

Original Research Article

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Qualitative and Quantitative Genetic Variations in the F₂ Inter Varietal Cross of Rice (*Oryza sativa* L.) under Aerobic Condition and Parental Polymorphism Survey

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ABSTRACT

Keywords

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Currently available rice varieties contain low percent of protein and many deficiency symptoms are predominantly seen in rice eating population are observed. To improve the efficiency of breeding for total grain protein in rice, a thorough understanding of the genetics of the trait concerned is essential. In order to address this problem we have identified promising local *indica* rice, (HPR14), which possesses relatively higher protein than cultivated rice. The rice protein normally posses 7-8 percent while the donor genotype identified has an average of 14.1 percent total protein. The initial results on the segregation for protein content indicated 3.5-18 percent of protein variation among the 1267 F₂ segregating lines. In order to transfer these valuable traits into popular rice variety BPT – 5204, crosses were made and F₂segregating lines were developed. The parental plants were surveyed using 402 rice SSR markers, out of which 69 (17.20%) showed polymorphism on agrose gel, 81 (20.00%) on PAGE and 252 were monomorphic (indicating homology between the parents). In F₂ field evaluation, we could observed clear cut segregation and top hundred lines were selected based on yield and segregation for protein content.

Introduction

As a pivotal crop in cereal, rice provides the staple food for more than 50% of the world's population. It supplies 23 per cent of global per capita energy and 16 per cent of protein. The consumption of rice is declining in developing countries because of its own limitation *viz.*, low protein, fat and micronutrients especially Iron and Zinc. Globally, rice is grown on about 150 m ha and Asian countries account for 90 percent of its area. India ranks first in area (44.8 m ha) and second in production (90 mt) among rice producing countries, in terms of productivity India ranks 9th (Anonymous, 2007). Grain

Protein content (GPC) is the macro nutrients essential for building up the human body. They are called macro nutrients because they form the bulk of the food. Many proteins are enzymes that catalyze biochemical reactions and are vital to metabolism. Proteins also have structural or mechanical functions, such as actin and myosin in muscle and the proteins in the cytoskeleton, which form a system of scaffolding that maintains cell shape.

After the achievement of sufficient yield by developing high yielding varieties, the

demand for grain quality is increasing day by day among the predominantly rice consuming peoples. In the early 1960's (green revolution era) primary attention was given to increasing rice yield. Even as late as 1970's when widespread drought and floods drastically reduced food grain levels, the world primary emphasis was on the quantity of food produced and not on its quality. Earlier decades of rice breeding started with a sole objective of increasing yield and developing disease and pest resistant types, and now a days is currently devoting increasing attention to grain quality. Most rice varieties developed so far are high grain yield with low protein ranging from 7 to 8 percent. Breeding for high yield in rice is mainly focused on production than the nutritional enhancement to feed the large rice eating population. As such protein deficiency is predominant in rice consuming population hence; enhancement of total protein in rice is of immense importance for nutritional security as food security. Hence the current study was conducted to develop high grain protein segregating line as a sole objective.

Materials and Methods

Plant materials

Diverse genetic back ground of parents BPT 5204 (good grain qualities and high yield) and HPR 14 (high protein content; Shailaja Hittalmani, 1990) were crossed and developed One thousand two hundred and sixty seven segregating lines and selection were carried out for high protein line with good grain quality parameters in F₂ (Table 1).

Experimental site and layout

The experiment was laid out in augmented design at Farmer's field, Devanahalli, Bengaluru North Taluk during *Kharif*- 2006 and the observations were recorded on selected individual plants and used for

statistical analysis. Twenty one days nursery seedlings were transplanted in main experimental field with 20cm X 20 cm spacing and minimum of five plants were maintained in each line. The crop was raised in aerobic condition with regular irrigations once in 5-7 days. Recommended cultural practices for Aerobic rice were carried out to ensure uniform crop stand as per the package of practices (Anonymous, 2004).

Phenotypic characterization and estimation of quantitative, qualitative, genotypic and phenotypic components of F₂ segregating lines

1267 lines were evaluated for various phenotypic/morphological, grain qualities, major and minor nutrient parameters as per the standard procedures and the details are given below.

