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High protein rice: A source to protein energy malnutrition (PEM) in India

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Abstract
The nutritional well-being and health of people are vital prerequisites for the development of societies. The fortification of salt with iodine has been a global success story, but other micronutrient supplementation schemes have yet to reach vulnerable populations sufficiently. Malnutrition, with its 2 constituents of protein–energy malnutrition (PEM) and micronutrient deficiencies continues to be a major health burden in developing countries particularly in India. Globally, 740 million peoples are deficient in iodine, about 2 billion in zinc, 1 billion in iron and 250 million in Vitamin A deficiency, mainly young children's and pregnant women's in developing countries. In India, an additional concern is that many patients with severe malnutrition are also infected with HIV (Müller and Krawinkel, 2005). Therefore supplementing recommended amount of protein and nutrients in regular human diet is prerequisite. Protein is an important part of good nutrition at every meal. Vitamins and minerals can fulfill nutrient needs on a once-per-day basis but for protein the body has no ability to store a daily supply. Protein need become more important during periods of reduced food intake such as weight loss or during periods of recovery after illness or during aging. To maintain healthy muscles and bones for adults, at least 30 g of protein should be consumed at more than one meal (Donald, 2009). Rice is the staple food for more than half of the world's population and is a major source of *energy*, as well as containing essential amino-acids (Lysine, Tryptophan, Methionine, Leucine, Isoleucine and Valine) are essential to human health, but which the body cannot synthesize. Hence, enrichment of protein in rice would have a positive impact on millions of poor and malnourished people in developing countries particularly in India. Researchers have been trying to boost the protein content in rice for five decades, only few groups found success in the development of high protein rice. Total grain protein (TGP) content is the prime most important parameter in rice quality production, TGP is a complex trait controlled by several genes and also it is tightly linked with grain yield. Therefore, the segregating generations is a good breeding material to know the genetics of inheritance pattern of several traits as well as to break the tight linkage if it is required. Keeping these in mind, PhD research work was carried out (thesis submitted in 2010) with an objective of development of high protein rice (Bio fortification of rice - alleviating malnutrition through the introgression of high grain protein content). Recombinant inbred lines (RIL's) were developed with higher protein content of 12.55 % under aerobic situation using BPT 5204 as female parent (popularly grown fine grain variety in Karnataka, also called Sonamasuri) and HPR 14 as male parent (Local land race showing 14.1% of protein). We are able to boost around 4.5 % of total protein from present day cultivating varieties without affecting to the regular yield as much of BPT 5204 along with medium to fine quality as well as moderate to higher content of micronutrients (Iron, Zinc, Molybedinum and Copper) in the selected promising RIL's. We have also identified nine microsatellite SSR markers significantly associated with TGP as per regression method. The present outcome of the study will be helpful in developing countries particularly in India for children with PEM problems and pregnant women's, so pediatricians and obstetricians may prescribe the consumption of this variety of rice for regular consumption. Increase in quantity of rice production was made possible through the green revolution during 1960's and consequently it helped to alleviate hunger and poverty in developing countries of the world. Now, the world particularly developing counties like India urgently needs one more green revolution particularly quality green revolution to alleviate PEM and micronutrient deficiencies. Hence, different fields like agriculture and medicine as well as other related fields need to collaborate and work together in a forum to fight against malnutrition problems as it is a global issue.

Keywords: Total grain protein (TGP), High protein rice (HPR), Malnutrition, Protein energy malnutrition (PEM), Rice

Introduction

Importance of Protein in human diet

Grain protein content and nutrients in rice are important parameters in normal human diet and also are essential for balanced nutrition in plants and animals (Welch and Graham, 1999; Graham *et al*., 2000) [33, 9]. Micronutrients also play vital role in abiotic stress tolerance in rice plants. Protein deficiency and amino acid imbalance are known to cause specific health disorders and to affect growth and brain development. Human protein requirements are met

mainly from plant sources. While, animals contribute only a small fraction of the total need. Further, plant proteins are cheaper and easier to transport than animal's protein; the latter; however have a well-balanced amino acid composition, while plant protein does not. Therefore considerable effort has been directed at improving the protein content and quality of food crops. Green revolution laid emphasis on increased grain yield, which led to reduced availability of micronutrients in food (Welch *et al*., 2000) [34]. This factor combined with the poor bio-availability of micronutrients is one of the main reasons for malnutrition of major rice consumers of Asia. Malnutrition and micronutrients deficiency causes several problems in developmental stages of children as well as in pregnant women's.

Malnutrition problems in India and developing countries

Poverty is the main underlying cause of malnutrition and its determinants. The degree and distribution of protein–energy malnutrition (PEM) and micronutrient deficiencies in a given population depends on many factors: the political and economic situation, the level of education and sanitation, the season and climate conditions, food production, cultural and religious food customs, breast-feeding habits, prevalence of infectious diseases, the existence and effectiveness of nutrition programs and the availability and quality of health services. It is globally the most important risk factor for illness and death, with millions of pregnant women and young children particularly affected. Apart from marasmus and kwashiorkor (the 2 forms of PEM), deficiencies in iron, iodine, vitamin A and zinc are the main manifestations of malnutrition in developing countries. In these communities, a high prevalence of poor diet and infectious disease regularly unites into a vicious circle. Although treatment protocols for severe malnutrition have in recent years become more efficient, most patients (especially in rural areas) have little or no access to formal health services and are never seen in such settings. Interventions to prevent PEM range from promoting breast-feeding to food supplementation schemes, whereas micronutrient deficiencies would best be addressed through food-based strategies such as dietary diversification through home gardens and small livestock.

Rice may be a source to fight against malnutrition

Rice provides more calories per hectare than any other cereal crop. At the average world yields, a hectare of rice could sustain 5.7 persons for a year compared with 5.3 for maize and 4.1 for wheat. The nutritional level of rice is high among cereals and other grains. Among cereals, it has a comparatively high content of essential amino-acids. Though the protein content of rice is lesser than that of wheat, the true digestible protein and the biological value of rice protein are the highest in comparison to wheat and other cereals. Rice grain contains hundred percent edible portion, and 6.8g of protein, 0.5g of fat, 78.2g of carbohydrate and 345 K cal of energy per hundred grams of rice (Juliano, 1985) [12].

