Genetic variation in segregating generations of F5 and F6 in rice (*Oryza sativa* L.) for grain protein content (GPC), seed nutrient and yield

N Shashidhara and Shailaja Hitstalmani

Abstract

Protein is an important component in human nutrition and is superior in lysine content than wheat, corn and sorghum (Hegsted, 1969) in rice (*Oryza sativa* L.). Present investigation was carried out to increase total grain protein (TGP) content in rice using two diverse parents viz., BPT – 5204 (fine grain with low protein) and HPR – 14 (bold grain with high protein). In F5, protein was ranged from 7.10 to 18.06 percent, however it was decreased in F6 (8.15 to 12.92%). The wide range indicates that the alleles are segregating the traits under consideration and stabilizing in F6. The other quality (grain length, grain width, grain length to breadth ratio, kernel length, kernel breadth, and kernel length to breadth ratio), nutrients (nitrogen, phosphorous, potassium, zinc, manganese, copper and iron), yield and yield attributing (days to flowering, days to maturity, biomass, number of tillers, number of panicles, panicle length, seed fertility percentage, grain yield, test weight and harvest index) parameters also recorded the same trend as TGP.

Keywords: Protein, total grain protein (TGP), rice, *Oryza sativa*, segregating lines

Introduction

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 (Hegsted, 1969) Rice has a 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) [11]. Therefore, in the improvement of rice storage protein, the main target has been to improve the quantity and nutritional quality of the protein in rice. 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. Kido and Yanatori (1965) and Tanaka and Tamura (1968) discussed the relationship between the protein content of unpolished, i.e. brown rice and polished rice. They suggested that the protein content of polished rice was highly correlated with that of brown rice, but no further information has yet been found.

Materials and Methods

Plant Materials

With the intention of combining good grain qualities and high yielding ability characters of BPT 5204 with high protein content of HPR 14 and because of their diverse genetic background, these parents were used for developing mapping population.

<table>
<thead>
<tr>
<th>Character</th>
<th>BPT - 5204</th>
<th>HPR – 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Plant colour</td>
<td>Green</td>
<td>Purple</td>
</tr>
<tr>
<td>Leaf colour</td>
<td>Green</td>
<td>Purple</td>
</tr>
<tr>
<td>Sheath colour</td>
<td>Green</td>
<td>Purple</td>
</tr>
<tr>
<td>Plant stature</td>
<td>Short (60-70cm)</td>
<td>Tall (above 90cm)</td>
</tr>
<tr>
<td>Tillerling ability</td>
<td>High (20)</td>
<td>Low (10 - 16)</td>
</tr>
<tr>
<td>Number of panicles</td>
<td>More (15 - 18)</td>
<td>Less (10 - 14)</td>
</tr>
<tr>
<td>Grain yield</td>
<td>High (26g/plant)</td>
<td>Medium (23g/plant)</td>
</tr>
<tr>
<td>Grain type</td>
<td>Fine</td>
<td>Bold</td>
</tr>
<tr>
<td>Protein content</td>
<td>Low (7.00 to 8.10)</td>
<td>High (13.9 to 14.1)</td>
</tr>
</tbody>
</table>
Table 2: Experimental Site and season in which experiment was undertaken

<table>
<thead>
<tr>
<th>Generation</th>
<th>Season and year</th>
<th>Experimental site</th>
</tr>
</thead>
<tbody>
<tr>
<td>F6</td>
<td>Summer - 2009</td>
<td>K-Block, GKVK, UAS, Bengaluru (Aerobic situation)</td>
</tr>
</tbody>
</table>

The experiment was laid out in augmented design at experimental sites, GKVK, Bengaluru 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, 2003).

Fig 1: F6 field view at different stages at K-Block, GKVK, UAS, Bengaluru (Summer– 2009)

Phenotypic characterization of F2 segregating lines

3657 lines were evaluated for various phenotypic characters are listed below.

Morphological characters

Morphological and grain yield attributing 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 as suggested by Donald, 1962) [5].

Grain quality characters

Grain quality parameters such as protein [%- determined by Standard micro Kjeldhal method and also 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).

Major and micro nutrient

Nutrient parameters such as nitrogen (%-determined by Standard micro Kjeldhal method), phosphorous [%- estimated by vanadomolybdophosphoric yellow colour method (Jackson, 1973)], potassium (%- Jackson, 1973), 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).

