*Biological Forum – An International Journal* **14(4a): 656-661(2022)**

**ISSN No. (Print): 0975-1130**
**ISSN No. (Online): 2249-3239**

# Analysis of Marker-trait Association of Backcross Population in Rice using SSR Markers for High Grain Protein Content

***Nutan Moharana[1], Bansidhar Pradhan[1*], Krishnendu Chattopadhyay[2], Shuvendu Shekhar Mohapatra[2], Tapash Kumar Mishra[1], Mihir Ranjan Mohanty[3] and Rajesh Kumar Kar[4]***

*[1]Department of Genetics and Plant Breeding, College of Agriculture,*
*Odisha University of Agriculture and Technology (OUAT), Bhubaneswar (Odisha), India.*
*[2]Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack (Odisha), India.*
*[3]Regional Research and Technology Transfer Sub-Station (RRTTSS),*
*Odisha University of Agriculture and Technology (OUAT), Jeypore (Odisha), India.*
*[4]Department of Genetics and Plant Breeding, Institute of Agricultural Sciences (IAS),*
*Siksha 'O' Anusandhan Deemed to be University, Bhubaneswar (Odisha), India.*

*(Corresponding author: Bansidhar Pradhan[*])*
*(Received 10 September 2022, Accepted 19 November, 2022)*
*(Published by Research Trend, Website: www.researchtrend.net)*

**ABSTRACT: Rice is the main staple food in the world. Despite having a lower nutritional quality than other cereals, rice contributes 15% of the world's population's protein intake per capita. Enhancing the nutritional quality of rice will improve the nutritional status of the malnourished improvised country, and for this, the detection of QTLs linked to grain protein content and markers associated with them need to be discovered to have an efficient breeding approach. The experiment was conducted at the Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha, India, during 2021. The current study looked at a marker trait association of rice grain protein content using a panel population of 96 derived lines of backcross population. Grain protein content (%) was phenotyped and genotyped using a few chromosome 1 polymorphic SSR markers. The mean grain protein content was found to be 9.03 per cent, while it ranged from 3.83 to 17.76 per cent. Polymorphic markers were then used to score the 96 derived lines. The single marker RM12107 was positively correlated with grain protein content and was found significant with an $R^2$ value > 0.05. It is clearly understood that this association is not consistent over seasons and needs to be studied on a large scale.**

**Keywords:** Grain protein, Marker-trait association, QTLs, Rice backcross population and SSR markers.

## INTRODUCTION

Rice (*Oryza sativa* L.) is a significant component of the primary human diet consumed globally (Juliano, 1999). 21% of all calories consumed globally per person come from rice. Despite having a lower nutritional quality than other cereals, rice contributes 15% of the world's population's per capita protein intake. In comparison to other significant cereals like wheat, rice had a better protein digestibility corrected amino acid score (PDCAAS), which showed the presence of necessary amino acids and overall protein quality (0.40). According to a screening of global germplasm collections, the protein content of farmed rice ranged from about 5% to 18%, with an average of 9.5%.This revealed the existence of genetic variability for this feature and suggested the viability of determining high-protein rice cultivars (Juliano *et al.,* 1968). Therefore, increasing the quantity and nutritional value of the protein in rice has been the main focus of efforts to increase rice storage protein.

Due to rising health concerns, rice breeders are now placing greater emphasis on the nutritional value of rice. After carbohydrates, protein is the second most crucial ingredient in milled rice. However, rice protein is particularly deficient in lysine, one of the required amino acids for human nutrition (Juliano, 1999). The average level of protein (PC) in milled rice is about 7%, and it is most frequently consumed. Important factors that determine the nutritional value of rice are its protein and lysine contents. Children in many impoverished nations face a major health risk from protein deficiency (Gearing, 2015). As a result, there is an urgent need for rice breeders to enhance additional proteins in rice. However, improving the PC of rice via the traditional breeding method is laborious and time-consuming. With the development of biotechnology and the availability of molecular markers, it is now simpler and more efficient to identify QTLs associated with protein content and to improve the trait (Sautter *et al.*, 2006). There are numerous papers on QTL mapping for protein content available. Strong efforts have been invested over the past five decades to improve the protein content of rice, mainly via conventional breeding techniques and induced mutations (Khush and Juliano 1984). Rice protein content has been quantified according to studies (Shenoy *et al.*, 1991). The heritability for protein content ranged from 0.130 to 0.372 due to the genotype ×

