



Characterising the diversity of grain nutritional and physico-chemical quality in Indian rice landraces by multivariate genetic analyses

Haritha Bollinedi, K. K. Vinod, Karishma Bisht, Archana Chauhan, S. Gopala Krishnan, Prolay K. Bhowmick, M. Nagarajan¹, D. Sanjeeva Rao², R. K. Ellur and A. K. Singh*

Division of Genetics, ICAR-Indian Agricultural Research Institute (IARI), New Delhi 110 012; ¹ICAR-IARI and Genetics Research Centre, Aduthurai, India, ²ICAR-Indian Institute of Rice Research (IIRR), Hyderabad

(Received: November 2019; Revised: January 2020; Accepted: February 2020)

Abstract

We evaluated a set of 190 genotypes for five grain-nutrient, five grain physico-chemical quality and four derived traits. Wide variability was observed for all the traits studied, with CV ranging from 9.33 to 51.89%. No correlation was observed between grain nutritional and physico-chemical quality traits. Iron (Fe) and Zinc (Zn) content in brown rice showed significant positive correlation. Zn content in brown and polished rice showed significant association however, the Fe content in brown and polished rice did not show significant association. Two principal components each in grain nutritional and physico-chemical traits explained 73.15 % and 62.82 % of the total variation, respectively. Cluster analysis grouped the accessions into two and three clusters on the basis of grain physico-chemical traits and nutritional traits, respectively. The study has led to the identification of promising donors for biofortification and grain quality improvement in rice.

Introduction

More than half of the global human population is dependent on rice as their major food staple. Rice is grown in an area of about 158 million hectares spread across more than hundred countries worldwide, accounting to an annual production of 700 million tonnes of paddy equivalent to 470 million tonnes of milled rice (Ricepedia, IRRI). About 90 % of the world's rice is produced as well as consumed in the South and Southeast Asian countries (Bollineni et al. 2014). Per capita consumption in the rice dependent nation is in the range of 62-192 kg of rice per year providing about 20% of the per capita energy and 13% of dietary protein. Among the rice growing countries, India stands next to China as the second largest producer with an annual production of 104 million metric tons of milled

rice harvested from an area of 44 million hectares.

Rice quality is judged by three components, (a) shape and appearance, (b) cooking and organoleptic characteristics and (c) nutritional quality. Shape and size are the two most important visual characteristics of the rice grains that influence consumers' preferences and thus are the primary selection criteria in varietal improvement programs. Grain size is primarily assessed by the grain length, while the grain shape is determined on the basis of the length-width ratio. Internationally, on the basis of kernel length, rice grains are classified as extra-long (>7.5mm), long (6.61-7.5mm), medium (5.51-6.6mm) and short (<5.5mm) as per the standard evaluation system (IRRI 2013). Further, the grain shape is grouped into four classes, as slender (>3.0), medium (2.1-3.0), bold (1.1-2.0) and round (<1.0) based on the length-to-width ratio (Dela Cruz and Kush 2000). Cooking and organoleptic characteristics include cooked kernel length, elongation ratio (ER), apparent amylose content (AAC), gel consistency (GC) and gelatinization temperature (GT). These parameters determine the important attributes of cooked rice that influence the repeated purchasing behaviour of the consumer. Cooked kernel length and elongation ratio decides the volume of cooked rice, a major determinant of the food quantity recovery from the raw grains. Amylose fraction of the starch is the primary regulator in determining the cooking and eating quality of rice. Higher amylose content (>25%) makes cooked rice hard upon cooling, less tender, dry and separate, while the cooked rice of low amylose varieties becomes sticky, soft and

*Corresponding author's e-mail: aks_gene@yahoo.com

glossy (Bao et al. 2006). The most preferred level of AAC is intermediate that ranges between 20-25%. The relationship between the firmness of the cooked rice texture and amylose content does not hold when the AAC is > 25 %. GC measures the cold paste-viscosity of milled rice flour on cooking. It complements AAC in distinguishing the cooked rice texture of high amylose rices (Cagampang et al. 1973; Juliano 1985; Juliano 1979).