Rice is a major protein source for most of the Asian rice growing countries. Rice protein is superior in lysine content to wheat, corn and sorghum (Hegested, 1969) [10] Rice has more balanced amino-acid profile. High-protein rice has the potential to enhance nutrition in poor rural families where rice serves as the staple food (Li *et al.,* 2004) [15]. Furthermore, it should be noted that the bran and outer layers of rice grain often are removed by grinding to meet the market demand for polished rice. Therefore, if a variety of rice contains protein only in the bran or outer layers of the grain, the protein content is actually discarded rather than used. Therefore, the main target has been to improve the quantity and nutritional quality of the protein in rice.

Materials and Methods

Work plan

Current research work was planned with an objective of Biofortification of rice - alleviating malnutrition through the introgression of high grain protein content. As rice is staple food for more than 50% of the world's population. Its higher yield and better taste are two major subjects for many breeding programs but, in contrast to disease and insect resistance, grain yield and quality are both controlled by quantitative trait loci (QTLs) showing continuous phenotypic variation in rice progeny (Yano and Sasaki, 1997). It is thus difficult for breeders to improve rice grain yield along with quality using conventional methods, due to lack of discrete phenotypic segregation in the progeny. As rice grain quality (includes TGP) is an endosperm trait, its inheritance can be more complicated because the genetic expression of an endosperm trait in cereal seeds is conditioned not only by the triploid endosperm trait endosperm genotype, but also by the diploid maternal genotype and other additional possible cytoplasmic differences (Pooni *et al.* 1992; Zhu and Weir, 1994; Mo, 1995) [21, 39, 17]. The nutritional quality (macro and micronutrients) parameters are an immense importance to the human diet. As India is suffering from PEM, present work was undertook to develop high protein rice (HPR). Hence the present study will help the Indian people (particularly low socio-economic condition population) to consume required amount of protein in their normal diet.

Study Location, Population, Observations, Design, and Analysis

Experimental Site and season

The experiments were conducted in different locations and seasons as presented in table 1.

Table 1: Experimental Site and season in which experiment was undertaken

Generation	Season and year	Experimental site
F_1	Summer 2006	Green House, MAS LAB, GKVK, UAS, Bengaluru.
F_2	*Kharif* - 2006	Farmer's field, Devanahalli, Bengaluru North Taluk.
F_3	Summer – 2007	Farmer's field, Devanahalli, Bengaluru North Taluk and Farmer's field, Vittarahatanahalli Village, Tumkur Taluk & district.
F_4	Summer – 2008	ZARS, Babbur farm Hiriyur.
F_5	*Kharif* – 2008	K-Block, GKVK, UAS, Bengaluru.
F_6	Summer - 2009	K-Block, GKVK, UAS, Bengaluru

The experiments was laid out in augmented design at different sites as shown in table 1 and the observations were recorded on selected individual plants, used for statistical analysis. 21 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 practiced to ensure uniform crop stand as per the package of practices (Anon, 2007) [3].

Plant materials

Based on the objective of biofortification of rice, Recombinant Inbred Lines (RILs) mapping population was developed using two diverse parents *viz*., BPT – 5204 as female parent (high yielder, low protein content and fine grain) crossed with HPR – 14 (high protein land race from south India) as male parent. Salient features of these varieties are presented in table 2.

Table 2: Salient features of parents selected for the present study

Character	BPT - 5204	HPR – 14
Parent	Female	Male
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	Fine	Bold
Protein content	Low (7.00 to 8.10)	High (13.9 to 14.1)

BPT - 5204 possesses many important quality parameters which are preferred by the consumers. HPR 14 on the other hand is an early-medium maturing with high grain protein content and also moderately resistant to drought. This provides an opportunity to transgress alleles that could generate a range of recombinants and broad spectrum of genetic variability that is required for mapping the trait of interest.

Observations recording

1. Phenotypic characterization

One thousand two hundred and sixty seven segregating lines were developed in F_2 and further in F_3, 3604 lines were generated by selfing and forwarded for RIL development (Fig 1), were evaluated for various phenotypic characters are listed below. Among these base population (date not shown) a subset of one hundred lines were selected based on individual plant yield and segregating patterns for quality parameters (data presented).

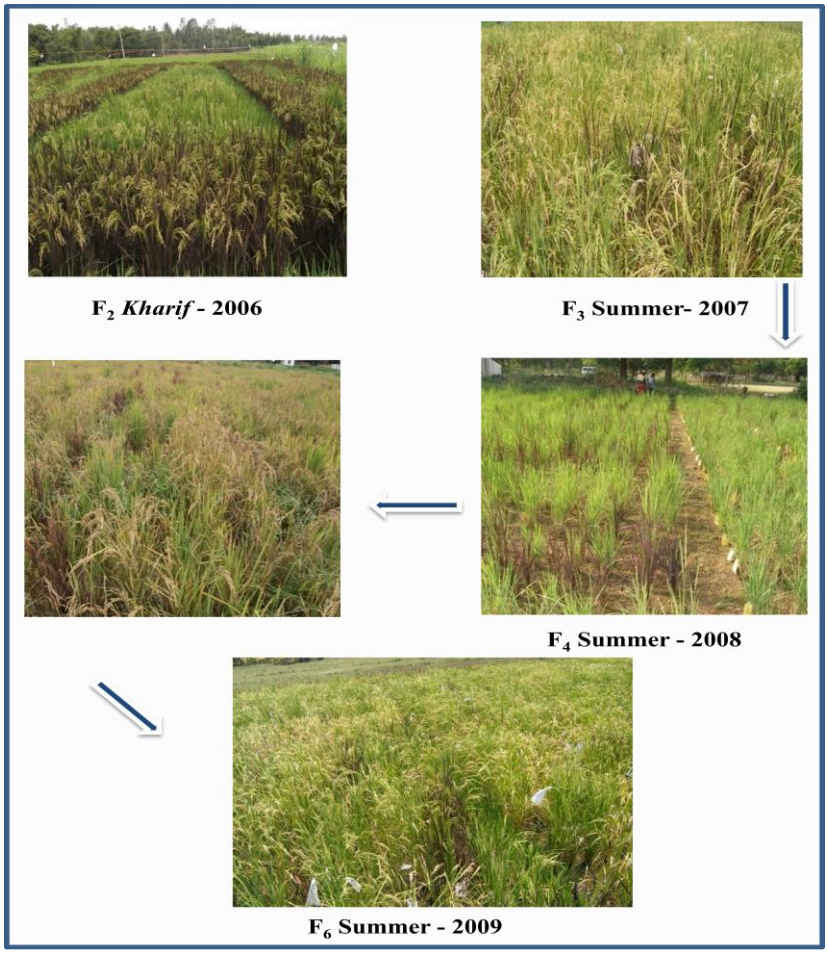


Fig 1: RILs development flow chart at reproductive stage (Field view)