environment (G × E) interaction effect. According to Shenoy *et al.* (1991) findings, protein content is 58.8% heritable. According to Lang and Buu (2005), genetic influences accounted for 25.9% of the variation in protein content. Even though several QTLs per se may influence the trait, previous inheritance studies revealed that the variation in GPC between two parents was caused by two main genes. Several crops have recently shown the potential of molecular marker-assisted selection in plant breeding (Huang *et al*., 1997; Reddy *et al*., 1997).

Several crops have recently shown the potential of molecular marker-assisted selection in plant breeding (Huang *et al*., 1997; Reddy *et al.*, 1997). Despite the development of molecular markers linked to dozens of genes affecting a number of economically significant features in this crop, their utility to plant breeders has not yet been proven in protein. More recent research has focused heavily on the protein content of rice, finding QTLs on all 12 of the rice chromosomes except for chromosome 9 (Aluko *et al.*, 2004; Hub *et al.*, 2004; Li *et al.*, 2004; Tan *et al*., 2001; Zhang *et al.*, 2008). Bao (2014) discovered roughly 43 QTLs from nine different rice types, covering all 12 chromosomes. Compared to the other chromosomes, chromosomes 1, 2, and 7 have a significant number of QTLs. Additionally, Zhang *et al.* (2005) found 2, 3, and 4 QTLs, respectively, for the protein fractions albumin, globulin, prolamin, and glutelin. The QTLs governing the differences in protein fraction composition may be located at the same chromosomal locus. Patindol and Wang (2003) discovered 18 chromosome segments for 19 distinct amino acids, with the group of 19 distinct quantitative trait loci at the base of chromosome 1 being somewhat more robust. Hu *et al.* (2004) found 12 quantitative trait loci on chromosomes 1, 4, 6, 7, and 11 for both the content of individual amino acids and the total amount. Eight amino acids were connected to the quantitative trait loci cluster on chromosome 1. These findings are crucial for finding the right genes and for marker-assisted breeding with the aim of increasing the amino acid content of rice for human consumption. The development of two high-yielding, protein-rich rice cultivars, CR Dhan 310 and CR Dhan 311, by the ICAR-National Rice Research Institute, Cuttack, India, through classical backcross breeding and release in 2015 and 2016, respectively, demonstrated that high protein could be combined with high grain yield in rice (Mahender *et al.*, 2016; NRRI Annual Report, 2015). Therefore, it is important that work on rice protein bio-fortification uses appropriate QTLs for grain PC improvement. The recognition of a genetic component for protein content has been enhanced by the recent identification of QTL for rice protein content (Aluko *et al.*, 2004; Hub *et al.*, 2004; Li *et al.*, 2004; Tan *et al.*, 2001; Guo *et al.*, 2007; Yu *et al.*, 2009). In order to increase the protein content of the grain, molecular approaches, such as marker-assisted selection, may be a more effective strategy than traditional breeding alone. The current study looked at a marker trait association of rice grain protein content using a panel population of 96 derived lines of backcross population.

## MATERIAL AND METHODS

The experiment was conducted at the Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, 753006, Odisha, India, during 2021.

**Plant material:** For the phenotyping of grain protein content (%), a backcross generated mapping population from the Swarna/ARC 10075 cross (ARC 10075: high protein donor) was employed and genotyped using a few chromosome 1 polymorphic SSR primers. For this investigation, 96 distinct $BC_3F_5$ lines were chosen to represent the grain protein content of this population.

**Phenotyping for grain protein content in rice**: Grain from the 96 backcross derived lines was harvested and dried to a moisture content of 12–13%. The rice huller (Satake, Japan) prepared approximately 10-15 g of brown rice from each type, which was then examined using near-infrared spectroscopy (NIRS). Using the software programme Win ISI-III Project Manager v.1.50e, the NIR spectroscope (model NIRS DS2500, FOSS Analytical, Sweden) reading was calibrated by fitting calibration equations 1, 4, 4, 1 for grain protein content. The grain protein content model for brown rice was proven to be reliable (Bagchi *et al.*, 2015).

