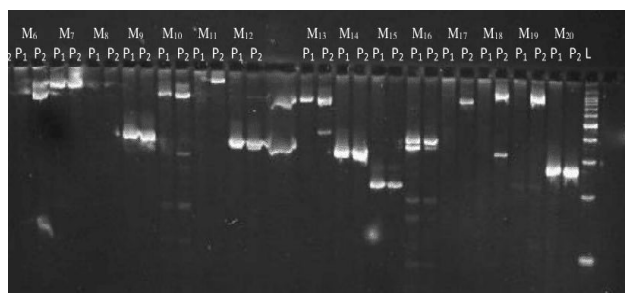
primers analyzed were monomorphic in nature. This might be attributed to the conservative nature of mitochondrial DNA (Fuji *et al.,* 2010; Khera *et al.,* 2012) across species and varieties. 15 *per cent* of polymorphism was observed on 6 per cent PAGE gel. These polymorphic markers could be utilized further to see the effect of cytoplasm in the progenies of both the crosses.

Total grain protein is quantitatively inherited and is sensitive to environmental conditions like the level

of nitrogen fertilizers and normal distribution for total grain protein (Harshiya Banu *et al.,* 2013; Uday *et al.,* 2014). Symmetric variation suggested complete ambi-directional tends to normality due to interaction of between epistatic gene action and G x E interaction effect. The study of distribution using skewness and kurtosis provides information about nature of gene action and number of genes controlling traits, respectively. (Ashwini Samak*et al.,* 2011; Mahadevaiah *et al.,* 2015). Since, no skewness in both the crosses for all micronutrient traits indicating that those traits are governed by large number of genes and quantitatively controlled.

High transgressive segregants for iron, zinc, amylose and phytic acid in reciprocal cross compared to direct cross is mainly due to the combination of favourable genes (positive alleles) from different parents and additive gene action. Low heritability and low genetic advance in reciprocal cross for protein, amylose, moisture and grain yield per plant indicating the involvement of large number of loci in the inheritance of these traits and the major role of non-additive genes. For such characters high proportion of phenotypic coefficient of variation as compared to genotypic coefficient of variation is mainly responsible for reduced heritability and selection could be ineffective.

Grain quality is an economically important character in rice varieties. Any knowledge of the genetic mechanism of rice grain quality will be of benefit to rice breeders. Therefore, the major and minor genes affecting rice grain quality should accelerate the process of breeding new rice varieties with both a higher yield and a better quality. Quality traits are dependent not only on the genotypic but also on an environmental influence, and are controlled by some minor genes with additive effects. The inheritance of grain quality is complicated due to epistasis, cytoplasmic effects, and the triploid nature of endosperm (Pooni *et al.*, 1992). Some of the plants in the above two crosses may be further advanced to later generations so as to select the best segregants for high single plant yield coupled with more nutritive content and grain quality. This implies that the trait was controlled by additive gene action, which offers scope for further improvement through selection.

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