Days to 50 per cent flowering (days): Total number of days taken by genotype/line for flowering from the sowing day to opening of first flower of the plants.

Days to maturity (days): The number of days from the date of sowing to harvesting was recorded at the time of harvest by each genotype.

Biomass weight per plant (g): After harvesting the panicles and straw about 2-3 cm above ground level. It was sun dried and the weight was recorded in grams. The total weight of straw was considered as total biomass weight per plant.

Plant height (cm): The plant height was recorded by measuring total height from the base of the plant to the tip for the main panicle expressed in centimeters.

Number of productive tillers per plant: Number of productive tillers was recorded by

counting the tiller bearing panicles at the time of harvest.

Number of panicles per plant: The total number of panicles was counted per plant at harvest. This is also equal to the number of productive tillers per plant.

Panicle length (cm): The length of the panicle from its base to tip in centimeters excluding awns was measured at the time of harvest recorded.

Number of fertile spikelets per panicle: The number of filled grains per panicle was counted and recorded after harvest.

Grain weight per plant (g): Total weight of all the filled grains per plant was estimated and expressed in grams.

Test weight (g): In each of the segregating lines, 1000 well filled grains were counted and their weight was recorded in grams as 100 grain weight.

Harvest index: The proportion of grain yield to biological yield of a plant as suggested by Donald (1962) was computed to calculate harvest index.

$$\text{Harvest index} = \frac{\text{Grain yield per plant}}{\text{Total biomass weight (g)}}$$

Length of paddy grain (mm): Ten paddy grains of each line were arranged lengthwise, for the cumulative measurement of length in centimeters of ten grains. Average length of the paddy grains was recorded as paddy grain length.

Breadth of paddy grain (mm): Ten paddy grains of each line were arranged breadth wise, for the cumulative measurements of

breadth in centimeters of ten grains. Average breadth of ten paddy grains was recorded as paddy grain breadth.

Length to Breadth (L/B) ratio of paddy grain: Ratio of length to breadth (L/B) of paddy grain was obtained by dividing the length of each grain by its corresponding breadth.

$$L/B \text{ ratio} = \frac{\text{Mean length of grains}}{\text{Mean breadth of grains}}$$

Length of rice kernel (mm): Ten dehusked and polished rice kernels of each line were arranged lengthwise for the cumulative measurement of length in centimeter of ten grains. Average length of the rice kernels recorded as rice kernel length.

Breadth of rice kernel (mm): Ten dehusked and polished rice kernels of each line were arranged breadth wise for the cumulative measurement of breadth in centimeter of ten grains. Average breadth of the rice kernels recorded as rice kernel breadth.

Length to Breadth (L/B) ratio of rice kernel: The ratio of length to breadth (L/B) of dehusked and polished grain was obtained by dividing the length of each grain by its corresponding breadth.

$$L/B \text{ ratio of rice kernel} = \frac{\text{Mean length of rice kernel}}{\text{Mean breadth of rice kernel}}$$

Protein (%): Standard micro Kjeldhal method was followed for determining Nitrogen content in the selected lines under study and correction factor 6.25 is multiplied to get crude protein percentage.

Total Nitrogen (%): Standard micro Kjeldhal method was followed for determining Nitrogen content.

Phosphorus (%): Phosphorus was estimated using a suitable aliquot of the above extract by vanodomolybdophosphoric yellow colour method (Jackson, 1973).

Potassium (%): Potassium content in plant was estimated by feeding the digested extract, after suitable dilution using flame photometer (Jackson, 1973).

Micronutrients (ppm): Micronutrients (Zn, Fe Cu and Mn) were estimated by feeding the digested extract after suitable dilutions, using Atomic Absorption Spectrophotometer (Perkin Elmer model Analyst-400).

Phenotypic Variance (V_p): Phenotypic variance was calculated by using the following formula.

$$V_p = \frac{\sum x^2 - \frac{(\sum x)^2}{N}}{N-1}$$

Where, \sum = Summation; X = an observation; X² = Square of an observation; N = Number of observation.

