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## Dayanand Navrang

Department of Plant Molecular Biology and Biotechnology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur, Chhattisgarh, India

## Vinay Premi

Department of Plant Molecular Biology and Biotechnology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur, Chhattisgarh, India

## Shrikant Yankanchi

Department of Plant Molecular Biology and Biotechnology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur, Chhattisgarh, India

## Ajit Kumar Manmade

Department of Plant Molecular Biology and Biotechnology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur, Chhattisgarh, India

## Corresponding Author:

### Ajit Kumar Manmade

Department of Plant Molecular Biology and Biotechnology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur, Chhattisgarh, India

## Phenological variability among RILs for grain protein content in rice indigenous to central India

Dayanand Navrang, Vinay Premi, Shrikant Yankanchi and Ajit Kumar Manmade

### Abstract

Phenological variability study was conducted to evaluate the mapping population for grain protein levels and morphological traits. The experimental material consisting of a mapping population having 192 lines derived from a cross between CGZR-1 X CGR-77 in which CGZR-1 is high yielding and grain zinc rich and CGR-77 is a germplasm with higher grain protein content. Protein content was estimated by modified Micro-Kjeldahl method and then further confirmed by Lowry's method. Results showed that 28.8% lines recorded common for higher grain yield and grain protein content. Pearson's correlation coefficients showed a positive correlation between grain protein content and most of all the traits under study i.e. PH, PL, ENT, GPC etc. The highest and lowest coefficient of variance was showed for panicle length (43.23) and grain protein content (2.75) respectively. Lines in the Mapping population showed normal distribution for all the traits recorded. Performance of all the lines were evaluated using scatter plot in which line numbers 27, 140, 152 and 153 recorded with higher grain yield and grain protein levels. Overall results suggest that the current mapping population is feasible for future breeding programs to develop high grain protein lines.

**Keywords:** Rice, bio-fortification, mapping population, grain protein content

### Introduction

Rice is one of the chief grains of the world including many developing countries in Asia, Africa and Latin America. Approximately 11% of the world's arable land is planted annually to rice. India is the world's second largest producer of rice, and the largest exporter of rice in the world, production increased from 53.6 million tons in financial year 1980 to 120 million tons in FY 2020-21 (Annual report 2020-21). Rice is the important cereal and a source of calories for one-third of the world population. Bio-fortification refers to the development of micronutrient-dense staple crops using the best traditional breeding practices and modern biotechnology. About 800 million people in developing world are undernourished suffering from either protein energy or other micro nutrient (Vit-A, Vit-C, Iron and Zinc) therefore staple crop like rice may play a major role in reducing hidden hunger and malnutrition problems in developing countries which usually affect children and especially pregnant women. Nearly, half of the death in children under the age of five is mainly attributed to nutrient deficiency of micro element (protein). It causes greater risk in children and pregnant woman by common infections. It may increase in frequency and severity which ultimately leads to death of the patients (Keiss *et al.* 2017) [6]. It is caused when the body is short of protein and energy foods. Protein is necessary for key body functions and energy buildup including provision of essential amino acids and development and maintenance of muscles. Therefore we suffer from diseases such as marasmus and kwashiorkor. Effect the marasmus disease Child gets sick and is unable to move and Physical growth of the child is stopped and kwashiorkor effect is Hair becomes red and frizzy, the mental and physical growth of the child is stopped Stomach becomes swollen with water, the skin gets discolored, Muscles waste away. India has the highest burden of malnutrition in the world. According to hunger index 2021 India ranks 101 among 116 nations, "Bio-fortification" refers to the development of micronutrient- dense staple crops using the best traditional breeding practices and modern biotechnology (Gregorio 2002; Pfeiffer and McClafferty 2007) [4, 10]. Rice supplies abundant carbohydrate as its kernel constitutes mainly of starch (>80%) but protein (7-8%) is the source of concern. However, the protein quality measured by protein digestibility index and amino acid composition is the best among cereals, which makes it preferable for the food and feed industries. Efforts were made during past three decades by rice breeders to improve the protein

content in rice grain. The majority of the undernourished population in the world subsists on diets based on cereals. Human beings require at least 49 nutrient elements to meet their metabolic needs, inadequate consumption of even one of these nutrients will result in adverse metabolic disturbances leading to sickness, poor health and impaired development in children and large economic costs of society (Bronca and Ferrari, 2000; Ramakrishna *et al.*, 1999). Protein is necessary for key body functions and energy buildup including provision of essential amino acids and development and maintenance of muscles. Seed storage proteins can be divided into four classes based on their solubility properties. Albumins are water soluble, globulins are salt soluble, prolamines are soluble in aqueous alcohol solutions, and glutelins are soluble in alkali or acid (Shotwell and Larkin's, 1989) [13]. Rice (*Oryza sativa* L.), a staple food crop for millions of people worldwide, has a seed protein content ranging from 5 to 12% (Villarreal and Juliano, 1978) [21]. The genetic basis of the accumulation of grain protein in the grain and mapping of QTL provide the basis for preparing the strategies and enhancement of the protein content in rice (Mahendra *et al.* 2016) [7]. The major storage proteins found in rice are the *oryzin*, 80% or more of the total seed protein (Tecson *et al.*, 1971; Juliano, 1972; Villarreal and Juliano, 1978) [16, 5, 21]. In the present investigation with the objective find out the grain protein content in CGZR-1 X CGR-77 derived mapping population containing 192 genotypes

shortlisted through phenotyping of protein content was studied for genetic diversity, population structure and association mapping of grain protein content in rice.