Morphological characters

Morphological and grain yield attributing characters such as, days to 50 per cent flowering (DF- number of days from sowing to opening of first flower of plant), days to maturity (DM- number of days from sowing to harvesting), Plant height (cm- total height from the base of the plant to the tip for the main panicle), Biomass (g- total weight of straw was considered as total biomass weight per plant), number of

tillers (NOT- by counting tillers bearing panicle at the time of harvest), number of productive tillers (NPT- by counting panicle bearing tillers), panicle length (cm- measured from its base to tip excluding awns), seed fertility percentage (%- by counting number of filled grains per panicle), grain yield (GY-in gram, by weighing total weight of all the filled grains per plant), test weight (TW- 1000 grains were counted and their weights were recorded in grams as 100 grain weight), harvest index (HI- computed by grain yield to biological yield of a plant) recorded as suggested by Donald, 1962 [6].

Grain quality characters

Grain quality parameters such as protein [%- determined by Standard micro Kjeldhal method for F2 and F3 samples and remaining samples were analyzed in Near infrared reflectance spectroscopy (NIR system, FOSS, Denmark)], grain length (mm- measured by arranging length wise ten paddy grains), grain breadth (mm- measured by arranging breadth wise ten paddy grains), grain length: breadth ratio (grain L: B- obtained by dividing the length of each grain by its corresponding breadth), kernel length (mm- measured by arranging length wise ten rice grains), kernel breadth (mm- measured by arranging breadth wise ten rice grains), kernel length: breadth ratio (kernel L; B- obtained by dividing the length of each grain by its corresponding breadth) and visual score (1 to 5) of grains were taken phenotypically by observing seeds like, 1 is for BPT- 5204 types, 2 for Slender grains like BPT – 5204, 3 for Intermediate types, 4 for Bold seeded grains like HPR – 14 and 5 for HPR - 14 types.

Major and micro nutrient

Nutrient parameters such as nitrogen (%-determined by Standard micro Kjeldhal method), phosphorous %- estimated by vanodomolybdophosphoric yellow colour method (Jackson, 1973) [11], potassium (%- Jackson, 1973) [11], copper (ppm), zinc (ppm), manganese (ppm) and iron (ppm) were recorded along with parents (Micronutrients like Zn, Fe, Cu and Mn were estimated by feeding the digested extract after suitable dilutions, using Atomic Absorption Spectrophotometer (Perkin Elmer model Analyst-400) for F2 and F3 samples and remaining samples were analyzed in Near infrared reflectance spectroscopy (NIR system, FOSS, Denmark).

2. Genotypic characterization

Parental polymorphism survey and SSR marker validation

402 Simple Sequence Repeats (SSRs) were checked for parental polymorphism on Agarose Gel Electrophoresis (AGE) and Poly Acrylamide Gel Electrophoresis (PAGE). After determining polymorphism between parents, eighteen SSR markers validated on the F_6 population.

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 [29]. Linear regression (One way ANOVA) and t- test (Sokal and Rohlf, 1995) [28] were used to test the significant association of trait with the marker for eighteen SSR markers validated on the F_6 population.

Results and Discussion

Conventional breeding for quality improvement is time consuming and difficult. Since protein content is polygenic character and environmental influence on the trait is very high. DNA markers linked to protein content help in screening of large number of genotypes with a short span of time for the trait of interest in seedling stage itself. Microsatellite markers are highly polymorphic, not affected by the environment and co-dominant in nature. Utilization of already mapped specific markers linked to protein content helps in selection of high protein alleles in the genotypes.

Estimation of TGP, nutrients and grain quality parameters

The phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) 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 (h^2) in broad sense coupled with higher genetic advance as percent mean (GAPM) indicated the more of additive gene action with fast and effective selection for the trait under consideration.

The wide range of TGP content in selected F_2 hundred lines was recorded with a range of 5.25% to 22.83% with an average of 12.01% and moderate PCV (19.57%) and GCV (15.63%) with high h^2 of 63.79% coupled with high GAPM of 25.72% was recorded. However, in F_6 generation, it was ranges from 8.44% to 12.55% with an average of 10.39% and the lowest PCV (6.57%) and GCV (6.24%) with high h^2 (49.13%) coupled with moderate GAPM of 10.83% Table 3). TGP content shows decrease trend of mean variability, heritability coupled with genetic advance from selected lines of F_2 to F_6 segregating lines were recorded. Whereas, h^2 was decrease from F_2 to F_5 but in case of F_6 it showed higher h^2 indicating that the improved h^2 could be because of increasing homozygosity for alleles segregating for TGP content in these segregating lines. This higher h^2 coupled with higher GAPM in all selfing generation indicating that more of additive gene action and selection is effective for the trait under consideration. The distribution of TGP content in the selected RILs (segregating lines) showed almost a normal curve indicating that both additive and non-additive genes were segregate in equal amount for the expression of trait, providing a fast and effective selection of this trait in the population (fig 2). Obtained results are with the lines of Raju *et al*. (2004) [22], Vanaja and Luckins (2006) [31], Das *et al*. (2005) [5], Sarkar *et al*. (2007) [24].