Parental polymorphism survey

402 Simple Sequence Repeats (SSRs) were checked for parental polymorphism check 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 [20].

Results and Discussion
Evaluation of inbreeding generations (F5 and F6) of BPT 5204 X HPR 14 were undertaken during different growing seasons as presented earlier and the genetic variability parameters for grain quality like total grain protein percentage, grain length (cm), grain breadth (cm), grain LB ratio, rice kernel length (mm), breadth (mm), rice kernel length to breadth ratio presented herein.

Estimation of genetic variability parameters in selected lines
Estimation of total grain protein content, major and micro nutrients
Grain protein content and nutrient parameters in rice are important parameters in normal human diet. Grain protein and micronutrients are essential for balanced nutrition in plants and animals (Welch and Graham, 1999; Graham et al., 2000) [7, 24]. Micronutrients also play vital role in abiotic stress tolerance in rice plants. Green revolution laid emphasis on increased grain yield, which led to reduced availability of micronutrients in food (Welch et al., 2000) [25]. 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.

The mean percent total grain protein (TGP) content in F5 was 10.16 percent with an average of 7.39 to 12.81 percent and the moderate phenotypic coefficient of variation (PCV) was 11.07 percent and lower genotypic coefficient of variation (GCV) was 7.65 percent with higher percent heritability (h2) of 57.83 percent coupled with moderate genetic advance as percent mean (GAPM) of 10.90 percent in selected lines. Whereas, in case of F6 generation, mean TGP was decrease up to 10.39 percent with an average of 8.44 to 12.55 percent and lower PCV (6.57%), GCV (6.24%) and moderate h2 (49.13%) coupled with lower GAPM (10.83%). This little increase in protein percentage could be due to environmental influence prevailing during the crop growth period as well as soil conditions in which crop established otherwise this little difference indicates that the population is stabilizing for the trait under consideration. From the estimates of h2 and GAPM, indicated that the protein content mainly controlled by additive gene action and higher heritability coupled with higher GAPM in selfing generations indicating that more of additive gene action and selection is effective for the trait under consideration (Table 3 & 4).

The micronutrients was recorded in the acceptable range compared to BPT – 5204, mean copper content in F5 was recorded 5.89 ppm with a range of 3.58 to 17.58 ppm and higher genetic parameters of PCV, GCV and h2 was 22.98, 10.09 and 60.43 percent with higher GAPM of 36.16 percent. However, in case of F6 mean was recorded up to 5.52 with a range of 3.40 to 17.87 percent and the higher PCV, GCV, h2 was 20.07, 16.89 and 60.00 percent respectively coupled with a higher mean GAPM of 27.57 percent. Mean zinc in F5 was recorded up to 17.36 and the range of 8.64 to 30.51 percent and the higher PCV, GCV and h2 was 26.15, 26.09 and 99.49 percent coupled with higher GAPM of 53.60 percent. However, in case of F6 mean were 15.50 with a range of 4.58 to 37.80 percent and the higher PCV, GCV and h2 of 27.80, 24.85, and 94.44 percent respectively with higher GAPM of 44.51 percent. Mean magnesium content in F5 was recorded up to 7.40 with a range of 5.94 to 16.94 ppm and 12.18, 11.03 and 79.71 percent of moderate PCV, GCV and higher h2 respectively coupled with 30.64 percent of higher GAPM. Whereas, in case of F6, mean magnesium content was 7.86 with a range of 3.66 to 16.80 and the moderate to lower PCV, GCV and h2 of 12.53, 9.18 and 77.60 percent respectively coupled with higher GAPM of 30.26 percent. F5 iron content was ranged from 28.62 to 68.84 ppm with an average of 50.51 and the higher genetic parameters of PCV, GCV and h2, 20.13, 19.61 and 94.95 percent respectively coupled with 39.37 of higher GAPM. 55.73 of mean iron content was recorded with an average of 24.67 to 66.43 and the higher genetic parameters of PCV, GCV and h2, 22.58, 22.03 and 95.11 respectively coupled with higher GAPM of 32.09 percent in F6 segregating lines (Table 3 & 4).