### Materials and Methods

The experimental materials consist of 192 lines of cross population CGZR-1 X CGR-77 seed harvested from the mapping population (selected protein rich) lines were used to raise the mapping population plant derived from the cross between CGZR-1 (high yielding and zinc rich) and CGR-77 (germplasm line with higher protein). Both the parent used was developed at Indira Gandhi Agriculture University, Raipur (C.G.) the plant materials were planted in the field for recording the phenotyping observations during the wet season 2018. The seed were sown on date 30 June 2018 and transplanted on 17 July. The plant to plant 15 cm and row to row spacing was 20cm. The NPK fertilizer was applied @ 100:60:40 kg per hectare screening, field observation and evaluation of parents population were taken.

### Field evolution and observations recorded on various physio morphological traits

The observations of 11 yield contributing phenotyping traits were recorded at specific stage during their maximum tillering stage, vegetative stage, maturation stage and post-harvest observations were also recorded by following standard evaluation system (SES/IRRI, 2002).



**Fig 1:** Kharif season mature 2019 F5 (Drone picture) (CGZR-1XCGR-77)

The crop was raised during the Kharif season 2018 at agricultural institutional cum research farm, Indira Gandhi Agriculture University, Raipur (C.G.) which falls under tropical wet and dry climate, located in the center –east Raipur is situated at 21° 11'N latitude, 81° 36'E longitude and at an altitude of 289.56 meter from the mean sea level. The average data of fifty year showed that the annual rainfall range between 1200-1600 mm data will received mostly from the middle of June to end of September. The mapping population (192) lines were sown in a single row of 2 m length in nursery beds. After 19- 21 days, seedlings were transplanted in the main field with plant to plant spacing of 15 cm and row to row spacing of 20 cm. Data were recorded for various and grain protein and yield relate traits, Plant height (cm), Effective no. of tillers/plant, Panicle length (cm), Per plant grain yield (g), test weight(100) (g), 1000 – grain weight (g), protein content( $\mu\text{g/g}$ ), Seed width (SD), Days of 50% flowering (DLF), seed length (SL) and L:B ratio (SLB).

### Protein Estimation by micro-Kjeldahl method and Lowry method

#### Digestion process

About 0.5 gm of rice grain was transferred into the digestion tube and 5-7 gm of  $\text{K}_2\text{SO}_4$  and  $\text{CuSO}_4$  mixture was added. 10 ml of concentrated Sulphuric acid was added and digestion tubes were placed on the digestion block with temperature set at 400 °C. After 2 to 3 hours when the samples color turned to light green, the digestion tubes were taken out of digestion block. The tubes were permitted to cool at room temperature for 10 minute.

#### Distillation process

Digested samples were subjected to Pelican make distillation unit and Distillation of samples were carried using 4% Boric acid and 40% Sodium hydroxide. 10 ml of Boric acid was then taken in conical flask, to which 2-4 drops of mixed indicator dye was added. The flask was beneath the condenser

with the delivery tip immersed in the solution. The digested samples were transferred to distillation apparatus and 8-10 ml of 40% Sodium hydroxide was added to it. Around 20 ml of distillate was collected in a conical flask. A blank was always run containing the same quantities of the entire reagent but without the sample for every set of nitrogen determination.

### Titration process

The distilled samples were titrated against the 0.05 N Sulfamic acid until the first appearance of violate color as the end point. The titer value was used to calculate percent Nitrogen, which is then used to estimate total protein content by using conversion factor 5.95 (Julliano, 1993).

$$\text{Nitrogen (\%)} = \frac{(\text{Vol. of Sulfamic acid} - \text{Vol. of blank}) \times \text{Normality} \times 14 \times 100}{\text{Sample weight (gm)} \times 1000}$$

### Protein Estimation by Lowry's Method

Protein can be estimated by different methods as described by Lowry and also by estimating the total nitrogen content. Hydrolyzing the protein and estimating the amino acids alone will give the exact quantification. The method developed by (Lowry *et al.*) is sensitive enough to give a moderately constant value and hence largely followed. Protein content of enzyme extracts is usually determined by this method.

### Principle

The blue colour developed by the reduction of the phosphomolybdic-phosphotungstic components in the folin-ciocalteau reagent by the amino acid tyrosine and tryptophan present in the protein plus the color developed by the biuret reaction of the protein with the alkaline cupric tartrate are measured in the Lowry method.

### Materials

- 2% Sodium Carbonate in 0.1 N Sodium Hydroxide pellet (Reagent A)
  - a. 0.1 N Noah in 100 ml autoclaved distilled water.
  - b. 2 g of Na<sub>2</sub>CO<sub>3</sub> in 100 ml 0.1 in Noah.
- 0.5% copper sulphate (CuSO<sub>4</sub>.5H<sub>2</sub>O) in 1% potassium sodium tart rate (Reagent B)
  - a. 1% (1g) (PST) in 100 ml Autoclaved distilled water (Reagent B1)
  - b. 0.5g (CuSO<sub>4</sub>.5H<sub>2</sub>O) in 100ml H<sub>2</sub>O (Reagent B2)
- Alkaline Copper Solution: Mix 48 ml Of reagent A and 1 ml B1, 1ml B2 total volume 50 ml prior to use(Reagent C)
- Foline – ciocalteau reagent (Reagent D): 1 N
- Phosphate Buffer Saline(PBS)use for crushing to sample gently (1x pH 7.4)

### Working Standard

Dilute 10 ml of the stock solution to 50 ml with distilled water in a standard flask. One mL of this solution contains 200 µg proteins.