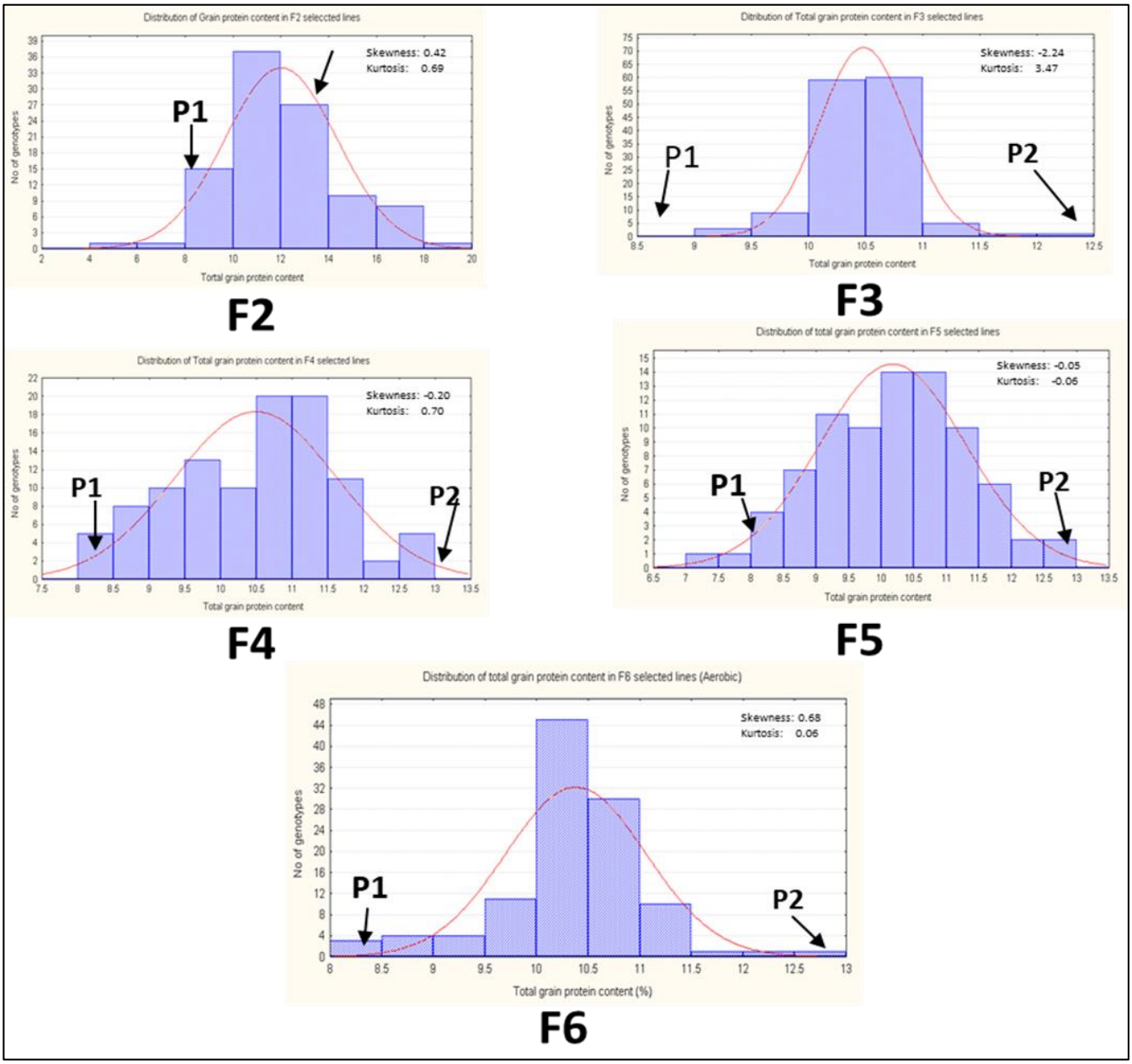


Fig 2: Frequency distribution in RILs of BPT – 5204 X HPR – 14 for TGP content in segregatin population

In successive generations of RILs development, variability (PCV and GCV), and h^2 coupled with GAPM showed almost decreased trend from F_2 to F_6 generations for all major (Nitrogen, phosphorus and potassium) and micro (Copper, Manganese, Zinc and Iron) nutrients. PCV and GCV, h^2 coupled with GAPM showed high value for all nutrient parameters studied indicating more of additive gene action and that selection is effective. However, a little variation of increase or decrease in F_5 and F_6 generation observed could be due to genotype and environment (G × E) interaction during the crop growth period. Normal distribution for nitrogen, potassium, copper, manganese and iron indicated both additive and non-additive gene action for the traits considered indicating that selection for this trait is fast and effective; whereas, for phosphorous and zinc, platykurtic and left skewed distribution indicating that the involvement of less number of segregating genes with some of them showing decreasing effects for the trait under consideration. Similar results were obtained by Zeng *et al*. (2006) [37], Abdual (2008) [1]. Olivera *et al*. (2009) [20] indicated that nutrient content was high in *Japonica* when compared to Indica types.

In accordance with the TGP content, other grain quality parameters like grain breadth, grain L: B ratio, kernal length and kernal L: B ratio also recorded mean decrease in trend from F_2 to F_6 segregating generations. Higher variability (PCV and GCV) coupled with higher genetic parameters (h^2 and GAPM) indicate more of additive gene action for these traits under consideration and that selection will be more effective. In F_6 selected lines, leptokurtic and right skewed distribution for grain breadth and kernal breadth indicated the involvement of more number of segregating genes with a majority of them having increasing effects. The selection is more effective as variability (PCV and GCV) and h^2 coupled with GAPM recorded moderate to high. For grain L: B ratio and kernal L: B ratio, platykurtic and left skewed distribution indicated the involvement of less number of genes with decreasing effects with higher variability (PCV and GCV) and h^2 coupled with GAPM indicating more of additive gene action for selection of trait under consideration. Present findings are in accordance with Roy *et al*. (2001) [23], Chand *et al*. (2004), Vanaja and Luckins (2006) [31], Das *et al*. (2005) [5] and Sarkar *et al*. (2007) [24].

Lower PCV and GCV and higher h^2 coupled with moderate to high GAPM was recorded for grain length and kernal length indicating non-additive gene action for these traits under consideration and selection is not effective with low co-efficient of variation indicating less variability of the characters. Thus, it can be used for exploitation of heterosis for this particular trait. However, in F_5, higher variability, h^2 coupled with moderate to high GAPM were recorded which could be due to more of genotype and environment (G × E) interaction prevailing during the crop growth period.