Estimation of grain quality parameters
Grain quality parameters are the important parameters which determine the market demand and consumers acceptance. BPT type’s varieties are more accepted by most of the consumers than bold seeded rice. Rice grain quality includes the milling, appearance, cooking and nutritional qualities. Among these, people pay more attention to the appearance and cooking quality (Huang et al., 1998) [10]. The appearance quality is often judged in china by the percentage of grain with a white core and a square of white core. The cooking quality is judged by the amylose content, alkali spreading score and gel consistency (Pooni et al., 1992) [15]. Hence,
Mean grain length in F5 was recorded up to 6.68 mm with a range of 4.00 to 7.00 mm and the higher genetic variability parameters of PCV, GCV and h2 of 19.34, 17.00 and 98.20 percent respectively, coupled with higher GAPM of 27.22 percent. Whereas, in F6 segregating lines, the mean 5.98 with a range of 5.60 to 6.70 mm and higher PCV, GCV and h2 of 17.00, 16.80 and 94.29 percent respectively, coupled with higher GAPM of 22.73 percent. F5 mean grain breadth of 2.83 mm with a range of 2.00 to 3.20 mm and the higher genetic parameters of PCV, GCV and h2 of 26.15, 21.23 and 74.79 percent respectively coupled with moderate GAPM of 18.74 percent. However, in F6 up to 2.56 mean grain breadth with a range of 1.40 to 3.20 and the higher genetic variability parameters of PCV, GCV and h2 recorded 26.21, 24.23 and 76.88 percent respectively coupled with higher GAPM of 24.20 percent. The grain length to breadth ratio was ranged from 1.38 to 3.45 with an average of 2.49 and higher PCV, GCV and h2 of 24.70, 22.19 and 37.66 percent respectively coupled with moderate GAPM of 14.25 percent in F5 segregating lines. And in case of F6 the mean was recorded 2.41 with a range of 1.75 to 3.71 and higher PCV (24.26%), moderate GCV (20.01%) and h2 (37.93%) coupled with moderate GAPM (11.91%) as shown in table 3 & 4. Kernel length in F5 segregating lines was ranged from 4.10 to 6.80 mm with an average of 5.41 mm and lower PCV (7.96%) & GCV (7.59%), higher h2 (90.83%) coupled with moderate GAPM (14.90%). Whereas, in F6 segregating lines, it was ranged from 4.20 to 6.10 mm with an average of 5.30 and lower genetic variability like PCV (7.46%), GCV (6.98%) and higher h2 of 87.88 percent coupled with moderate GAPM (12.69%). Kernel breadth in F5 segregating lines was ranged from 1.10 to 2.50 mm with an average of 2.01 mm and the moderate PCV (11.18%), GCV (10.01%) and higher h2 (80.19%) coupled with moderate to high GAPM (18.47%). Whereas, it was ranging from 1.10 to 2.42 mm with an average of 2.01 mm and the moderate to low PCV (12.00%), GCV (10.91%) and higher h2 (82.76%) coupled with moderate GAPM (18.92%). The kernel length to breadth ratio in F5 lines ranges from 1.96 to 4.82 mm with an average of 2.73 mm and the moderate PCV (14.11%), GCV (12.60%) and higher h2 (79.71%) coupled with higher GAPM (23.17%). Whereas, in case of F6 it was ranged from 2.00 to 4.82 mm with an average of 2.75 and moderate to moderate PCV (14.72%), GCV (13.32%) and higher h2 (81.71%) coupled with higher GAPM (23.29%). Lower to moderate phenotypic and genotypic co-efficient of variation and higher heritability coupled with moderate to 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) [13], Das et al., (2007) [4] and Abdul (2008) [1]. However, Vanajja and Luckins (2006) [22] reported low values of PCV and GCV for grain length (Table 3 & 4).