### Determination of protein from rice sample

Extraction is usually carried out with buffers used for the enzyme assay. Weigh 500 mg of the sample and grind well with a pestle and mortar in 5-10 ml of the (PBS) buffer. Centrifuge and use the supernatant for protein estimation.

### Estimation of protein

1. Pipette out 0.2, 0.4, 0.6, 0.8 and 1mL of the working standard into a series of test tubes.
2. Pipette out 0.1 mL and 0.2 mL of the sample extract in two other test tubes.
3. Make up the volume to 1mL in all the test tubes. A tubes with 1 mL of the serves as the including blank.
4. Add 5 mL of reagent C to each tube including the blank. Mix well and allow standing for 10 minute.
5. Then add 0.5 mL of reagent D, mix well and incubate at room temp. in the dark for 30 minute. Blue color is developed.
6. Take the readings at 660 nm. Draw a standard graph and calculate the amount of protein in the sample.

The data was subsequently analysis using SPSS (statistical package for the social science) analysis for software using for phenotypic normal distribution analysis for all the protein and yield related traits. OPSTAT to determine the phenotyping variability correlation coefficient analysis and Microsoft excel data analysis software used and mean value were taken from the measurement of 3 replicates and standard error of the mean value was calculated. Difference between means was determined by one way ANOVA. According to Fisher and Yates (1963) the correlation coefficient analysis are used to (<0.01 and <0.05) significance with (n-2) degree of freedom as per the procedure describe.

### Result and Discussion

#### Protein estimation method.

All the 192 lines check two different types of protein estimation method show to fifteen highest protein and yield lines of the data. In which four such line show to higher like 140, 152, 153 and 27 proteins and yield data (Table 1). Mean performance of the traits showed large variation for all the studied traits (Table 2). The analysis revealed of cross population range of variation existed among the genotype for all major traits related to grain protein content and yield in rice plant among eleven traits high variation was obtained 43.23 and lowest was found in 2.75 as shown by coefficient of variation (Table) out of 192, the maximum yield was observed in 23.21 gram per plant (PPY) and their coefficient of variation was found in 43.21. Maximum Grain width was in found 2.6 and lowest was 2.4 in all cross population. The three majored traits (Grain size, width and length, and their L: B ratio and per plant yield was directly associated with rice grain productivity and these traits strongly depends on the genetic potential and related to grain protein content in rice. L: B ratio was recorded maximum in 3.79 and minimum in 3.26 and their coefficient of variation in 12.3 found. Earliest 50% flowering (DLF) was observed 89 days of highest and lately 81 days and coefficient was found in 17.23. Maximum grain length was recorded in 9.2 and lowest grain length was recorded in 8.5 and their coefficient of variation was found as shown by 21.32. Maximum test weight was recorded in 2.66 whereas lowest test weight observed in 2.35 and harvest index (1000 wt) observed 26.58 and lowest 23.46(g) found. Maximum plant height was recorded in 142.62(cm) and lowest plant height 78.68 (cm) Maximum Grain protein content was recorded in 10.37(cm) and lowest plant height 5.83 (cm) and their cv is recorded 2.75.



**Table 1:** Study of protein estimated by Kjeldhal method on fifteen lines show high grain protein and yield

S.N.	Lines Season	Protein content/yield	
		Kjeldhal%	Yield/tones/haq
1	CGZR-1X CGR-77(50)	9.8 ± 1.1	4.7
2	CGZR-1X CGR-77(64)	9.2 ± 1.0	4.9
3	CGZR-1X CGR-77(120)	9.4 ± 1.1	5.3
4	CGZR-1XCGR-77(135)	9.6 ± 1.2	5.8
5	CGZR-1XCGR-77(140)	10.4 ± 1.3	6.2
6	CGZR-1XCGR-77(149)	9.2 ± 1.4	5.4
7	CGZR-1XCGR-77(152)	10.0 ± 1.0	6.1
8	CGZR-1XCGR-77(153)	10.0 ± 0.9	5.9
9	CGZR-1XCGR-77(161)	9.9 ± 0.8	4.3
10	CGZR-1XCGR-77(179)	9.5 ± 0.7	4.6
11	CGZR-1XCGR-77(189)	9.2 ± 1.2	4.2
12	CGZR-1XCGR-77(204)	9.0 ± 1.1	4.3
13	CGZR-1XCGR-77(27)	10.4 ± 1.2	6.04
14	CGZR-1XCGR-77(41)	9.0 ± 1.1	5.3
15	CGZR-1XCGR-77(56)	9.5 ± 1.4	5.6

**Table 2:** Protein estimation by micro –Kjeldahl method then further confirmation by Lowry's method

S.N.	Lines Season	Protein content	
		Kjeldhal	Lowry's
1	CGZR-1XCGR-77(140)	10.4±1.3	10±0.7
2	CGZR-1XCGR-77(152)	10±1.0	10.2±1.10
3	CGZR-1XCGR-77(153)	10.3±0.9	9.9±0.9
4	CGZR-1XCGR-77(27)	10.4±1.2	10.1±1.1
	SE(m)		0.144
	SE(d)		0.203
	C.V.		1.998

Further there four lines for validated for protein content using Lowry's method. Results are mentioned in (table 3) and there correlation coefficient of variation 1.998, SD was show 0.203 and standard error of mean is 0.144.