Table 3: Estimated genetic parameters for total grain protein content and nutrient parameters in segregating generations (RILs) of BPT – 5204 X HPR – 14 in selected lines

Generations	Genetic parameters	TGP (%)	N (%)	P (%)	K (%)	Cu (ppm)	Mn (ppm)	Zn (ppm)	Fe (ppm)
F2	Mean	12.01	1.98	0.16	0.15	5.56	7.73	26.74	42.99
	Range	5.25 - 22.83	0.73 - 2.96	0.07 - 0.27	0.08 - 0.27	3.31 - 15.50	3.69 - 11.29	2.88 - 30.17	24.14 - 61.43
	PCV (%)	19.57	48.69	26.59	28.46	22.65	19.36	26.72	26.15
	GCV (%)	15.63	47.28	26.08	27.82	18.20	17.40	26.14	25.61
	h2 (%)	63.79	98.98	85.00	90.00	64.56	80.81	95.69	95.87
	GAPM (%)	25.72	49.94	46.56	52.76	38.13	32.23	52.67	51.65
F3	Mean	11.49	1.21	0.15	0.12	5.39	7.91	26.23	45.61
	Range	7.10 – 18.06	0.84 – 2.20	0.11 – 0.21	0.16 - 0.25	3.18 - 17.28	5.80 - 16.54	6.62 - 37.8	23.21 - 65.40
	PCV (%)	17.25	49.96	31.73	28.00	23.20	16.43	24.51	25.21
	GCV (%)	12.81	48.90	27.00	27.72	22.87	13.00	21.45	23.31
	h2 (%)	67.10	98.33	89.90	99.33	67.22	80.00	98.00	93.00
	GAPM (%)	29.35	40.43	44.83	52.47	46.46	33.36	53.80	41.08
F4	Mean	10.46	1.44	0.13	0.13	5.88	7.24	20.30	49.59
	Range	8.15 - 12.92	0.89 - 2.56	0.07 - 0.22	0.05 - 0.24	3.58 - 17.58	3.60 - 10.35	8.64 - 30.51	28.62 - 70.38
	PCV (%)	10.70	42.83	29.00	35.81	24.03	12.39	25.66	21.32
	GCV (%)	7.36	41.02	25.81	23.78	11.41	11.26	25.15	20.81
	h2 (%)	57.33	91.74	89.09	90.79	66.07	82.60	96.06	95.33
	GAPM (%)	10.43	40.64	47.25	55.97	39.10	31.36	50.78	41.86
F5	Mean	10.16	1.97	0.12	0.12	5.89	7.40	17.36	50.51
	Range	7.39 - 12.81	0.89 - 2.32	0.03 - 0.24	0.05 - 0.26	3.58 - 17.58	5.94 - 16.94	8.64 - 30.51	28.62 - 68.84
	PCV (%)	11.07	37.49	35.33	24.18	22.98	12.18	26.15	20.13
	GCV (%)	7.65	33.98	27.64	22.19	10.09	11.03	26.09	19.61
	h2 (%)	57.83	86.13	86.48	89.25	60.43	79.71	99.49	94.95
	GAPM (%)	10.90	39.85	47.05	52.97	36.16	30.64	53.60	39.37
F6	Mean	10.39	1.36	0.40	0.19	5.52	7.86	15.50	55.73
	Range	8.44 - 12.55	0.84 - 2.58	0.07 - 0.21	0.13 - 0.23	3.40 - 17.87	3.66 - 16.80	4.58 - 37.8	24.67 - 66.43
	PCV (%)	6.57	32.30	20.23	24.66	20.07	12.53	27.80	22.58
	GCV (%)	6.24	30.77	20.19	23.94	16.89	9.18	24.85	22.03
	h2 (%)	49.13	83.54	89.63	85.00	60.00	77.60	94.44	95.11
	GAPM (%)	10.83	38.50	42.16	53.12	27.57	30.26	44.51	32.09

Table 4: Estimated genetic parameters for grain quality parameters in segregating generations (RILs) of BPT – 5204 X HPR – 14 in selected lines

Generations	Genetic parameters	GL	GB	GLBR	KL	KB	KLBR
F2	Mean	6.88	2.92	2.58	5.51	2.02	2.71
	Range	5.60 - 7.90	2.0 - 3.6	2.00 - 3.65	4.1 - 6.0	1.60 - 2.50	1.96 - 3.65
	PCV (%)	18.79	23.21	25.50	8.13	10.52	11.95
	GCV (%)	13.50	18.45	14.42	7.69	9.28	10.11
	h2 (%)	96.71	76.52	22.50	89.64	77.78	71.43
	GAPM (%)	24.38	13.06	18.23	15.01	18.85	27.59
F3	Mean	6.53	2.81	2.55	5.40	1.99	2.78
	Range	5.00 - 7.40	1.6 - 3.2	1.79 - 3.31	4.0 - 5.6	1.10 - 2.50	1.96 - 4.82
	PCV (%)	18.28	22.26	25.23	7.84	12.09	15.11
	GCV (%)	14.96	19.43	17.85	7.35	11.24	13.93
	h2 (%)	90.00	71.14	26.67	88.89	83.33	83.33
	GAPM (%)	22.70	15.15	18.37	14.35	20.75	25.95
F4	Mean	6.80	2.80	2.48	5.17	1.94	2.68
	Range	6.10 - 7.10	2.1 - 3.3	1.66 - 3.50	4.5 - 6.5	1.50 - 2.30	2.23 - 3.42
	PCV (%)	17.81	25.43	26.38	7.32	10.51	10.78
	GCV (%)	14.77	19.52	19.89	6.88	8.00	8.62

	h2 (%)	97.23	76.77	36.49	88.15	75.74	74.02
	GAPM (%)	25.99	24.40	12.31	13.30	18.84	24.21
F5	Mean	6.68	2.83	2.49	5.41	2.01	2.73
	Range	4.00 - 7.00	2.0 - 3.2	1.38 - 3.45	4.1 - 6.8	1.10 - 2.50	1.96 - 4.82
	PCV (%)	19.34	26.15	24.70	7.96	11.18	14.11
	GCV (%)	17.00	21.23	22.19	7.59	10.01	12.60
	h2 (%)	98.20	74.79	37.66	90.83	80.19	79.71
	GAPM (%)	27.22	18.74	14.25	14.90	18.47	23.17
F6	Mean	5.97	2.56	2.41	5.30	2.01	2.75
	Range	5.60 - 6.70	1.4 - 3.2	1.75 - 3.71	4.2 - 6.1	1.10 - 2.42	2.00 - 4.82
	PCV (%)	17.00	26.21	24.26	7.46	12.00	14.72
	GCV (%)	16.80	24.23	20.01	6.98	10.91	13.32
	h2 (%)	94.29	76.88	37.93	87.80	82.76	81.71
	GAPM (%)	22.73	24.20	11.91	12.69	18.92	23.29