**Estimation of yield and yield attributing parameters**

Yield is the prime most important factor in the entire breeding objective. In the present study along with the TGP, grain quality and nutrient parameters yield also considered significantly to get at least as BPT – 5204. Mean days to 50% flowering was recorded was 113.41 days among the selected F5 segregating lines with a range of 95 to 141 days and the lower PCV (6.11%), GCV (5.65%) with moderate h2 (35.39%) coupled with lower GAPM (8.89%). However in F6 it was ranged from 100 to 123 days with a mean of 118.36 days and lower PCV (6.67%) and GCV (5.18%) with moderate h2 (39.69%) coupled with lower GAPM (9.42%). Days to maturity was ranged from 112 to 181 days with an average of 162.00 days and lower PCV (7.01%) and GCV (6.11%) with moderate h2 (30.65%) coupled with lower GAPM (5.39%) in F5 segregating lines. However, in F6 it was ranged from 149 to 199 days with an average of 162.65 days and lower PCV (5.86%) and GCV (4.36%) with moderate h2 (50.24%) coupled with lower GAPM (5.44%). In F5, the mean plant height was recorded 85.47 cm with a range of 58 to 99 cm and moderate PCV (12.63%) and GCV (11.49%) with higher h2 (82.82%) coupled with higher GAPM (21.54%). Whereas, in case of F6 the mean plant height was decreased up to 84.73 cm with a range of 52 to 100 cm and moderate PCV (11.85%) and GCV (10.78%) with higher h2 (82.76%) coupled with higher GAPM (20.44%). Biomass was ranged from 20.00 to 63.00 g with an average of 30.01 g and the higher PCV (29.49%), GCV (25.64%) and h2 (75.58%) coupled with higher GAPM (445.91%) in F5 segregating lines. But in F6 generation it was decrease with a mean of 31.88 g with a range of 14.50 to 90.00 g and the higher PCV (30.82%), GCV (26.23%) and h2 (77.46%) coupled with higher GAPM (42.09%). The mean number of tillers was ranged from 8 to 27 with a mean of 19.70 and higher PCV (36.12%), GCV (26.34%) and h2 (66.54%) coupled with higher GAPM (44.56%) in F5 segregating lines. In F6, the mean was 14.81 with a range of 5 to 26 and higher PCV (31.19%) and GCV (24.99%) with moderate h2 (64.20%) coupled with higher GAPM (38.48%). Mean numbers of panicles in F5 were recorded up to 14.01 with a range of 6 to 24 and the higher PCV (38.83%), GCV (29.69%) and h2 (67.76) coupled with higher GAPM (58.20%). In case of F6, it was ranged from 2 to 19 with an average of 10.78 and higher PCV (36.86%), GCV (29.14%) and higher h2 (62.48%) coupled with higher GAPM (44.54%). Panicle length was ranged from 10 to 18 cm with an average of 14.16 cm and moderate PCV (14.90%), GCV (13.84%) and higher h2 (97.64%) coupled with higher GAPM (26.91%) in F5 segregating lines. However, it was ranging from 12 to 21 with an average of 17.80 and the moderate PCV (16.10%) and GCV (15.84%) with higher h2 (98.45%) coupled with higher GAPM (29.52%) as indicated in table 3 & 4. The obtained results are in line of Shivapiya (2000) [19], Manjunath (2003) [12], Ganapathy et al. (2007) [6] and Abdual (2008) [1].

In F5 segregating lines seed fertility percentage was ranged from 45.35 to 98.00 percent with an average of 79.47 percent and the moderate PCV (12.08%) and GCV (11.10%) with higher h2 (84.47%) coupled with higher GAPM (21.02%). Whereas, in case of F6, it was ranging from 45.83 to 98.00 percent with an average of 82.35 percent and moderate PCV (13.37%) and GCV (12.56%) with higher h2 (88.19%) coupled with higher GAPM (20.43%), 24.67 g of mean grain yield per plant with a range of 17.91 to 27.10 g and higher PCV (24.29%), GCV (23.58%) and h2 (74.18%) coupled with higher GAPM (32.61%) in F5 segregating lines. Whereas in F6, it was ranging from 12.87 to 28.70 g with an average of 24.15 g and the higher PCV (28.47%), GCV (22.10%) and h2 (76.19%) coupled with higher GAPM (35.35%). Test weight...
in F5 was ranging from 13.00 to 22.00 g with an average of 19.36 g and moderate PCV (14.55%) and GCV (12.25%) with moderate h2 (49.99%) coupled with moderate GAPM (19.12%). But little decrease in test weight in F6 lines was ranging from 12.00 to 22.20 g with an average of 18.48 g and moderate PCV (15.17%), GCV (13.77%) and h2 (50.29%) coupled with higher (20.24%). 0.45 harvest index with a range of 0.05 to 0.49 and the higher PCV (32.69%), GCV (30.81%) and moderate h2 (43.73%) coupled with higher GAPM (23.27%) was recorded in F5 segregating lines. Whereas, in F6 it was ranging from 0.22 to 0.50 with an average of 0.37 and higher PCV (31.24%) and GCV (27.48%) with moderate h2 (41.22) coupled with higher GAPM (21.34%) as shown in table 3 & 4. It was increased in F6 with recorded mean of 0.34 ranging from 0.12 to 0.49 and the higher PCV (33.68%) and GCV (28.37%) with moderate h2 (44.55%) coupled with higher GAPM (22.32%). Similar findings were reported by earlier workers like Yadav (1992) [26]; Hajare (1993) [18]; Nandarajan and Rajeshwari (1993) [14]; Ahmed and Das (1994) [12]; Shivapriya (2000) [19] and Sharma and Hazarika (2007) [18].