Environmental Variance (V_e): Environmental variance for each character was estimated from the mean variance of non segregating parental populations. Environmental variance (V_e) was calculated by using the following formula.

$$V_e = \frac{V_{p1} - V_{p2}}{2}$$

Where, V_{p1} = Phenotypic variance of parent one; V_{p2} = Phenotypic variance of parent two

Genotypic Variance (V_g): Genotypic variance was separated from the total variance by subtracting the environmental variance as per the method formulated by Webber and Moorthy(1952).

$$V_g = V_p - V_e$$

Where, V_g = Genotypic variance; V_p = Phenotypic variance; V_e = Environmental variance

Phenotypic and Genotypic coefficient of variation: The phenotypic and genotypic coefficients of variation (PCV and GCV) were computed as per Burton and Dewane (1953) from the respective variances.

$$\text{Phenotypic Co-efficient of Variability (PCV\%)} = \frac{\sqrt{\text{Phenotypic variance}}}{\text{Grand mean}} \times 100$$

$$\text{Genotypic Co-efficient of Variability (GCV\%)} = \frac{\sqrt{\text{Genotypic variance}}}{\text{Grand mean}} \times 100$$

PCV and GCV were classified according to Robinson *et al.*, (1966) that,

- 0-10% : Low
- 10-20% : Moderate
- 20% and above : High.

Heritability (h²): Broad sense Heritability was calculated as ratio of genotypic variance to phenotypic variance as per the formula suggested by Johnson *et al.*, (1955) and Hanson *et al.*, (1956).

$$h^2 = \frac{\text{Genotypic variance}}{\text{Phenotypic variance}} \times 100$$

Where, h² = Heritability; V_g = Genotypic variance; V_p = Phenotypic variance
Heritability percentage was categorized as follows (Robinson *et al.* 1966)

- 0-30% : Low
- 30-60% : Moderate
- 60% and above : High

Genetic advance (GA): Genetic advance was calculated by using formula given by Johnson *et al.*, (1955).

$$GA = h^2 \times \sigma_p \times k$$

Where, h^2 = Heritability (Broad sense); σ_p = Phenotypic standard deviation

k = selection differential which is 2.06 at 5% intensity of selection (Lush, 1949).

To compare the extent of predicted genetic advances of different characters under selection, genetic advance as per cent of mean was computed as devised by Johnson *et al.*, (1955).

$$\text{GA as per cent of mean} = \frac{\text{GA}}{\text{Grand mean}} \times 100$$

The GA as per cent mean was classified (Johnson *et al.* 1955) as given below:

0-10 % : Low
10-20 % : Moderate
20% and above : High

Parental polymorphism survey

402 Simple Sequence Repeats (SSRs) were surveyed for parental polymorphism both on Agarose Gel Electrophoresis (AGE) and Poly Acrylamide Gel Electrophoresis (PAGE).

Statistical analysis

The obtained field data were subject STASTICA and SPAR1 to compute all the genetic parameters to partition the variance. Simple correlation coefficients were determined as reported by Sunderraj *et al.*, 1972.

Results and Discussion

The availability of genetic variability is prerequisite for crop improvement. Important quantitative characters like yield, GPC mainly influenced by large number of genes and also greatly influenced by environmental factors. The variability is the sum total of hereditary effects of concerned genes as well as environmental influence. Hence, the

variability is partitioned into heritable and non-heritable components with suitable genetic parameters such as genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (h^2) and genetic advance as percent mean (GAPM). The phenotypic coefficient of variation was higher than genotypic coefficient of variation for all the characters and the difference between these two was observed to be low, which indicated less influence of environment on the trait expression. High heritability coupled with higher GAPM indicated the more of additive gene action with fast and effective selection for the trait under consideration. The estimation of these variability parameters helps the breeder in achieving the required crop improvement by selection (Fig.1 and 2).

Variation for total grain protein content and grain quality parameters

Wide range of TGP content (5.25% to 18.43%) with an average of 11.85% was recorded in base population of F_2 segregating generation indicating there is wide potentiality to develop high protein lines using this segregating population. Moderate PCV (16.73%) and GCV (11.73%) with moderate h^2 (49.11%) coupled with moderate GAPM (16.93%) were recorded. However, in selected hundred lines, it ranges from 5.25 to 18.43% with an average of 12.01% with moderate PCV (19.57%) and GCV (15.63%) as well as high h^2 (63.79%) coupled with high GAPM (25.72%) was recorded (Table 2 and 3). These estimates of h^2 and GAPM, indicated that the GPC mainly controlled by additive gene action and higher h^2 coupled with higher GAPM in selfing generation indicating that more of additive gene action and selection is effective for the trait under consideration. Higher heritability and GAPM in selected lines indicated that both additive and non-additive gene action for the trait under consideration.