**Table 3:** Mean range maximum minimum and including coefficient of variance percentage for various traits and grain protein content CGZR-1 X CGR-77 mapping populations

S.N.	Traits	Mean ± S.Em	Maximum	Minimum	Cv (%)
1	Per Plant Yield(g) PPY	14.35 ± 0.26	35.59	7.55	43.21
2	Grain Width (CM)GW	2.52 ± 0.00	2.6	2.4	15.34
3	Grain Length (CM) GL	8.83 ± 0.01	9.2	8.5	21.32
4	100 Grain wt (TW)	2.5 ± 0.00	2.65	2.34	10.31
5	1000 Grain wt (TGW)	25.01 ± 0.04	26.57	23.4	15.04
6	Seed L:B Ratio(SLB)	3.50 ± 0.00	3.79	3.26	12.30
7	Plant Height(cm) PH	126.45 ± 0.98	158.26	89.4	27.05
8	Panicle Length(cm) PL	25.61 ± 0.14	36.94	18.4	43.23
9	Plant Tiller(cm) PT	6.69 ± 0.07	10.2	4.6	14.23
10	Days of 50% Flowering(DLF)	78.85 ± 0.19	83	75	17.23
11	Grain Protein Content(GPC)	8.08 ± 0.05	10.37	5.83	2.75

± indicate standard error; PH: Plant height (cm), PL: Panicle length(cm), ENT: Effective no. of tiller, PPY: Per plant yield(g), DLF: Days of 50% flowering, SW: Seed width(cm), SL: Seed length(cm), SLB: Seed L:B ratio, TW: 100-test weight(g), TGW: 1000- Grain weight(g), GPC: Grain protein content(%)

**Table 4:** Analysis of variance (ANOVA TABLE) for 11 yield and grain protein content attributing traits under field condition during kharif 2018

S.N.	Traits	DF	MSS	ESS	P-Value
1	Plant height(cm) PH	191	927.776	103.432	0
2	Panicle length(cm) PL	191	167.645	132.866	0.01
3	Plant tiller(cm) PT	191	4.653	3.274	0
4	Grain width (CM)GW	191	0.005	0.002	0
5	Grain Length (CM) GL	191	0.053	0.054	0.014
6	Days of 50% Flowering(DLF)	191	37.619	37.298	0.001
7	Per Plant Yield(g) (PPY)	191	68.327	41.221	0
8	100 Grain wt (TW)	191	0.004	0.002	0.054
9	1000 Grain wt (TGW)	191	0.418	0.0462	0.075
10	Length and Breadth(L:B)Ratio	191	0.016	0.013	0.098
11	Grain protein content (GPC)	191	1.044	0.050	0

The quantitative character of 192 rice mapping populations was analyzed using Randomized Block Design (RCBD) with two replication. The mean sum of squares values for all the eleven characters are presented in table 3. The mean sum of square was found to be significant for all traits. The indicated the existence of variability could be attributed to the diverse source of materials used in the study

**Table 5:** Pearson's correlation coefficient for various grain yield related traits and grain protein content in CGZR-1 X CGR-77 mapping population

Traits	PH	PL	ENT	PPY	DLF	SW	SL	SLB	TW	TGW	GPC
<b>Mapping population</b>											
PH	1										
PL	0.246**	1									
ENT	0.302**	-0.006	1								
PPY	0.291**	0.275**	0.115*	1							
DLF	0.035	-0.129	-0.231**	-0.101*	1						
SW	-0.067	-0.050	-0.035	0.250	0.294**	1					
SL	0.218	0.266	-0.094	0.202	-0.166	-0.073*	1				
SLB	0.055	0.081	-0.045	-0.231	-0.182	-0.093	0.169	1			
TW	0.203	0.142	0.209	0.279	0.054	0.182**	0.210	-0.059	1		
TGW	-0.001	0.241**	0.213	-0.179*	0.257**	0.080	-0.015	-0.061	0.099	1	
GPC	0.382**	0.402**	0.227**	0.297**	-0.081	0.182	0.427**	0.034	0.439	0.536**	1

Significant at ( $p < 0.01$ ) per cent LSD and \* significant at ( $p < 0.05$ ) per cent LSD; PH: Plant height(cm), PL: Panicle length(cm), ENT: Effective no. of tiller, PPY: Per plant yield(g), DLF: Days of 50% flowering, SW: Seed width (cm), SL: Seed length(cm), SLB: Seed L:B ratio, TW: 100-test weight(g), TGW: 1000- Grain weight(g), GPC: Grain protein content(%).