In general, the phenotypic coefficient of variation was comparatively higher than the corresponding genotypic coefficient of variation for the most of the characters studied indicating significant genotype by environment (G X E) interactions. The differences between genotype and phenotype coefficient variations were relatively low for some characters. Present findings are in the line of Barbara and Hazarika (1998) [3].

Estimation of yield and yield attributing parameters

Days to 50% flowering was ranged from 89 to 160 days with an average of 114.34 days and lower PCV (7.02%) and GCV (4.94%) with moderate h2 (48.83%) coupled with moderate GAPM (14.34%) in F5 segregating population. However, mean was increased up to 118.96 days with a range of 80 to 140 days and lower PCV (5.91%) and GCV (3.27%) with moderate h2 (49.57%) coupled with moderate GAPM (14.51%) in F6 segregating generation. In F5, 161.99 days of mean days to maturity were recorded with a range of 126 to 191 days and lower PCV (6.42%) and GCV (5.07%) with higher h2 (62.33%) coupled with lower GAPM (8.24%). Whereas, it was ranged from 124 to 200 days with an average of 161.88 days and lower PCV (5.75%) and GCV (4.20%) with moderate h2 (53.52%) coupled with lower GAPM (6.33%). Mean plant height in F5 were recorded up to 81.77 cm with a range of 60 to 108 cm and moderate PCV (18.90%) and GCV (18.20%) with higher h2 (92.72%) coupled with higher GAPM (36.09%). However it was ranged from 65 to 119 cm with an average of 78.94 cm and lower moderate PCV (17.59%) and GCV (16.77%) with higher h2 (90.98%) coupled with higher GAPM (32.96%) in F6. F5 base population mean biomass was 29.81 g with a range of 12.00 to 100.00 g and highest PCV (34.25%), GCV (31.63%) and h2 (79.29%) coupled with higher GAPM (60.18%). Whereas, it was decrease in F6 up to 30.29 g with a range of 16.00 to 99.50 g and higher PCV (30.40%), GCV (26.93%) and h2 (78.46%) coupled with higher GAPM (59.14%). Number of tillers was ranged from 10 to 34 with an average of 18.70 and the higher PCV (29.91%) and GCV (25.99%) with highest h2 (65.56%) coupled with higher GAPM (46.55%) in F5 were recorded. Whereas, in F6, it was ranging from 4 to 25 with an average of 13.69 and higher PCV (32.87%) and GCV (25.94%) with h2 (62.26%) coupled with higher GAPM (42.16%). Number of panicle was ranged from 6 to 26 with an average of 13.90 and the higher PCV (30.59%), GCV (26.57%) and higher h2 (65.41%) coupled with higher GAPM (57.52%) in F5 were recorded. Whereas, in F6, it was ranging from 3 to 22 with an average of 10.08 and higher PCV (31.44%), GCV (23.89%) and h2 (57.71%) coupled with higher GAPM (47.38%). 14.10 cm of mean panicle length was recorded with a range of 8 to 20 cm and moderate PCV (13.04%) and GCV (12.95%) with higher h2 (98.57%) coupled with higher GAPM (26.48%). But, it was up to 17.10 cm with a range of 8 to 25 cm and moderate PCV (15.14%) and GCV (15.09%) with higher h2 (99.39%) coupled with higher GAPM (31.00%) as indicated in table 5 &6. The obtained results are in line of Shivapriya (2000) [19],

~ 1999 ~
Mean seed fertility percentage in F5 was recorded up to 79 percent with a range from 59.50 to 99.01 percent and moderate PCV (11.05%) and GCV (10.07%) with higher h2 (83.15%) coupled with moderate GAPM (18.92%). However, in F6 it was increased up to 82.00 percent and ranged from 52.31 to 99.15 percent with moderate PCV (11.62%) and GCV (10.70%) with higher h2 (84.69%) coupled with higher GAPM (20.28).