**Markers used in the study**: Out of 140 markers located on chromosome 1 used for the polymorphic study of the backcross parents ARC10075 and Swarna, thirteen markers were found to be polymorphic. Details of the polymorphic markers used in the study are given in Table 1.

**DNA isolation and selection of markers:** For the isolation of genomic DNA, leaves from 21-day-old seedlings of 96 backcross-derived lines were collected. The samples were broken up into minute pieces and powdered in liquid nitrogen before being extracted with CTAB (2% CTAB, 100 mM Tris-Cl (pH 8.0), 20 mM EDTA (pH 8.0), 1.3 M NaCl), then extracted again with phenol-chloroform-isoamyl alcohol, RNase treatment, and ethanol precipitation. The amount of DNA in the solution was verified before diluting it to 30 ng/l.

**Rice grain protein content markers amplification and visualisation**: In a 20-l PCR reaction, 1U Taq polymerase, 1.5 mM Tris HCl (pH 8.75), 50 mM KCL, 2 mM $MgCl_2$, 0.1% TritonX-100, 200 M each of dATP, dCTP, dTTP, and dGTP, 4 pmol of forward and reverse primers, and 30 ng of genomic DNA were used. The temperature profile for the PCR amplification was initial denaturation at 94°C for 4 min, followed by 35 cycles of 94°C for 1 min, annealing at 55–62 °C for 1 min, extension at 72 °C for 1 min 30 s, and then a final extension for 10 min at 72 °C.Electrophoresis was carried out using 2.5-35% agarose gel containing 0.8 g/mL ethidium bromide, depending on the projected amplicon size. On the gel, 10 µl of PCR products were placed. To determine the amplicon size, a 50-bp DNA ladder was put into at least one lane. A Gel-Doc System was used to take pictures of the gel after it had been run in 1X TBE buffer (pH 8.0) at 120 volts/cm for 2 hours.

**Table 1: Details of polymorphic markers used in the study.**

| Sr. No. | Marker name | Position on chromosome | QTLs region | Forward sequence | Reverse sequence | Annealing temperature (°C) |
|---|---|---|---|---|---|---|
| 1. | RM5062 | 42548123 | *qPC1.1/ OsAAP6* | AAGCAAGCGAGTGCTTTGTACCG | GGTGTGGCTAGAAACACACG | 55 |
| 2. | RM12109 | 40422250 | *qPC1.1/ OsAAP6* | CAATTAATCCTCTCCCTAGCAAGC | GAGTTCATGAGTTGAGTTGTGTGG | 55 |
| 3. | RM12107 | 40420644 | *qPC1.1/ OsAAP6* | CTGATAGTGATGTCCATTGTGTGAGG | CTTTCCTCTATGCCTAGAAACACACG | 55 |
| 4. | RM12102 | 40325807 | *qPC1.1/ OsAAP6* | GACGGAGGGAGTGCTTTGTACCG | GCATCTGGCGTGGTTCAGTTGC | 55 |
| 5. | RM6141 | 42410706 | *qPC1.1/ OsAAP6* | AAGCTTCCCCAATCTGGAAC | TAGCTTAGCTGCTGCTGCTG | 55 |
| 6. | RM12209 | 41941678 | *qPC1.1/ OsAAP6* | TTGCCATCCATTCGATTTCACC | GAGACCACCAGATCGCTTCACC | 55 |
| 7. | RM12207 | 41898956 | *qPC1.1/ OsAAP6* | GGTCTATGCATGGGACAACACG | AGCTGTGGGAGCGAGACTGC | 55 |
| 8. | RM8147 | 43500000 | *qPC1.1/ OsAAP6* | GATCGCTTCACCACAGCCTTCC | CGATTTCACCACAGAGAGC | 55 |
| 9. | RM10048 | 744440 | *qGPC1.1* | CAAGCAGTGATCATACAGCCTTCC | GCCATGGCTGAGAACAGAGAGC | 55 |
| 10. | RM3148 | 746085 | *qGPC1.1* | GACTATTGCTCGAACACTTTG | TTGTCTGCTTTGGTATTTGC | 55 |
| 11. | RM12135 | 40570183 | *qPC1.1/ OsAAP6* | GGGACAGATAGAATCCCATAGCC | CTGCGACTTGCTCTTCCTCTACC | 55 |
| 12. | RM8099 | 4085000 | *qPC1.1/ OsAAP6* | CATGGGCCAGAATTAAGAGG | CATCCACTTTCCTCTCCTGC | 55 |
| 13. | RM12146 | 40717930 | *qPC1.1/ OsAAP6* | AGTATGCCCTGCCCACTACACTAGG | CAGCGAATGGCAAGAGCAACC | 55 |