Table.1 Salient features of parents selected for the present study

Character	BPT – 5204	HPR – 14
Parent	Female	Male
Protein content	Low (7.90 to 8.10%)	High (14.1%)
Plant colour	Green	Purple
Leaf colour	Green	Purple
Sheath colour	Green	Purple
Plant stature	Short (60-70cm)	Tall (above 90cm)
Tillering ability	High (20)	Low (10 - 16)
Number of panicles	More (15-18)	Less (10 - 14)
Grain yield	High (26g/plant)	Medium (23g/plant)
Grain type	Short Fine	Short Bold

Table.2 Genetic parameters estimated in F₂ segregating lines in base population

Parameters	Mean	Range		PCV (%)	GCV (%)	h ² (%)	GAPM
		Minimum	Maximum				
Protein	11.85	5.25	18.43	16.73	11.73	49.11	16.93
GL	6.15	3.70	7.00	13.69	11.83	94.65	21.06
GB	2.69	2.00	3.20	11.53	10.80	68.33	18.86
GLBR	2.21	1.42	3.30	18.24	12.62	29.00	25.32
KL	5.43	4.50	6.80	7.80	7.35	88.83	14.26
KB	1.99	1.10	2.52	11.52	7.35	88.83	14.26
KLBR	2.77	1.96	3.54	14.86	13.50	82.35	25.22
Nitrogen	1.87	0.73	3.96	30.49	27.04	78.53	49.32
Phosphorous	0.16	0.07	0.38	27.41	25.45	85.00	48.00
Potassium	0.16	0.08	0.38	29.48	27.16	90.00	54.65
Copper	5.61	3.30	18.30	22.99	18.75	66.52	31.50
Zinc	26.67	2.88	35.17	27.31	26.73	95.78	53.89
Manganese	7.83	3.74	11.49	19.77	17.91	82.04	33.41
Iron	44.92	24.67	66.43	23.02	22.45	95.12	45.11
DF	118.88	89	199	11.66	10.47	80.66	19.38
DM	163.85	126	205	6.54	5.22	63.66	8.58
PH	85	61	155	16.47	15.74	91.22	30.96
Biomass	48.71	20.21	111.74	46.49	45.00	93.70	79.74
NOT	22.25	12	30	19.63	15.19	59.94	24.23
NOP	17.00	8	26	43.81	39.44	76.57	64.67
PL	18.00	12	28	19.22	15.01	91.00	21.34
SFP	83.37	37.74	98.63	11.53	10.42	87.70	23.03
GY	20.1	10.24	31.59	45.13	35.56	95.58	36.76
TW	15.20	10.70	20.90	33.38	30.13	62.86	34.12
HI	0.34	0.10	0.45	30.23	27.52	72.84	38.45

Key: TGP – Total grain protein (%); KL - Kernel length (mm); PH – Plant height (cm); Fe – Iron (ppm); GYP – Grain yield per plant (g); KB – Kernel breadth; DF – Days to 50% flowering; N - Nitrogen (%); GL - Grain length (mm); KLBR – Kernal L: B ratio; P - Phosphorous (%); GB - Grain breadth (mm); DF – Days to 50% flowering; K - Potassium (%); GLBR – Grain L: B ratio; DM – Days to maturity; Zn – Zinc (ppm).