Micronutrient malnutrition, also known as hidden hunger is a global problem, because of its adverse effect on societal health (Welch and Graham 2004). Lack of dietary supply of mineral micronutrients *viz.* iron (Fe) and zinc (Zn) are notably the most serious micronutrient deficiencies affecting two billion individuals worldwide (FAO 2013). This issue is more rampant in India (Ritchie et al. 2018) as it is home for nearly half of the world's micronutrient deficient population with its 58.5% children anaemic (IIPS 2016) and about 74% are at the risk of anaemia (FAO 2013). Rice though a staple food, has low level of Fe and Zn making the rice eaters to suffer from by Fe and Zn deficiencies. Hence, enhancing grain Fe and Zn content in rice can provide a sustainable solution to hidden hunger. Therefore, profiling nutritional quality of rice grain for Fe, Zn, protein and lipids is an important to identify potential donors for use in breeding programme.

India is one of the centres of diversity for rice both at inter- and intraspecific levels. However, the rapid spread of semi-dwarf, input responsive, high yielding rice varieties resistant to pests and diseases during the green revolution had led to the replacement of several locally adapted low yielding landraces leading to erosion of genetic diversity. Continuous breeding and selection from the crosses of genetically related parents had further augmented this narrowing down. Green revolution primarily focussed on poverty alleviation, through improving the yield potential of rice and little importance was given to the grain physico-chemical and nutritional quality. Due to this, most of the early green revolution rice cultivars had poor quality grains and low nutrient status. Although, the grain physicochemical traits were improved subsequently, much focussed attention is needed for improvement of nutrient content. Successful genetic improvement for any trait relies on the variability in the crop gene pool. Analysis of genetic diversity helps in the identification of suitable parents for breeding programme, as well as in the maintenance and utilisation of desirable variation in breeders' activities. Besides, it helps in enhancing our understanding on

the crop evolutionary pattern. In this study, we have chosen to evaluate a set of 190 rice accessions uncharacterised for the grain quality parameters and sourced from different parts of India, for assessing the magnitude and pattern of genetic variability.

Material and methods

A set of 190 genotypes that include five check varieties *viz.*, IR64, Swarna, Pusa Sugandh 5, Kalanamak and Chittimutyalu was used in the evaluation. The genotypes are coded as GP1 to GP190 serially as per details given in the Supplementary Table 1. The germplasm included local landraces collected from various places across the country, and being maintained in the Division of Genetics, ICAR-Indian Agricultural Research Institute (ICAR-IARI), New Delhi. All the accessions were grown in field in an augmented randomized complete block design (Federer 1956), at

Table 1. Descriptive statistics for the grain nutritional and physico-chemical quality traits based on 190 rice accessions

Variable	Minimum	Maximum	Mean	SD	CV %
Nutritional quality traits					
Fe-BR	6.50	23.10	12.77	3.30	25.84
Fe-PR	0.80	12.30	3.70	1.92	51.89
Fe-Ret	5.39	96.25	30.29	15.59	51.47
Zn-BR	13.00	46.20	22.85	5.73	25.07
Zn-PR	8.20	40.90	17.97	5.37	29.90
Zn-Ret	57.30	96.80	78.05	7.84	10.05
GPC	3.43	10.84	7.57	1.14	15.11
Physicochemical traits					
KLBC	3.33	8.00	5.33	0.83	15.61
KWBC	1.33	2.37	1.73	0.16	9.33
LW Ratio	2.00	5.10	3.11	1.33	16.62
KLAC	5.23	17.22	7.99	0.36	14.54
KWAC	1.67	3.27	2.44	0.20	13.48
ER	1.02	2.33	1.51	21.87	40.09
GC	21.00	122.50	54.54	0.58	18.54

Fe-BR = Fe content of brown rice in µg/g; Fe-PR = Fe content of polished rice in µg/g; Fe-Ret = Fe content that is retained in polished grains in %; Zn-BR = Zn content of brown rice in µg/g; Zn-PR = Zn content of polished rice in µg/g; Zn-Ret = Zn content that is retained in polished grains in %; GPC = grain protein content in %; KLBC = kernel length before cooking in mm; KWBC = kernel width before cooking in mm; LWR = length-width ratio; KLAC = kernel length after cooking in mm; KWAC = kernel width after cooking in mm; ER = elongation ratio; GC = gel consistency in mm; SD = standard deviation; CV = coefficient of variation in %

the research farm of ICAR-IARI, New Delhi during *Kharif* 2017. The experimental field was divided into four blocks and each block consisted of 46 test genotypes and 6 checks. To ensure uniform germination and establishment, the seeds were sown on a raised seedbed and subsequently 30-day-old seedlings were transplanted into the experimental field. Each genotype was grown in a single row of 4 m length with a row to row spacing of 20 cm and plant to plant spacing of 15 cm. Recommended agronomic package of practices was followed for the proper field management during the experiment.