**Data analysis:** Based on the presence or absence of bands, marker allele data were generated for each genotype and marker and were then organised in matrix form for each genotype-marker pair. The association analysis was carried out between the marker and grain protein content using single marker regression analysis in Microsoft Excel.

## RESULTS AND DISCUSSION

**Phenotyping for grain protein content:** The grain protein content of 96 backcross derived lines from the Swarna/ARC 10075 cross was estimated using NIR spectroscopy. The experimental mean grain protein content was 9.03%, while the mean grain protein content ranged from 3.83% to 17.76% (Fig. 1 and Table 2).

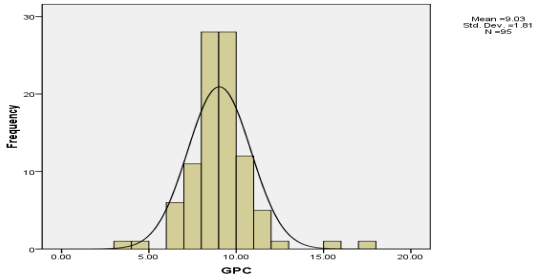


**Fig. 1.** Grain Protein content (GPC) frequency distribution.

**Table 2: Grain protein content information.**

| Parameters | GPC (%) |
|---|---|
| Mean protein content | 9.03 |
| Range | 3.83- 17.76 |
| SD | 1.81 |

Compared to high yielding varieties like Swarna, ARC10075 (high protein donors) has a much greater protein content. The distribution of protein content in brown rice was found to be normal. Phenotyping of a backcross-derived mapping population revealed a significant variation in GPC (3.83 to 17.76%), which allowed for the identification of introgression lines in high yielding backgrounds with QTLs for grain protein content in rice, the possibility of further improving this trait, and the potential for generating new QTLs for grain protein content in rice.

**Association mapping:** The marker–trait association was done with the grain protein content of *kharif*, 2021. Out of 140 markers, located in chromosome 1 used for the polymorphic study of the backcross parents ARC10075 and Swarna, thirteen markers were found to be polymorphic. Polymorphic markers were then used to score the 96 derived lines (Fig. 2).

Although it was widely believed that the amount of milling and environmental factors, such as nitrogen fertilizer and growth duration, had a significant impact on protein content (Blumenthal *et al.*, 2008), the recent discovery of QTL for rice protein content has strengthened the notion that there is a genetic component to protein content (Aluko *et al.*, 2004; Hub *et al.*, 2004; Li *et al.*, 2004). Thirteen of the 140 primers tested between ARC10075 and Swarna for polymorphism were found to be polymorphic, exhibiting a distinct and reproducible banding pattern. With this set of primers, which were linked to multiple QTLs/genes previously found, low and high grain protein-containing lines could not be identified in the current investigation (Aluko *et al.*, 2004; Yu *et al.*, 2009; Shi-yong *et al.*, 2006; Huang *et al.*, 2015).

The marker–trait associations (Fig. 3) were filtered at less than 5% error, i.e., 95% confidence (p value < 0.05) on the analysis of protein content of the backcross ARC10075/Swarna population, $R^2$ value ranged from 0.000316 to 2.2642 and the F value from 4.0034 to 17.1167 as analyzed through single marker regression analysis.