Table.3 Genetic parameters estimated in F₂ segregating lines in selected population

Parameters	Mean	Range		PCV (%)	GCV (%)	h ² (%)	GAPM
		Minimum	Maximum				
Protein	12.01	5.25	22.83	19.57	15.63	63.79	25.72
GL	6.88	5.60	7.90	18.79	13.50	96.71	24.38
GB	2.92	2.0	3.6	23.21	18.45	76.52	13.06
GLBR	2.58	2.00	3.65	25.50	14.42	22.50	18.23
KL	5.51	4.1	6.0	8.13	7.69	89.64	15.01
KB	2.02	1.60	2.50	10.52	9.28	77.78	18.85
KLBR	2.71	1.96	3.65	11.95	10.11	71.43	27.59
Nitrogen	1.98	0.73	2.96	48.69	47.28	98.98	49.94
Phosphorous	0.16	0.07	0.27	26.59	26.08	85.00	46.56
Potassium	0.15	0.08	0.27	28.46	27.82	90.00	52.76
Copper	5.56	3.31	15.50	22.65	18.20	64.56	38.13
Zinc	26.74	2.88	30.17	26.72	26.14	95.69	52.67
Manganese	7.73	3.69	11.29	19.36	17.40	80.81	32.23
Iron	42.99	24.14	61.43	26.15	25.61	95.87	51.65
DF	120.96	95	158	8.97	7.42	68.43	12.65
DM	162.92	137	189	6.82	5.81	64.05	8.83
PH	85.36	61	113	16.88	16.16	91.63	21.86
Biomass	43.64	16.25	144.00	36.10	35.91	88.92	63.57
NOT	21.77	12	29	22.08	19.04	74.38	33.83
NOP	17.05	9	25	46.27	32.67	74.44	65.29
PL	18.62	12	28	20.35	16.11	83.93	20.26
SFP	85.05	65.52	99.1	18.26	17.51	91.90	34.57
GY	25.17	2.2	31.59	38.27	36.63	94.19	44.67
TW	20.78	11.7	24.2	20.04	18.00	60.87	25.13
HI	0.15	0.05	0.41	40.00	38.49	70.00	32.73

Table.4 DNA markers used for detecting parent polymorphism of BPT 5204 and HPR 14

Marker type	No. of markers	Number of bands			Average number of bands			Percent polymorphism
		Poly morphic	Mono morphic	Total	Poly morphic	Mono morphic	Total	
SSR (3% agarose)	402	69	333	402	0.17	0.82	1.00	17.20
SSR (12% PAGE)	402	81	321	402	0.20	0.80	1.00	20.00

Fig.1 Some of the selected genotypes in F₂ population along with parents (BPT-5204 and HPR-14)

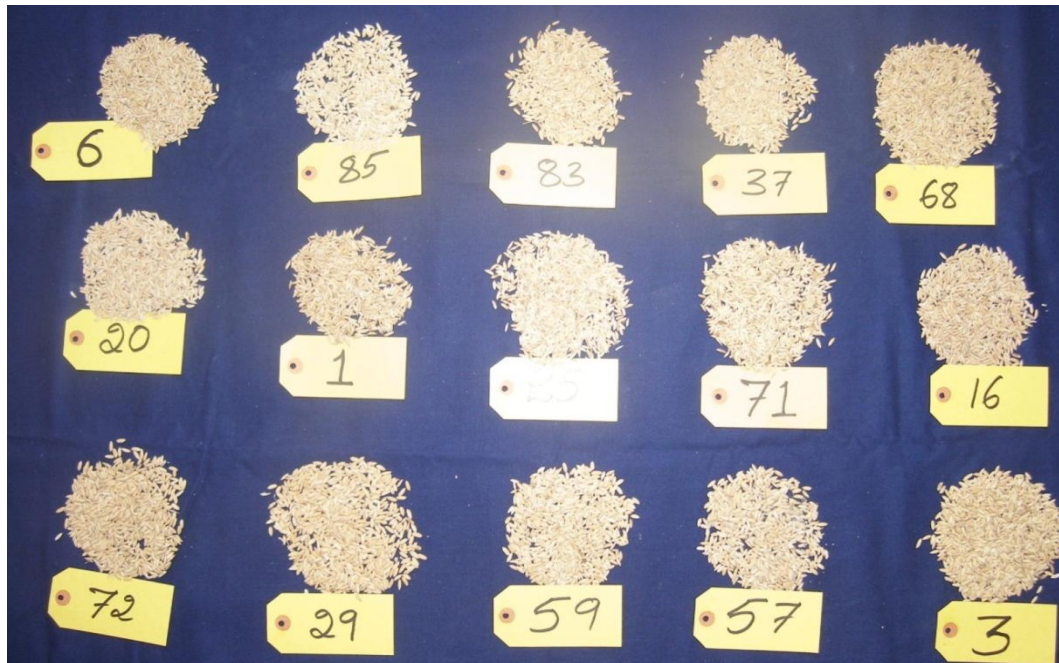


Fig.2(A) Frequency distribution for total grain protein content in F₂ segregation population of BPT – 5204 X HPR – 14 in base population and (B) in selected hundred lines