Table 5: Estimated genetic parameters for yield and yield attributing parameters in segregating generations (RILs) of BPT – 5204 X HPR – 14 in selected lines

Generations	Genetic parameters	DF	DM	PH	Biomass	SFP	GY	TW	HI
F2	Mean	120.96	162.92	85.36	43.64	85.05	25.17	20.78	0.15
	Range	95 - 158	137 - 189	61 - 113	16.25 - 144.00	65.52 - 99.10	2.2 - 31.59	11.7 - 24.2	0.05 - 0.41
	PCV (%)	8.97	6.82	16.88	36.10	18.26	38.27	20.04	40.00
	GCV (%)	7.42	5.81	16.16	35.91	17.51	36.63	18.00	38.49
	h2 (%)	68.43	64.05	91.63	88.92	91.90	94.19	60.87	70.00
	GAPM (%)	12.65	8.83	21.86	63.57	34.57	44.67	25.13	32.73
F3	Mean	124.70	163.25	89.83	40.27	81.29	20.75	16.18	0.31
	Range	111 - 162	149 - 199	70 - 110	14.50 - 100.00	51.48 - 98.7	11.4 - 28.97	12 - 20.8	0.22 - 0.45
	PCV (%)	7.66	5.91	11.16	34.56	17.98	36.19	17.36	41.10
	GCV (%)	5.89	4.43	10.14	32.20	17.37	30.04	14.05	32.44
	h2 (%)	59.20	56.19	82.69	86.80	93.30	83.29	59.24	62.29
	GAPM (%)	10.34	6.84	19.00	61.80	34.55	41.40	24.34	30.28
F4	Mean	123.58	161.34	87.10	30.76	84.62	24.65	18.89	0.34
	Range	111 - 162	125 - 199	60 - 125	15.00 - 75.00	52.27 - 99.78	5.04 - 30.98	16.40 – 21	0.12 - 0.49
	PCV (%)	6.33	5.43	11.11	31.92	14.69	23.47	17.02	33.68
	GCV (%)	3.96	3.72	10.33	26.03	13.99	20.67	14.05	28.37
	h2 (%)	39.19	46.88	81.99	76.49	90.73	72.29	55.05	44.55
	GAPM (%)	9.31	5.24	18.21	48.72	27.45	30.89	23.76	22.32
F5	Mean	113.41	162.00	85.47	30.01	79.47	22.67	19.36	0.45
	Range	95 - 141	112 - 181	58 - 99	20.00 - 63.00	45.35 – 98.00	17.91 - 27.10	13 - 22	0.05 - 0.49
	PCV (%)	6.11	7.01	12.63	29.49	12.08	24.29	14.55	32.69
	GCV (%)	5.65	6.11	11.49	25.64	11.10	23.58	12.25	30.81
	h2 (%)	35.39	50.65	82.82	75.58	84.47	74.18	49.99	43.73
	GAPM (%)	8.89	5.39	21.54	45.91	21.02	32.61	19.12	23.27
F6	Mean	118.36	162.65	84.73	31.88	82.35	24.15	18.48	0.37
	Range	100 - 123	149 - 199	52 - 100	14.50 - 90.00	45.83 – 98.00	12.87 - 28.70	12.00 - 22.20	0.22 - 0.50
	PCV (%)	6.67	5.86	11.85	30.82	13.37	28.47	15.17	31.24
	GCV (%)	5.18	4.36	10.78	26.23	12.56	22.10	13.77	27.48
	h2 (%)	39.69	50.24	82.76	77.46	88.19	76.19	50.29	41.22
	GAPM (%)	9.42	5.44	20.46	42.09	20.43	35.35	20.24	21.34

Estimation of yield and yield attributing parameters

Availability of genetic variability is prerequisite. Yield being a quantitative character mainly influenced by large number of genes that are greatly controlled 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 (GA). The estimation of these variability parameters helps the breeder in achieving the preferred crop improvement by selection.

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. As yield is the

prime most important factor in the entire breeding programme. In the present study along with the TGP, grain quality and nutrient parameters yield also considered significantly to get at least as BPT – 5204.

Low PCV and GCV were observed for days to 50% flowering and days to maturity in all the segregating generations and decreasing trends were observed as the generation is forwarded towards F_6 and moderate h^2 coupled with low GAPM indicating more of non-additive gene action. Hence, further it can be used for exploitation of heterosis. Leptokurtic and right skewed distribution for days to 50% flowering in F_6 selected lines indicated that involvement of fewer segregating genes with majority having decreasing effects. Hence, more number of genes segregates for this trait indicating that selection is more effective. Platykurtic and left skewed distribution for days to maturity in F_6 selected lines indicated the involvement of more number of minor genes segregates with majority of them having increased effects. Hence, the selection will not be more effective. In other words it indicates slow selection response. These low co-efficient of variation indicates less variability for the characters. Similar observations were reported by Karim *et al* (2007) [13] and Abdual (2008) [1].

Moderate PCV and GCV high h^2 coupled with high GAPM were recorded for plant height, panicle length and seed fertility percentage in all the segregating generations except in case of F_5 generation, it could be due to more genotype and environment (G × E) interaction during the more of additive gene action and selection is effective. Leptokurtic and right skewed distribution for plant height, panicle length and seed fertility percentages in F_6 selected lines indicated the involvement of fewer segregating genes with major decreasing effects. Hence, more number of genes segregates for this trait indicating that selection is more effective. The obtained results are in line with the observations of Shivapriya (2000) [25], Manjunatha (2003) [16], Ganapathy *et al*. (2007) [8] and Abdual (2008) [1]. High PCV and GCV was observed for Biomass, number of tillers, number of panicles and grain yield per plant indicating considerable amount of variability for these characters among the inbreeding generations. High h^2 coupled with high GAPM indicated more of additive gene action for the gene segregation of these characters and that selection is effective. Platykurtic and left skewed distribution for Biomass and grain yield per plant in F_6 selected lines indicated the involvement of more segregating genes with a majority of them having increased effects. Hence, more number of minor genes segregates for these traits and the selection will not be more effective. In other words it indicates slow selection response. Normal distribution for number of tiller and number of panicles indicated the provenance of both additive and non-additive gene action. Hence the selection of these characters in these segregating generations is effective. Present findings supported by the results obtained by Kumar *et al*. (2001) [14], Sinha *et al*. (2004) [27] and Sharma and Sharma (2007) [26]. Low PCV and GCV for test weight and harvest index were recorded indicating that narrow range of variability found for these traits and lower heritability coupled with low GAPM for these traits except for harvest index in selected lines indicated that the non-additive gene action. Hence, the selection will not be effective for these characters in this segregating generation. However, contrasting results reported for harvest index by Nath and Tulukardar (1997) [19] and supporting results reported by Sharma and Sharma (2007) [26].