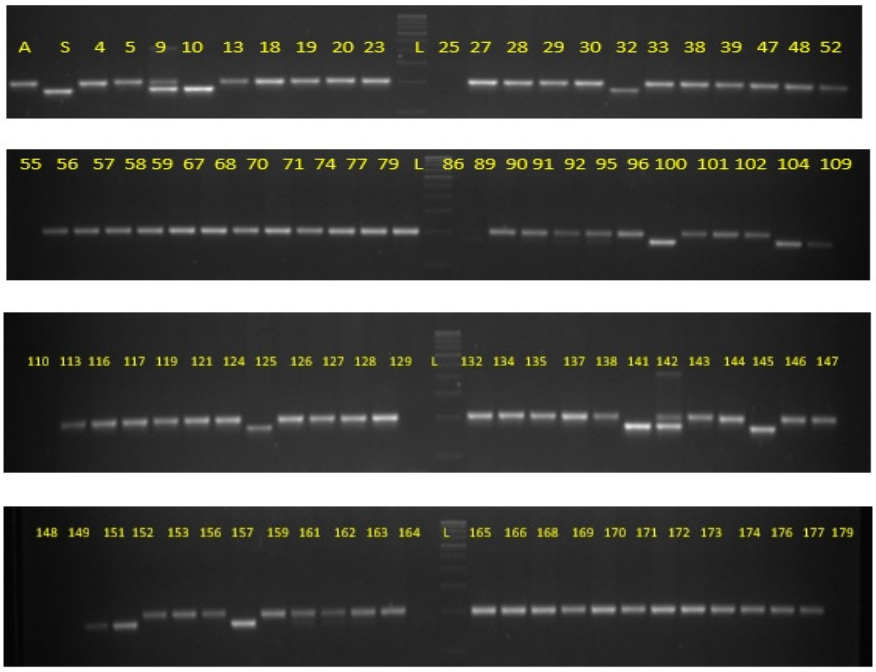


**Fig. 2.** Gel picture depicting primer RM12107 showing polymorphism among the backcross derived lines; L-100bp ladder; A-ARC10075; S-Swarna.

The single marker RM12107 was positively correlated with grain protein content and was found to be significant. This significantly associated marker had an $R^2$ value > 0.05. It is clearly understood that this association is not consistent over seasons and needs to be studied on a large scale. To investigate any linkage between the GPC characteristic in rice, it may be necessary to test a larger number of polymorphic primers linked to known QTLs or genes. One of the upcoming research programmes for increasing the GPC trait in rice is the analysis of mapping populations for the discovery of additional QTLs/genes responsible for GPC.

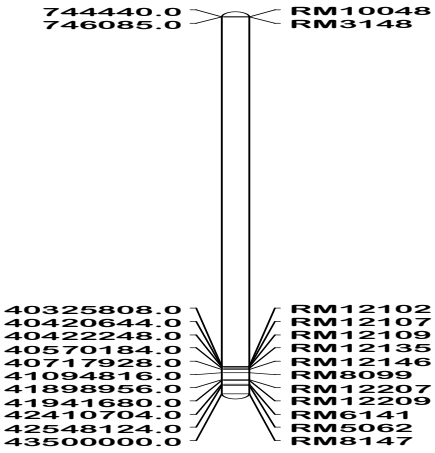


**Fig. 3.** SSR markers associated with Quantitative trait loci (QTLs) of protein.

Chattopadhyay *et al.* (2019) used a 40K Affymetrix custom SNP array to genotype a BC3F4 mapping population derived from a cross between grain protein donor ARC10075 and high-yielding cultivar Naveen and identified one stable QTL, qGPC1.1, explaining 13% PVE. This QTL is located in a 0.7 Mb region of chromosome 1. So, SSR markers have been taken of this region. But no significant marker-trait association was detected. As a result, more markers will be collected in this region in the future in order to obtain tightly linked markers with GPC for use in marker-assisted selection. On the other hand, another QTL for GPC qPC1/OsAAP6 located in a 42 Mb region on chromosome 1 was cloned (Peng *et al.*, 2014). However, the OsAPP6 expression level is found to be associated with GPC variation only in indica accessions, and no correlation between the OsAAP6 expression level and GPC variation was detected in the japonica genetic background. It seems that OsAAP6 could only be used as a target gene to regulate GPC in *indica* breeding programs. In our experiment, we have selected polymorphic markers in this region. One marker, RM12107, was found to be associated with GPC. So after validation using another mapping population or through association mapping, this marker can be utilized in marker-assisted breeding for the development of high-protein elite lines.