Found and 27.05 as shown by coefficient of variation. Maximum plant tiller 19 and lowest tiller 6.2 and maximum panicle length were recorded 30.38 and lowest 19.72(cm) in experiment populations. The quantitative character of 192 rice mapping populations was analyzed using Randomized Block Design (RCBD) with two replication. The mean sum of squares values for all the eleven characters are presented in table 5. The mean sum of square was found to be significant for all the traits. The indicated the existence of variability could be attributed to the diverse source of materials used in the study. Plant height was highly positively correlated with Grain protein content ( $r = 0.382^{**}$ ). However significant positively correlation with panicle length ( $r = 0.246^{**}$ ), per plant yield ( $r = 0.291$ ) and effective no. of tillers per plant ( $r = 0.302^{**}$ ) was observed. \*\* Panicle length was highly positively correlated with grain protein content ( $r = 0.402^{**}$ ) However positively correlation with per plant yield ( $r = 0.275^{**}$ ) and 1000- grain weight ( $r = 0.241^{**}$ ) was observed. Effective no. of tillers /plant was highly positive correlated with grain protein content ( $r = 0.227^{**}$ ). However positively and negatively correlated with per plant yield ( $r = 0.115^{*}$ ) and days of 50% flowering ( $r = -0.231^{**}$ ). Per plant yield was highly positively correlation with grain protein content ( $r = 0.297^{**}$ ). However negatively correlated with days of 50% flowering ( $r = -0.101^{*}$ ) and 1000-grain weight ( $r = -0.179^{*}$ ) was observed. Days of 50% flowering was positively correlated with seed width ( $r = 0.294^{**}$ ) and 1000- grain weight ( $r = 0.257^{**}$ ) was observed. Seed width was highly positively correlated with test weight ( $r = 0.182^{**}$ ) and negatively correlated with seed length ( $r = -0.073^{*}$ ) was observed. Seed length was positively correlated with grain protein content ( $r = 0.427^{**}$ ) was observed. 1000- Grain weight was highly positively correlated with grain protein content ( $r = 0.536^{**}$ ) was observed.

#### Qualitative traits observation analysis of mapping population

Out of 192 mapping population lines, 30 percent present light purple basal leaf sheath color, 66percent green color and 4 percent of purple line of basal leaf sheath color in a population, 9 percent present light leaf intensity of green color, 29 percent dark color and 62 percent of medium leaf intensity of green color in a population, 4 percent present week leaf pubescence, 28 percent strong pubescence and 68 percent of medium pubescence present in a population, 3

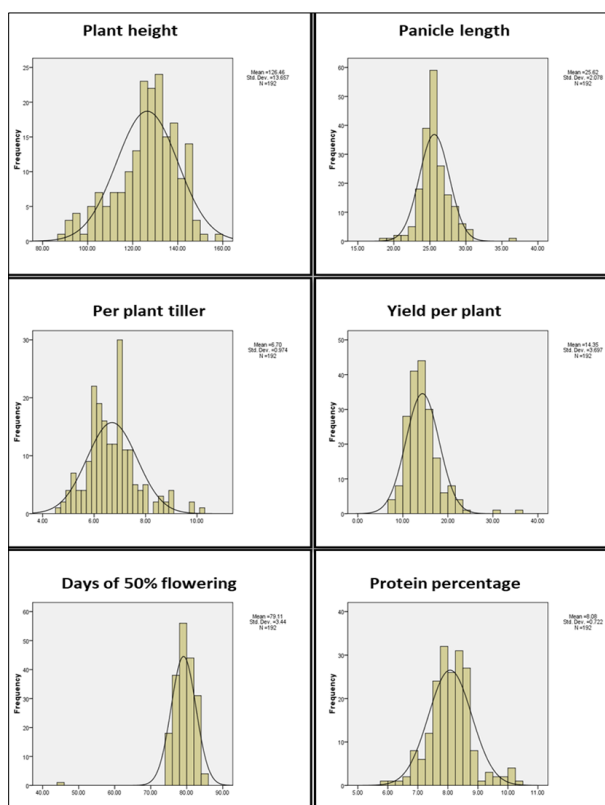
percent absent leaf auricle in a population 97 percent present of leaf auricle in a population, 50 percent present Leaf anthocynin colorations of collar, 31percent absent Leaf anthocynin colorations of collar present in a population.5 percent present purple color leaf ligules, 64percent light purple and 31 percent white color ligules color present in a population. 40 percent short length of blade and 60 percent present medium type of leaf length of blade in a entire population, 39 percent present narrow type of leaf width, 61percent medium type of leaf width present in a population. 38percent present semi erect of Culm attitude, 62percent open type of Culm attitude present in a population, 1percent present purple color, 60 percent light purple and 39 percent colorless type of anthocynin present in a auricles present in a population, 39 percent present thin type of stem, 61 percent medium type of stem thickness are reported, 14 percent present late type of panicle heading, 34 percent early type and 52 percent of panicle heading are medium type, 39 percent present stem anthocynin colorations, 61 percent stem anthocynin colorations are reported.38 percent short panicle length of main axis (PnL)and 62 percent of medium panicle length of main axis.88 percent panicle AWNS have been present of mapping population and 12 percent panicle AWNS have been absent of populations, 23 percent leaf anthocynin colorations have been present of mapping population and 77 percent leaf anthocynin colorations have been absent of populations.