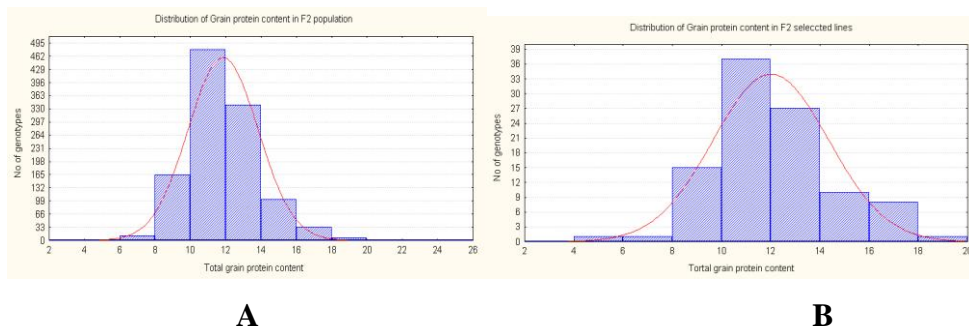
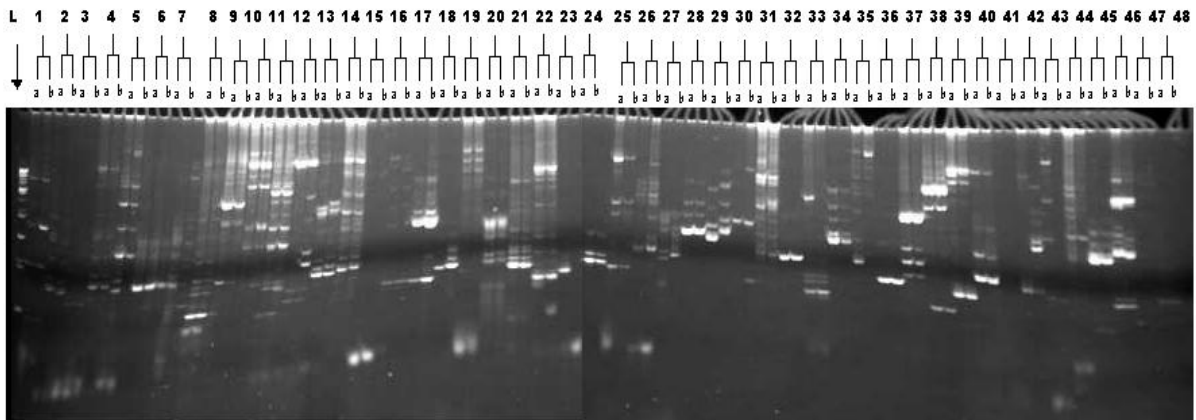


Fig.3 Parental polymorphism using SSR markers for the parents BPT 5204 (a) and HPR 14 on 9% PAGE gel



Key:

L – 100 bp ladder	10 - RM 3496	20 – RM 4455	30 – RM 500	40 – RM 552
1 – RM 3376	11- RM 3808	21 – RM 5352	31 – RM 503	41 – RM 456
2 – RM 3866	12 – RM 3912	22 – RM 3668	32 – RM 463	42 – RM 484
3 – RM 4348	13 – MRG 4568ARS	23 – RM 3625	33 – RM 147	43 – RM 245
4 – RM 1335	14 - RM 3515	24 – MRG 1734RG	34 – RM 431	44 – RM 454
5 – RM 1959	15 – RM 3025	25 – RM 5599	35 – RM 14	45 – RM 548
6 – RM 2819	16 – RM 5055	26 – RM 3283	36 – RM 522	46 – RM 558
7 – RM 2878	17 – RM 166	27 – RM 5128	37 – RM 535	47 – RM 457
8 – RM 3153	18 – RM 2197	28 – RM 544	38 – RM 556	48 – RM 27
9 – RM 3508	19 – RM 2224	29 – RM 555	39 – RM 288	

The distribution frequency for GPC in the segregating population showing an expected normal in both base as well as selected population, providing a fast and effective selection for the trait under consideration in this population. Obtained results are in line of Raju *et al.*, (2004), Vanaja and Luckins (2006), Das *et al.*, (2007), Sarkar *et al.*, (2007) and Abdual (2008).