## CONCLUSIONS

Using a panel population of 96 derived lines from a backcross population, the current study examined a marker trait relationship of rice grain protein content. Using a few chromosome 1 polymorphic SSR markers, grain protein content (%) was phenotyped and genotyped. It was found that the average grain protein content was 9.03%, ranging from 3.83 to 17.76%. The 96 generated lines were then scored with polymorphic markers. The one marker, RM12107, had a significant $R^2$ value of greater than 0.05 and was positively linked

with grain protein content. It is clearly understood that this association is not consistent over seasons and needs to be studied on a large scale.

## FUTURE SCOPE

Testing more polymorphic primers connected to known QTLs or genes may be necessary to look into any association between the GPC trait in rice and any other trait. The examination of mapping populations for the finding of additional QTLs/genes responsible for GPC is one of the next research programmes for improving the GPC trait in rice.

**Acknowledgement.** The authors acknowledge the Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha, India.
**Conflict of Interest.** None.

## REFERENCES

Aluko, G., Martinez, C., Tohme, J., Castano, C., Bergman, C. and Oard, J. H. (2004). QTL mapping of grain quality traits from the interspecific cross *Oryza sativa* × *O. glaberrima*. *Theoretical and Applied Genetics*, *109,* 630-639.

Bagchi, T. B., Sharma, S. G. and Chattopadhyay, K. (2015). Development of NIRS models to predict protein and amylose content of brown rice and proximate compositions of rice bran. *Food Chemistry*, *191*, 21–27.

Bao, J. (2014). Genes and QTLs for rice grain quality improvement, rice - germplasm, genetics and improvement, Dr. Wengui Yan (Ed.), In Tech.

Blumenthal, J. M., Baltensperger, D. D., Cassman, K.G., Mason, S. C. and Pavlista A. D. (2008). Importance and effect of nitrogen on crop quality and health. In: Hatfield J. L. and Follett R. F (eds.) Nitrogen in the environment; sources, problems and management, USA: Academic Press- Elsevier.

Chattopadhyay, K., Behera, L. and Bagchi, T. B. (2019). Detection of stable QTLs for grain protein content in rice (*Oryza sativa* L.) employing high throughput phenotyping and genotyping platforms. *Scientific Reports*, *9*(1), 3196.

Gearing, M. E. (2015). Good as gold: Can golden rice and other biofortified crops prevent malnutrition? Science in the News, Harvard University. http://sitn.hms.harvard.edu/

Guo, Y., Mu, P., Liu, J., Lu, Y. and Li, Z. (2007). QTL mapping and QxE interaction of grain cooking and nutrient qualities in rice under upland and lowland environments. *Journal of Genetics and Genomics*, *34* (5), 420-428.

Hub, Z. L., Li, P., Zhou, M. Q., Zhang, Z. H., Wang, L. X., Zhu, L. H. and Zhu, Y. G. (2004). Mapping of quantitative trait loci (QTL) for rice protein and fat content using doubled haploid lines. *Euphytica*, *135,* 47-54.

Huang, N., Angeles, E. R., Domingo, J., Magpantay, G., Singh, S., Zhang, G., Kumaravadivel, N., Bennett, J. and Khush, G. S. (1997). Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. *Theoretical and Applied Genetics*, *95,* 313-320.

Huang, Y., Sun, C., Min, J., Chen, Y., Tong, C. and Bao, J. (2015). Association mapping of quantitative trait loci for mineral element contents in whole grain rice (*Oryza sativa* L.). *Journal of Agricultural and Food Chemistry*, *63*(50), 10885–10892.

Huang, X., Zhao, Y., Wei, X., Li, C., Wang, A., Zhao, Q. and Li, W. (2012). Genome wide association study of flowering time and grain yield traits in a Worldwide collection of rice germplasms. *Nature Genetics*, *44*, 32-41.

Hu, Z. L., Li, P., Zhou, M. Q., Zhang, Z. H., Wang, L. X., Zhu, L. H. and Zhu, Y. G. (2004). Mapping of quantitative trait loci (QTLs) for rice protein and fat content using doubled haploid lines. *Euphytica*, *135*, 47–54.

Juliano, B. O., Ignacio, C. C., Panganiban, V. M. and Perez, C. M. (1968). Screening for high protein rice varieties. *Cereal Science Today*, *13*, 299.