#### Discussion

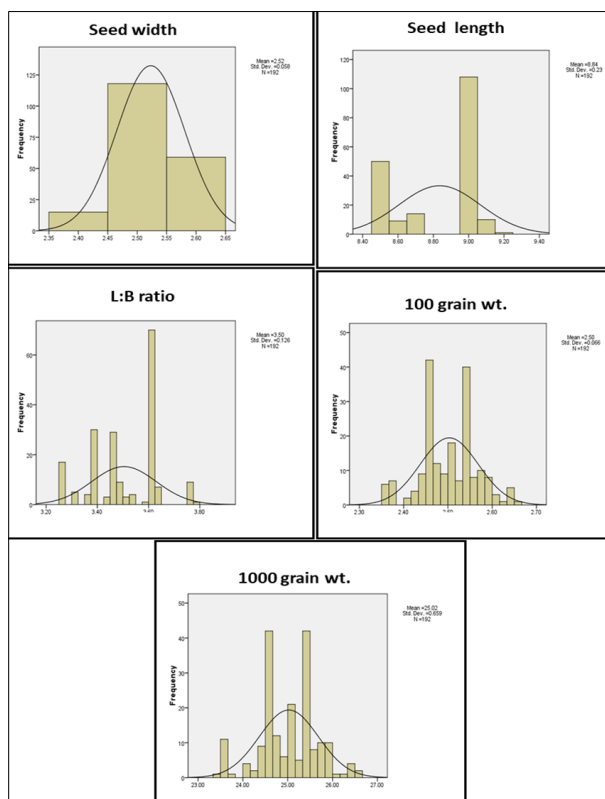
Earlier reports confirmed existence of genetic and phenology variability in grain protein content in rice (Tan *et al.* 2001; Aluko *et al.* 2004; Weng *et al.* 2008; Zhang *et al.* 2008; Yu *et al.* 2009; Banerjee *et al.* 2011; Zhong *et al.* 2011; Oko *et al.* 2012; Patil *et al.* 2014; Yun *et al.* 2014; Bagchi *et al.* 2015; Mahender *et al.* 2016; Verma andrivastava 2017) [15, 22, 23, 2, 25, 9, 1, 7, 20]. The protein content estimated from rice grains of germplasm line ARC10063 was 16.41% (Mahender *et al.* 2016; Mohanty *et al.* 2011) [7]. However, according to earlier report protein content among the recombinant inbred lines was found to vary from 1.53 to 22.42% (Uday *et al.* 2014) [14]. Thus, through protein biofortification it is feasible to enhance PC in high-yielding popular rice varieties forum developed and developing countries. But, by the classical breeding approach, a very limited success could be achieved in this context, Earlier report confirm grain protein content in rice

(Upadhyay *et al.* 2021) Furthermore, on assessing the impact of cooking on protein levels there was significant increase in protein content which varies from 50%-70% among low protein to high protein genotypes. These results open up new avenues in nutritional research for Comparison of grain

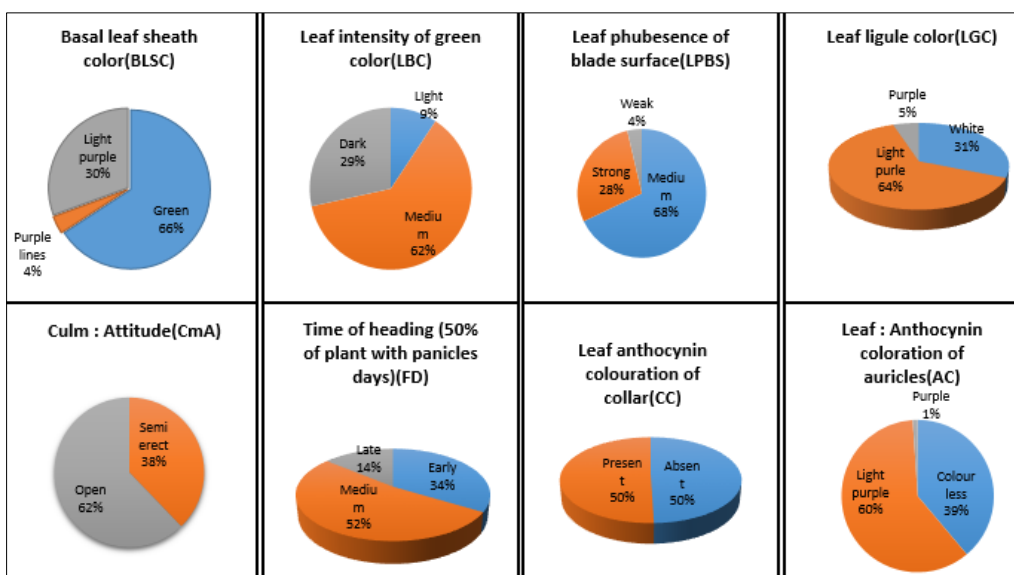
protein content (%) in brown, polish and cooked rice genotypes (b) % potential increase in GPC in high and low protein polished genotypes after cooking. addressing the problem of malnutrition in vulnerable population whose sustenance depends on staples.