Grain quality parameters in this segregating population were also recorded as the same trend of inheritance of GPC and recorded almost same as the BPT – 5204 characteristics, which encourages us for further development in these lines.

Moderate to higher variability (PCV and GCV), h^2 and genetic advance indicating that additive gene action for these traits under consideration and selection will be effective.

Moderate to higher co-efficient of variation indicates more variability for the characters intern it will helps us to carryout the selection process effectively for most of the traits both in base as well as selected population. However, lower phenotypic and genotypic co-efficient of variation and higher heritability coupled with moderate to high GAPM was recorded for grain length and kernal length indicating that non-additive gene action for these traits under consideration and selection is not effective with low co-efficient of variation indicates less variability for the characters intern it can be used for exploitation of heterosis for this particular trait. Similar results were reported by Mini (1989), Das *et al.*, (2007) and Abdual (2008). However, Vanaja and Luckins (2006) reported low values of PCV and GCV for grain length.

Variation for major and minor nutrients

Since, population derived from *indica* parents, all micronutrients content were high in F₂ segregating lines. Similarly, higher micronutrient content was reported by Zeng *et al.*, (2005, 2006). They indicated that the micronutrients like zinc, iron, manganese, copper content were high in *japonica* followed by *indica* types.

Moderate to high phenotypic and genotypic variability, high heritability coupled with high genetic advance was observed for all nutrients studied except copper and manganese which were showed moderate heritability with moderate genetic advance. Hence, these indicates that the additive gene action playing for the traits, therefore selection is effective in these segregating population for nutrient parameters except for copper and manganese.

Variation for yield and yield attributing traits

The range in mean value reflects the extent of phenotypic variability present in breeding material. The values include genetic, environmental and genotype x environmental interaction components. So, the estimation of genetic (heritable) and environmental (non-heritable) components of the total variability was required to identify the probable parents. Thus, in the present study coefficient of variability, heritability and predicted genetic advance was compound in respect of growth, yield and its components.

The phenotypic coefficient of variation was comparatively higher than the corresponding genotypic coefficient of variation for the most of the morphological characters studied indicating significant genotype by environment (G X E) interactions. This difference between genotype and phenotype coefficient variations was relatively low for some of the characters. Higher heritability

coupled with moderate to higher GAPM recorded for all the parameters both in base as well as selected population indicating there is a potential to select good segregating lines for the trait under consideration, except days to maturity recorded lower GAPM. Recorded results are in the line of Nandarajan and Rajeshwari (1993) and Ahmed and Das (1994).

DNA marker validation for parental polymorphism

Molecular markers are efficient tools for selecting good genotype in plant breeding. Seventeen rice microsatellites markers specific to protein were already mapped in different mapping population by various workers (Wang *et al.*, 2008; Zhang *et al.*, 2008; Tan *et al.*, 2001). Utilization of already mapped specific markers for protein helps in selection of high protein alleles in the genotypes.

Totally 402 rice microsatellite (SSR) markers screened on BPT - 5204 and HPR-14 genotypes. The amplified products were resolved on 3% agarose and 12 % PAGE gel. The number of total and polymorphic bands generated on agarose and PAGE. Out of 402 markers, 69 were polymorphic on 3% agarose and 81 were polymorphic on 12% PAGE. On an average, 17.20 percent on 3 percent agarose and 20.00 percent polymorphism on PAGE (Table 3 and Fig. 3).

In conclusion the initial results on the segregation for protein content indicated 3.5-18.0 percent of protein variation among the 1267 F₂ segregating lines developed using BPT-5204 and HPR-14. And also the developed F₂ population is highly potential to develop high protein lines and showed clear cut segregation pattern for the trait under consideration and fine mapping can be done to select the high protein genotype.

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