Juliano, B. O. (1999). Rice in human nutrition. *FAO food and nutrition series*, *26,* 5461.

Khush, G. S. and Juliano, B. O. (1984). Status of rice varietal improvement for protein content at IRRI. In: Nuclear Techniques for Cereal Grain Protein Improvement; proceedings of research coordination meeting: IAEA, Vienna, Austria, p. 199–202.

Lang, N. T. and Buu, B. C. (2005). Tag genes controlling grain protein content (GPC) using microsatellite markers in rice. *Omonrice*, *13*, 12-18.

Li, J. M., Xiao, J., Grandillo, S., Jiang, L., Wan, Y., Deng, Q. and McCook, S. R. (2004). QTL detection for rice grain quality trails using an interspecific backcross population derived from cultivated Asian (*O. saliva* L.) and African (*O. glaberrima* S.) rice. *Genome, 47,* 697-704.

Mahender, A., Anandan, A., Pradhan, S. K. and Pandit, E. (2016). Rice grain nutritional traits and their enhancement using relevant genes and QTLs through advanced approaches. *Spring plus*, *5*(1), 2086.

NRRI Annual Report (2015). ICAR-national rice research institute (NRRI). Cuttack, India.

Patindol, J. and Wang, Y. J. (2003). Fine structures and physicochemical properties of starches from chalky and translucent rice kernels. *Journal of Agricultural and Food Chemistry*, *51*, 2777–2784.

Peng, B., Kong, H., Li, Y., Wang, L., Zhong, M. and Sun, L. (2014). OsAAP6 functions as an important regulator of grain protein content and nutritional quality in rice. *Nature Communications*, *5,* 5847.

Reddy, J. N., Baraoidan, M. R., Bernardo, M. A., George, M. L. C. and Sridhar, R. (1997). Application of marker assisted selection in rice for bacterial blight resistance. *Current Science*, 73, 873-875.

Sautter, C., Polite, S., Zhang, P. and Gruissem, W. (2006). Biofortification of essential nutritional compounds and trace elements in rice and cassava. Proceedings of Natural Society, *65*, 153–159.

Shenoy, V.V., Seshu, D. V. and Sachan, J. K. S. (1991). Inheritance of protein per grain in rice. *Indian Journal of Genetics and Plant Breeding*, *51*(2), 214–220.

Shi-yong, S., Wei, H. and Hong-xuan, L. (2006). Identification of QTLs for cooking and eating quality of rice grain. *Rice Science*, *13,* 161–169.

Tan, Y. F., Sun, M., Xing, Y. Z., Hua, J. P., Sun, X. L., Zhang, Q. F. and Corke, H. (2001). Mapping quantitative trait loci for milling quality, protein content and color characteristics of rice using a recombinant inbred line population derived from an elite rice hybrid. *Theoretical and Applied Genetics*, *103*(6,7), 1037–1045.

Yu, Y. H., Li, G., Fan, Y. Y., Zhan, K. Q., Min, J., Zhu, Z. W. and Zhuang, J. Y. (2009). Genetic relationship between grain yield and the contents of protein and fat in a recombinant inbred population of rice. *Journal of Cereal Science*, *50,* 121-125.

Zhang, W., Jingcui, Bi., Xie, K., Zhao, Z., Wang, Y., Liu, L. L., Jiang, L. and Wan, J. (2008). QTL mapping for crude protein and protein fraction contents in rice (*Oryza sativa* L.). *Journal of Cereal science*, *48*, 539-547.

Zhang, S., Ni, D., Yi, C., Li, L., Wang, X., Wang, Z. and Yang, J. (2005). Lowering grain amylose content in backcross off-springs of indica rice variety 057 by molecular marker assisted selection. *Rice Science*, *12*, 157–162.

**How to cite this article:** Nutan Moharana, Bansidhar Pradhan, Krishnendu Chattopadhyay, Shuvendu Shekhar Mohapatra, Tapash Kumar Mishra, Mihir Ranjan Mohanty and Rajesh Kumar Kar (2022). Analysis of Marker-trait Association of Backcross Population in Rice using SSR Markers for High Grain Protein Content. *Biological Forum – An International Journal, 14*(4a): 656-661.