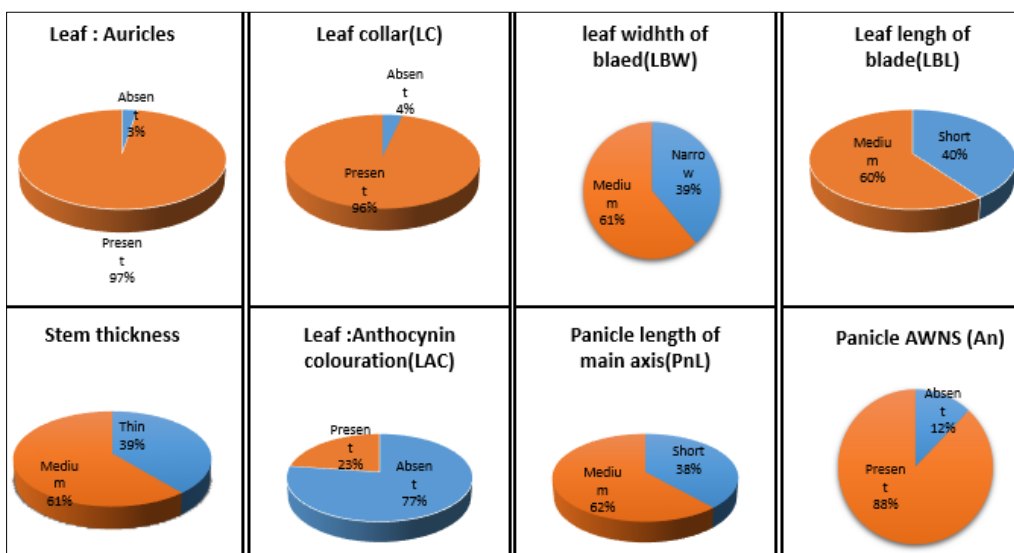
**Fig 3:** Normal curve, (A) Plant height, (B) Panicle length, (C) Number of tiller and (D) Yield per plant. (E) Days of 50% flowering. (F) Protein percentage



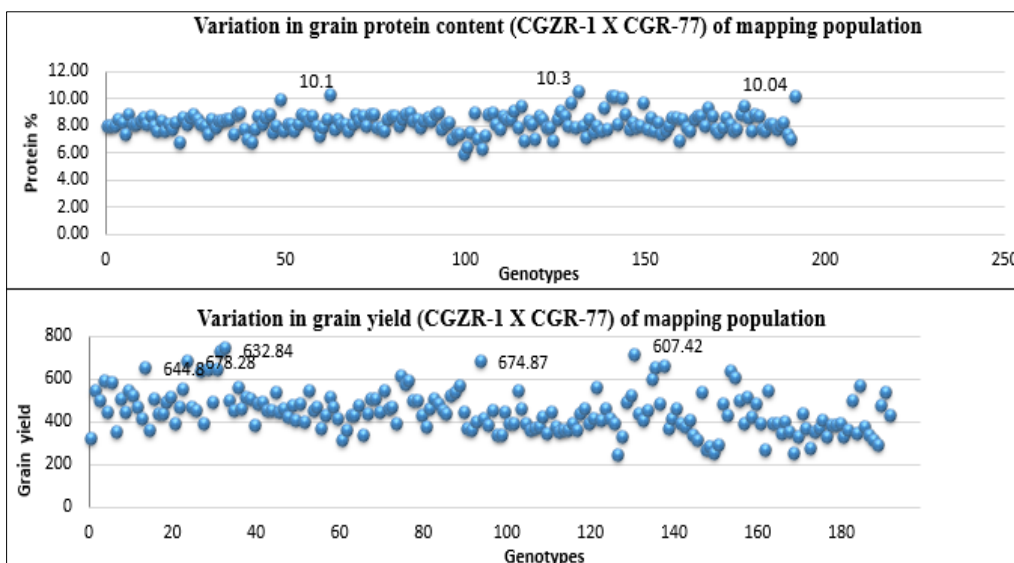
**Fig 4:** Normal curve, (G) Seed length, (H) Seed width (I) L:B Ratio, (J) 100 Grain weight, (K) 1000 Grain weight



**Fig 5:** Qualitative traits of mapping population- Basal leaf sheath color, leaf intensity of green color, leaf pubescence of blade surface, leaf ligules, Culm attitude, time of 50% of plant with panicle days, leaf anthocyanin coloration of collar and leaf anthocyanin coloration



**Fig 6:** Qualitative traits of mapping population- Leaf auricles, leaf collar, leaf width of blade, leaf length of blade, stem thickness, leaf anthocyanin coloration, panicle length main axis and panicle AWNS



**Fig 7:** Performance of mapping population variation in Grain protein content and Grain yield (CGZR-1 X CGR-77) mapping populations

A scatter plot displays a relationship between two sets of data. A scatter plot can also be called a scatter gram or a scatter diagram. In a scatter plot, a dot represents a single data point. With several data points graphed, a visual distribution of the data can be seen. Depending on how tightly the points cluster together, you may be able to discern a clear trend in the data. The closer the data points come to forming a straight line when plotted, the higher the correlation between the two variables, or the stronger the relationship. If the data points make a straight line going from near the origin out to high y-values, the variables are said to have a positive correlation. If the data points start at high y-values on the y-axis and progress down to low values, the variables have a negative correlation. The set of data represent higher and lower yield of populations and represent upon higher and lower grain protein content in a populations.

### Conclusion

The significant variation for protein content in mapping population suggests the existence of genetic potential to increase the concentration of this micronutrient in rice grain. The Pearson's correlation coefficients showed protein content and grain yield/plant were positively correlated to all the studied traits. The distribution curves showed normal parabolic distribution for effective number of tillers/plant and grain protein content. This also indicated that available populations is feasible to plan a breeding and biotechnology program to develop high-yielding, protein rich rice genotypes and identify genomic location for micronutrient content. However, more research is required to understand molecular and biochemical mechanism for minerals uptake and transport using such novel diverse populations.