Table 3: Genetic parameters estimated in F5 segregating population in selected lines

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean</th>
<th>Range</th>
<th>PCV (%)</th>
<th>GCV (%)</th>
<th>h2 (%)</th>
<th>GAPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>10.16</td>
<td>7.39</td>
<td>12.81</td>
<td>11.07</td>
<td>6.56</td>
<td>57.83</td>
</tr>
<tr>
<td>GL</td>
<td>6.68</td>
<td>4.00</td>
<td>7.00</td>
<td>19.34</td>
<td>17.00</td>
<td>98.20</td>
</tr>
<tr>
<td>GB</td>
<td>2.83</td>
<td>2.0</td>
<td>3.2</td>
<td>26.15</td>
<td>21.23</td>
<td>74.79</td>
</tr>
<tr>
<td>GLBR</td>
<td>2.49</td>
<td>1.38</td>
<td>3.45</td>
<td>24.70</td>
<td>22.19</td>
<td>37.66</td>
</tr>
<tr>
<td>KL</td>
<td>5.41</td>
<td>4.1</td>
<td>6.8</td>
<td>7.96</td>
<td>7.59</td>
<td>90.83</td>
</tr>
<tr>
<td>KB</td>
<td>2.01</td>
<td>1.10</td>
<td>2.50</td>
<td>11.18</td>
<td>10.01</td>
<td>80.19</td>
</tr>
<tr>
<td>KLBR</td>
<td>2.73</td>
<td>1.96</td>
<td>4.82</td>
<td>14.11</td>
<td>12.60</td>
<td>79.71</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>1.97</td>
<td>0.89</td>
<td>2.32</td>
<td>37.49</td>
<td>33.98</td>
<td>86.13</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>0.12</td>
<td>0.03</td>
<td>0.24</td>
<td>35.33</td>
<td>27.64</td>
<td>86.48</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.12</td>
<td>0.05</td>
<td>0.26</td>
<td>24.18</td>
<td>22.19</td>
<td>89.25</td>
</tr>
<tr>
<td>Copper</td>
<td>5.89</td>
<td>3.58</td>
<td>17.58</td>
<td>22.98</td>
<td>20.09</td>
<td>60.43</td>
</tr>
<tr>
<td>Zinc</td>
<td>17.36</td>
<td>8.64</td>
<td>30.51</td>
<td>26.21</td>
<td>26.09</td>
<td>99.49</td>
</tr>
<tr>
<td>Manganese</td>
<td>7.40</td>
<td>5.94</td>
<td>16.94</td>
<td>12.18</td>
<td>11.03</td>
<td>79.71</td>
</tr>
<tr>
<td>Iron</td>
<td>50.51</td>
<td>28.62</td>
<td>68.84</td>
<td>20.13</td>
<td>19.61</td>
<td>94.95</td>
</tr>
<tr>
<td>DF</td>
<td>113.41</td>
<td>95</td>
<td>141</td>
<td>6.11</td>
<td>5.65</td>
<td>35.39</td>
</tr>
<tr>
<td>DM</td>
<td>162.00</td>
<td>112</td>
<td>181</td>
<td>7.01</td>
<td>6.11</td>
<td>50.65</td>
</tr>
<tr>
<td>PH</td>
<td>85.47</td>
<td>58</td>
<td>99</td>
<td>12.63</td>
<td>11.49</td>
<td>82.82</td>
</tr>
<tr>
<td>Biomass</td>
<td>30.01</td>
<td>20.00</td>
<td>63.00</td>
<td>29.49</td>
<td>25.64</td>
<td>75.58</td>
</tr>
<tr>
<td>NOT</td>
<td>19.70</td>
<td>8</td>
<td>27</td>
<td>36.12</td>
<td>26.34</td>
<td>66.54</td>
</tr>
<tr>
<td>NOP</td>
<td>14.01</td>
<td>6</td>
<td>24</td>
<td>38.83</td>
<td>29.69</td>
<td>67.76</td>
</tr>
<tr>
<td>PL</td>
<td>14.16</td>
<td>10</td>
<td>18</td>
<td>14.90</td>
<td>13.84</td>
<td>97.64</td>
</tr>
<tr>
<td>SFP</td>
<td>79.47</td>
<td>45.35</td>
<td>98</td>
<td>12.08</td>
<td>11.10</td>
<td>84.47</td>
</tr>
<tr>
<td>GY</td>
<td>22.67</td>
<td>17.91</td>
<td>27.1</td>
<td>24.29</td>
<td>23.58</td>
<td>74.18</td>
</tr>
<tr>
<td>HI</td>
<td>0.45</td>
<td>0.05</td>
<td>0.49</td>
<td>32.69</td>
<td>30.81</td>
<td>43.73</td>
</tr>
</tbody>
</table>