Top five high protein lines were identified *viz*., HPR – 89-2 (12.55%), HPR-2-2-1 (12.19), HPR – 53-1 (1) (11.86%), HPR- 17 (11.47%) and HPR- 49-1 (11.45%) under aerobic situation (table 6). These lines can be further forwarded and tested in multi-location trials (MLTs), and then the best performing lines will be released as high protein rice variety in suitable zones/areas.

Table 6: High protein lines identified under aerobic situation in recombinant inbred lines

Genotypes	TGP (%)	GYP (g)	G (cm)	GB (cm)	GLBR	KL (cm)	KB (cm)	KLBR	DF	DM	PH (cm)	N (%)	P (%)	K (%)	Zn (ppm)	Fe (ppm)	VS
HPR - 89-2	12.55	35.91	6.1	2.5	2.44	5.5	2.1	12.55	112	151	85	1.78	0.14	0.14	5.84	65.55	3
HPR - 2-2-1	12.19	22.87	6.2	2.1	2.95	5.5	2.1	12.19	120	166	82	2.49	0.24	0.22	9.91	29.72	4
HPR - 53-1(1)	11.86	23.63	6.6	3.1	2.13	5.2	1.7	11.86	115	154	85	2.41	0.15	0.15	7.56	25.64	3
HPR - 17	11.47	24.00	5.8	3.2	1.81	5.4	2.1	11.47	110	199	89	2.39	0.14	0.34	4.69	55.92	2
HPR - 49-1	11.45	28.76	6.3	2.6	2.42	5.3	1.9	11.45	120	164	70	1.13	1.65	0.6	28.58	44.92	3

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 (%) VS – Visual score
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)

DNA marker validation

Molecular markers were efficient tools for selecting good genotype in plant breeding. The thirteen and 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) [32, 38, 30]. Utilization of already mapped specific markers for protein helps in selection of high protein alleles in the genotypes. The genotype showing HPR banding pattern with moderate yield can be selected and used in crop improvement programme.

DNA marker validation for parental polymorphism

Total of 402 rice microsatellite (SSR) markers used for screening BPT - 5204 and HPR - 14. The amplified products were resolved on 3% agarose and 12 % PAGE gel. Out of 402 markers, 69 were polymorphic on 3 percent agarose and 81 were polymorphic on PAGE. On an average, 17.20 percent on 3 percent agarose and 20.00 percent polymorphism on PAGE (Table 7 & Fig 3).

Table 7: 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

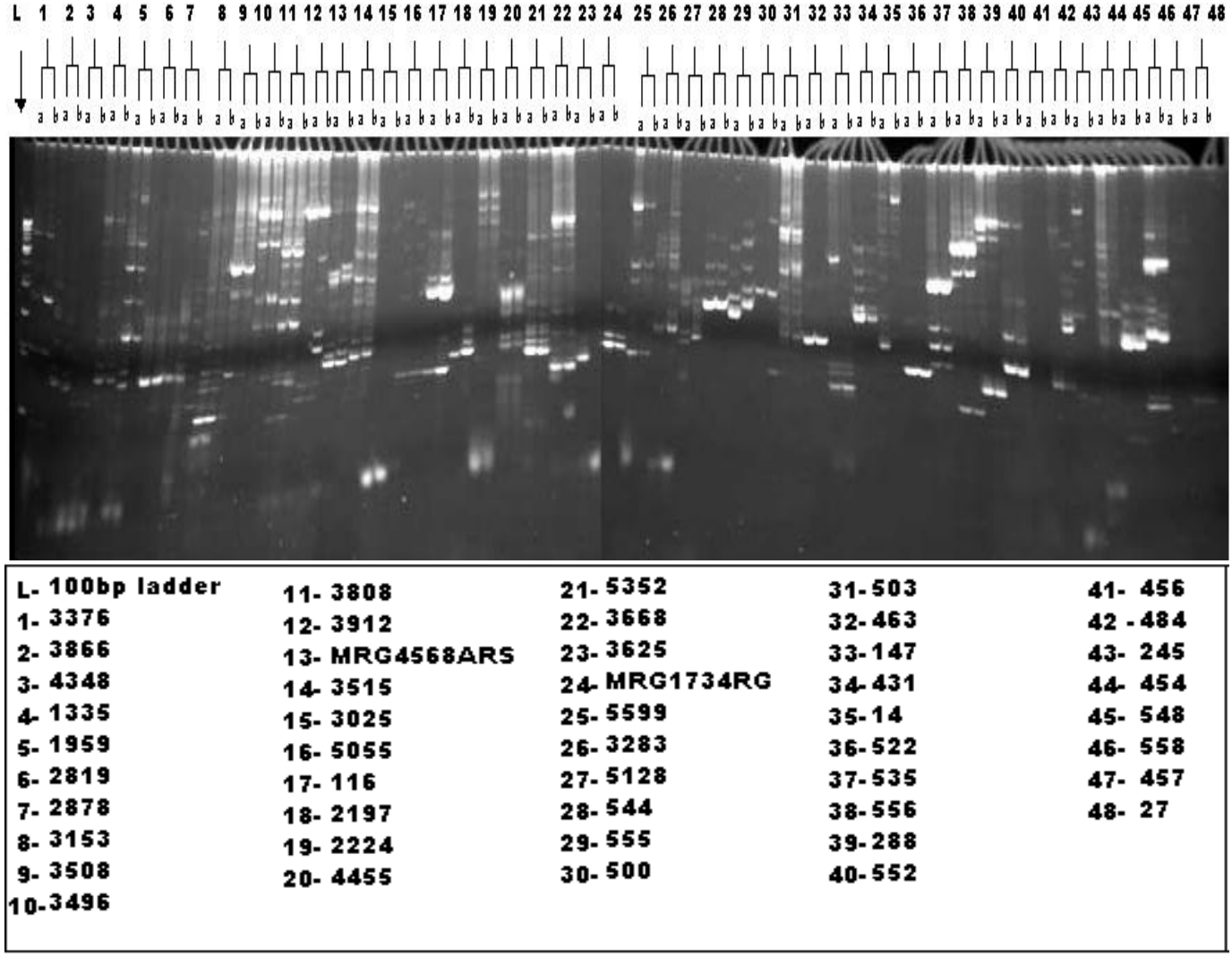


Fig 3: 9% PAGE gel of 1 X TBE for parental polymorphism BPT – 5204 and HPR – 14 genotypes using SSRs