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### References

1. Bagchi TB, Sharma SG, Chattopadhyay K. Development of NIRS models to predict protein and amylose content of brown rice and proximate compositions of rice bran. *Food Chem.* 2015;191:21-27. <https://doi.org/10.1016/j.foodchem.2015.05.038>.
2. Banerjee S, Chandel G, Mandal Meena NM, Saluja T. Assessment of nutritive value in milled rice grain of some Indian rice landraces and their molecular characterization. *Bangladesh J Agril Res.* 2011;36(3):369-380.
3. Chattopadhyay K, Behera L, Bagchi TB, Sardar SS, Moharana N, Patra NR, *et al.* Detection of stable QTLs for grain protein content in rice (*Oryza sativa* L.) employing high throughput phenotyping and genotyping platforms. 2019;9:3196.
4. Gregorio GB. Progress in breeding for trace elements in staple crops. *Journal of Nutrition.* 2002;132:500-502.
5. Juliano BO. The rice caryopsis and its composition. In DF Houston, *Rice: Chemistry and Technology.* American Association of Cereal Chemists, St. Paul, MN, 1972, 16-74pp.
6. Kiess L, Aldern N, Pee S, Bloem MW. Nutrition in Humanitarian Crises. In *Nutrition and Health in a Developing World* Springer International Publishing, 2017, 647-664.
7. Mahender A, Anandan A, Pradhan SK, Pandit E. Rice grain nutritional traits and their enhancement using relevant genes and QTLs through advanced approaches. *Springer plus* 5-2086, 2016.
8. Nilson A, Piza J. Food fortification: a tool for fighting hidden hunger *Food and Nutrition Bulletin.* 1998;19:49-60.
9. Patil AH, Premi V, Sahu V, Dubey M, Sahu GR, Chandel G. Identification of elite rice germplasm lines for grain protein content, SSR based genotyping and DNA fingerprinting. *Int J Agric For.* 2014;2(2):16-23.
10. Pfeiffer WH, McClafferty B. Harvest Plus: breeding crops for better nutrition. *Crop Science,* 2007;47:88.
11. Pippal A, Rajinder K, Jain Sunita, Kumar Sandeep, Bhussal. Phenotyping for grain mineral contents (iron and zinc) in PAU201 X PALMAN 579 F5 and BC1F4 populations in rice (*Oryza sativa* L.), 2018, 658pp.
12. Pradhan SK, Barik SR, Sahoo A, Mohapatra S, Nayak DK, Mahender A. Population structure, genetic diversity and molecular marker-trait association analysis for high temperature stress tolerance in rice. *PLoS One.* 2016;11:e0160027. <https://doi.org/10.1371/journal.pone.0160027>.
13. Shotwell MA, Larkins BA. The molecular biology and biochemistry of seed storage proteins. In *A. Marcused, The Biochemistry of Plants.* CA. 1989;15:297-345.
14. Stangoulis JC, Huynh BL, Welch RM, Choi EY, Graham RD. Quantitative trait loci for phytate in rice grain and their relationship with grain micronutrient content. *Euphytica.* 2007;154:289-294.
15. Tan YF, Sun M, Xing YZ, Hua JP, Sun XL, Zhang QF, *et al.* 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. *Theor Appl Genet.* 2001;103(6, 7):1037-1045.
16. Tecson EM, Esmama BV, Lontok LP, Juliano BO. Studies on the extraction and composition of rice endosperm glutelin and prolamin. *Cereal Chem.* 1971;48:169-181.
17. Tiwari VK, Rawat N, Chhuneja P, Neelam K, Aggarwal R, Randhawa GS, *et al.* Mapping of quantitative trait loci in rice. 2009;100:771-776.
18. Trijatmiko KR, Arines FM, Oliva N, Slamet-Loedin IH, Kohli A. Molecular analyses of transgenic plants. *Recombinant Proteins from Plants. Methods and Protocols,* 2016, 201-222pp.
19. Uday G, Murthy MK, Hittalmani S. Genetic analysis of recombinant inbred lines for total grain protein content and grain yield in rice (*Oryza sativa* L.). *Int J Agric Sci Res.* 2014;4(2):51-58.
20. Verma DK, Srivastava PP. Proximate composition, mineral content and fatty acids analyses of aromatic and non-aromatic Indian rice. *Rice Sci.* 2017;24(1):21-31.
21. Villareal RM, Juliano BO. Properties of glutelin from mature and developing rice grain. *Phytochemistry.* 1978;17:177-182.
22. Weng J, Gu S, Wan X, Gao H, Guo T, Su N, *et al.* Isolation and initial characterization of GW5, a major QTL associated with rice grain width and weight. *Cell*



Res. 2008;18:1199-1209.

23. Yu YH, Li G, Fan YY, Zhang KQ, Min J, Zhu ZW, *et al.* Genetic relationship between grain yield and the contents of protein and fat in a recombinant inbred population of rice. *J Cereal Sci.* 2009;50(1):121-125.
24. Zhang P, Li J, Li X, Liu X, Zhao X, Lu Y. Population structure and genetic diversity in a rice core collection (*Oryza sativa* L.) investigated with SSR markers. *PLoS One.* 2011;6:e27565.  
<https://doi.org/10.1371/journal.pone.20110027565> (pmid: 22164211).
25. Zhong M, Wang L, Yuan J, Luo L, Xu C, He YQ. Identification of QTL affecting protein and amino acid contents in rice. *Rice Sci.* 2011;18(3):187-195.