Table 4: Genetic parameters estimated in F6 segregating population in selected lines

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean</th>
<th>Range</th>
<th>PCV (%)</th>
<th>GCV (%)</th>
<th>h2 (%)</th>
<th>GAPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>10.39</td>
<td>8.44</td>
<td>12.55</td>
<td>6.57</td>
<td>6.24</td>
<td>49.13</td>
</tr>
<tr>
<td>GL</td>
<td>5.97</td>
<td>5.60</td>
<td>6.70</td>
<td>17.00</td>
<td>16.80</td>
<td>94.29</td>
</tr>
<tr>
<td>GB</td>
<td>2.56</td>
<td>1.4</td>
<td>3.2</td>
<td>26.21</td>
<td>24.23</td>
<td>76.88</td>
</tr>
<tr>
<td>GLBR</td>
<td>2.41</td>
<td>1.75</td>
<td>3.71</td>
<td>24.26</td>
<td>20.01</td>
<td>37.93</td>
</tr>
<tr>
<td>KL</td>
<td>5.30</td>
<td>4.2</td>
<td>6.1</td>
<td>7.46</td>
<td>6.98</td>
<td>87.80</td>
</tr>
<tr>
<td>KB</td>
<td>2.01</td>
<td>1.10</td>
<td>2.42</td>
<td>12.00</td>
<td>10.91</td>
<td>82.76</td>
</tr>
<tr>
<td>KLBR</td>
<td>2.75</td>
<td>2.00</td>
<td>4.82</td>
<td>14.72</td>
<td>13.32</td>
<td>81.71</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>1.36</td>
<td>0.84</td>
<td>2.58</td>
<td>32.30</td>
<td>30.77</td>
<td>83.54</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>0.40</td>
<td>0.07</td>
<td>0.21</td>
<td>20.23</td>
<td>20.19</td>
<td>89.63</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.19</td>
<td>0.13</td>
<td>0.23</td>
<td>24.66</td>
<td>23.94</td>
<td>85.00</td>
</tr>
<tr>
<td>Copper</td>
<td>5.52</td>
<td>3.40</td>
<td>17.87</td>
<td>20.07</td>
<td>16.89</td>
<td>60.00</td>
</tr>
<tr>
<td>Zinc</td>
<td>15.50</td>
<td>4.58</td>
<td>37.8</td>
<td>27.80</td>
<td>24.85</td>
<td>94.44</td>
</tr>
<tr>
<td>Manganese</td>
<td>7.86</td>
<td>3.66</td>
<td>16.8</td>
<td>12.53</td>
<td>9.18</td>
<td>77.60</td>
</tr>
<tr>
<td>Iron</td>
<td>55.73</td>
<td>24.67</td>
<td>66.43</td>
<td>22.58</td>
<td>22.03</td>
<td>95.11</td>
</tr>
<tr>
<td>DF</td>
<td>118.36</td>
<td>100</td>
<td>123</td>
<td>6.67</td>
<td>5.18</td>
<td>39.69</td>
</tr>
<tr>
<td>DM</td>
<td>162.65</td>
<td>149</td>
<td>199</td>
<td>5.86</td>
<td>4.36</td>
<td>50.24</td>
</tr>
<tr>
<td>PH</td>
<td>84.73</td>
<td>52</td>
<td>100</td>
<td>11.85</td>
<td>10.78</td>
<td>82.76</td>
</tr>
</tbody>
</table>
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) [23, 29]. 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. The number of total and polymorphic bands generated on agarose and PAGE. 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 2).