DNA marker validation of protein specific DNA markers in recombinant inbred lines (RILs)

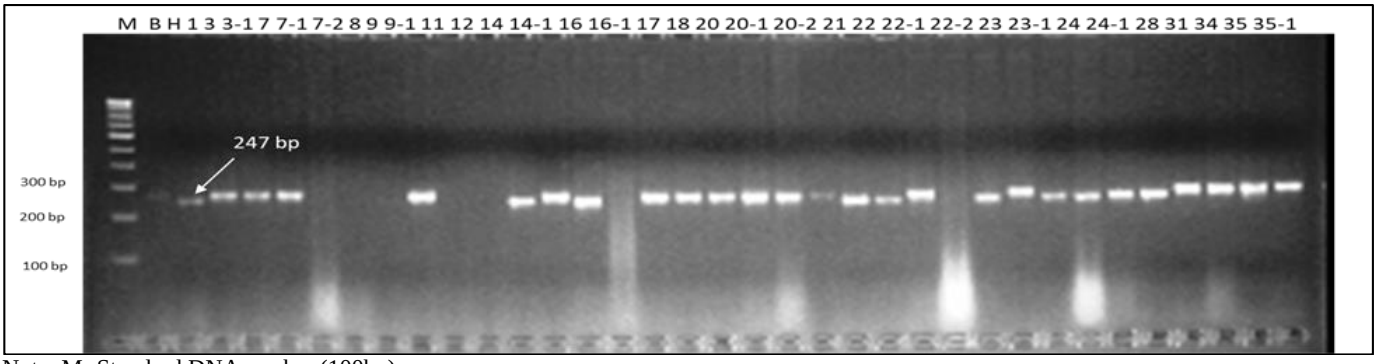
Selected segregating lines were used for the marker analysis with 18 primers (table. 8 & Fig 4). Marker analysis was done using single marker analysis with a help of linear regression method. Among 18 primers tested, nine were showing significant association with protein content using regression method. RM 253 had shown highest phenotypic variance (16.90%) with 0.360 additive effects, followed by RM 206 (13.52%), RM 228 (13.03%), RM 520 (10.00%), RM 555 (8.495%) and other markers did not showed significant association with protein content in this population. RM 253 located very near to a QTL qCP-2 as reported by Zhang *et al.* (2008) [38]. Whereas, Aluko *et al*. (2004) [2] reported 8.8 percent phenotypic variability in doubled haploid population. Whereas, Yoshida *et al.* (2002) [36] reported 12.4 percent and 11 percent phenotypic variation for protein content in rice. In accordance with protein QTL mapping, Wang *et al.* (2008) [32] reported 7.8 percent phenotypic variation by RM 520 using interval mapping in RILs. Whereas, we observed 10.00 % of phenotypic variation was observed in RILs of BPT – 5204 X HPR – 14.

RM228 significantly associated with TGP content with 13.03 percent phenotypic variation whereas, Tan *et al.* (2001) [30] reported epistasis with the other protein markers for the protein content. The phenotypic variability of 4.66% explained by RM 80 in RILs of BPT – 5204 and HPR - 14 genotypes whereas, non-significant results were reported by Wang *et al.* (2008) [32] for this marker. However, RM 555 showed 8.49 percent phenotypic variation and also the location of RM 555 was very near to a QTL qCP-2 as reported by Zhang *et al.* (2008) [38].

Table 8: Results of SSR markers analysis in RILs genotypes with reference to total grain protein content of cross BPT 5204 X HPR 14 in rice.

Sl. No	Markers name	Chromosome Number	R^2 (%)	Additive effect
1	RM 168	2	6.90*	0.215
2	RM 205	11	7.00**	0.225
3	RM 341	5	0.29	0.040
4	RM 520	8	10.00**	0.250
5	RM 206	9	13.52**	0.335
6	RM 253	11	16.90**	0.360
7	RM 447	6	1.00	0.075
8	RM 1369	6	0.20	-0.055
9	RM 1313	3	1.20	0.000
10	RM 209	10	1.87	0.020
11	RM 555	4	8.49**	0.240
12	RM 228	10	13.03**	0.335
13	RM 204	8	5.11	0.150
14	MRG2702	6	7.07**	0.220
15	RM 80	2	4.66	0.180
16	RM 304	3	0.09	0.010
17	RM 255	8	1.51	0.095
18	RM 6911	2	1.37	0.100

*- significant at 5%
**- significant at 1%
R^2- Phenotypic variability by the QTL



Note: M- Standard DNA marker (100bp)
B- BPT – 5204, HPR – 14

Fig 4: DNA amplified products of RM 520 for F_6 genotypes of BPT – 5204 X HPR – 14 cross and parents resolved on 3% agarose gel

Implications and Future Research

1. Identified genotypes for high TGP content further forwarded and tested in multiplication trial to confirm the present results and release as variety.
2. Government authorities to inform on high protein rice varieties to propagate and publicity of the high protein rice variety to farming community to cultivate this variety to replace the existing low protein rice varieties and to create the awareness to public for the consumption.
3. Pediatricians and obstetricians may prescribe this rice variety to mal-nourished [called as protein energy malnutrition (PEM)] peoples particularly for children's and pregnant women's.

Conclusion

The fortification of salt with iodine has been a global success story, but other micronutrient supplementation schemes have yet to reach vulnerable populations sufficiently. To be effective, all such interventions require accompanying nutrition-education campaigns and health interventions. Hence, interdisciplinary research approach (Agriculture, Medicine and other related disciplines) is needed to fight against malnutrition problems as it is a global issue.

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