Table 5: Genetic parameters estimated in F5 segregating lines in base population

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean</th>
<th>Range</th>
<th>PCV (%)</th>
<th>GCV (%)</th>
<th>h² (%)</th>
<th>GAPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass</td>
<td>29.81</td>
<td>12.00</td>
<td>100.00</td>
<td>34.25</td>
<td>31.63</td>
<td>79.29</td>
</tr>
<tr>
<td>NOT</td>
<td>18.70</td>
<td>10</td>
<td>34</td>
<td>29.91</td>
<td>25.99</td>
<td>65.56</td>
</tr>
<tr>
<td>NOP</td>
<td>13.90</td>
<td>6</td>
<td>26</td>
<td>30.59</td>
<td>26.57</td>
<td>65.41</td>
</tr>
<tr>
<td>PL</td>
<td>14.10</td>
<td>8</td>
<td>20</td>
<td>13.04</td>
<td>12.95</td>
<td>98.57</td>
</tr>
<tr>
<td>SFP</td>
<td>79</td>
<td>59.5</td>
<td>99.01</td>
<td>11.05</td>
<td>10.07</td>
<td>83.15</td>
</tr>
<tr>
<td>GY</td>
<td>19.55</td>
<td>9.55</td>
<td>29.10</td>
<td>28.45</td>
<td>25.59</td>
<td>76.76</td>
</tr>
<tr>
<td>TW</td>
<td>18.22</td>
<td>13</td>
<td>22</td>
<td>23.35</td>
<td>20.12</td>
<td>52.18</td>
</tr>
<tr>
<td>HI</td>
<td>0.40</td>
<td>0.20</td>
<td>0.49</td>
<td>22.49</td>
<td>21.50</td>
<td>64.38</td>
</tr>
</tbody>
</table>

Table 6: Genetic parameters estimated in F6 segregating lines in base population

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean</th>
<th>Range</th>
<th>PCV (%)</th>
<th>GCV (%)</th>
<th>h² (%)</th>
<th>GAPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass</td>
<td>30.29</td>
<td>16.00</td>
<td>99.50</td>
<td>30.40</td>
<td>26.93</td>
<td>78.46</td>
</tr>
<tr>
<td>NOT</td>
<td>13.69</td>
<td>4</td>
<td>25</td>
<td>32.87</td>
<td>25.94</td>
<td>62.26</td>
</tr>
<tr>
<td>NOP</td>
<td>10.08</td>
<td>3</td>
<td>22</td>
<td>31.44</td>
<td>23.89</td>
<td>57.71</td>
</tr>
<tr>
<td>PL</td>
<td>17.10</td>
<td>8</td>
<td>25</td>
<td>15.14</td>
<td>15.09</td>
<td>99.39</td>
</tr>
<tr>
<td>SFP</td>
<td>82</td>
<td>52.31</td>
<td>99.15</td>
<td>11.62</td>
<td>10.70</td>
<td>84.69</td>
</tr>
<tr>
<td>GY</td>
<td>24.34</td>
<td>9.40</td>
<td>29.70</td>
<td>27.06</td>
<td>26.63</td>
<td>77.84</td>
</tr>
<tr>
<td>TW</td>
<td>18.06</td>
<td>11.2</td>
<td>25</td>
<td>20.38</td>
<td>19.24</td>
<td>50.34</td>
</tr>
<tr>
<td>HI</td>
<td>0.37</td>
<td>0.21</td>
<td>0.50</td>
<td>21.64</td>
<td>19.13</td>
<td>59.66</td>
</tr>
</tbody>
</table>

Table 7: DNA markers used for detecting parent polymorphism of BPT 5204 and HPR 14

<table>
<thead>
<tr>
<th>Marker type</th>
<th>No. of markers</th>
<th>Number of bands</th>
<th>Average number of bands</th>
<th>Percent polymorphism</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSR (3% agarose)</td>
<td>402</td>
<td>69</td>
<td>333</td>
<td>402</td>
</tr>
<tr>
<td>SSR (12% PAGE)</td>
<td>402</td>
<td>81</td>
<td>321</td>
<td>402</td>
</tr>
</tbody>
</table>
Conclusion
Presently developed segregating population has the potential to develop the high protein genotype and showed clear cut segregation pattern for the trait under consideration and fine mapping can be done to select the high protein genotype.

Acknowledgement
Financial support by DBT, New Delhi and RKVY, GOK profoundly acknowledged.

References
19. Shivapiya. Phenotypic evaluation for agronomically useful traits, blast disease, DNA finger printing and diversity studies in local rices (Oryza sativa L.) of...