At the time of maturity, three individual plants per genotype were harvested and the grains were hand-threshed and dried to uniform moisture content both by open drying and subsequently in the hot air oven. The grains were cleaned free of impurities and ill-filled and discoloured spikelets and stored under aseptic conditions.

Evaluation of grain nutritional quality

The well-dried grains of uniform size and shape were dehusked using a non-ferrous and non-zinc de-husker. The brown rice was further polished with a Fe and Zn free polisher (Mini Lab Rice Polisher Model K-710, Krishi International). The polished rice kernels were thoroughly cleaned to remove residual bran using a non-shredding tissue paper. The broken kernels were also removed and only whole kernels were used for evaluation. The check varieties in each block were processed separately.

The rice kernels were scanned through X-ray fluorescence (XRF) for determining grain Fe and Zn concentrations. Prior to the analysis, all the samples were uniformly dried to keep the moisture content similar for all the test samples. Both raw and polished kernels were assessed separately. Polished rice kernels were thoroughly cleaned with tissue paper to remove any residual bran, before the analysis. In each case, 5g kernels were exposed to high energy X-rays (Rao et al. 2014) using the energy dispersive (ED) XRF spectrophotometer (OXFORD Instruments X-Supreme 8000). After excitation of the mineral ions in the grains, the characteristic secondary emission X-rays (fluorescence) was captured by the instrument and from the characteristic K lines for Fe and Zn in the fluorescence spectra the concentration of the elemental ions was estimated and displayed in parts per million unit (mg g^{-1}).

The total nitrogen content of brown rice was measured using the semi-micro-Kjeldahl method (CBS 1982) and was multiplied by a conversion factor of 5.95 to estimate total grain protein content (GPC). The samples were prepared by grinding the brown rice into a fine powder using a motor and pestle and 100 mg of the flour was weighed into a 50 mL Kjeldahl flask to which 3 mL of concentrated H_2SO_4 and 0.5 g of the catalyst were added and digested for two hours. The samples were cooled to room temperature; distilled water was added, mixed properly and finally transferred for colourimetric analysis.

Evaluation of grain physicochemical quality

Ten polished whole kernels per genotype were lined up end-to-end on a graph paper without any gap and placed under a 10x photo-enlarger for measuring the average kernel length before cooking (KLBC). Similarly, kernel width before cooking (KWBC) was also measured by aligning the kernels sidewise. The average length and width of 10 kernels in millimetre (mm) was used for analysis. The LW ratio was then calculated using the average values of KLBC and KWBC.

The kernels used in the procedure above were soaked in distilled water for 30 minutes and were allowed to cook in a water bath at 100°C for 10 minutes. The cooked kernels were then cooled to room temperature. After removing excess water, all the ten fully cooked kernels were lined up on a graph paper and measured for kernel length after cooking (KLAC) and kernel width after cooking (KWAC). The average length and width of 10 cooked kernels in millimetre (mm) was used for analysis. Kernel elongation ratio (KER) was then calculated by dividing the average KLAC with average KLBC for each genotype.

Polished kernels were finely powdered in a micronizer mill. 100 mg of flour was placed in a 20 mL slim borosilicate glass test tube (1.6 x 15 mm) and 0.2 mL of ethanol containing 0.25 % thymol blue was added followed by 2 mL of 0.2 N KOH. The flour was dispersed uniformly by mixing in a cyclone mixer. The test tubes were placed in a water bath at $90\text{--}100^\circ\text{C}$ for 8 min. The samples were cooled to room temperature, vortexed and then placed in a low-temperature water bath at $0\text{--}2^\circ\text{C}$ for 20 minutes. The tubes were wiped off moisture and then laid horizontally on a graph paper for one hour. The length of the blue coloured gel from the inside bottom of the test tube to the gel front was measured as gel consistency.

Statistical analyses

All the analyses were performed using the software packages PTools v.1.4 and STAR 2.0.1 (IRRI 2014 a,b), R Studio v.1.1.453 and Microsoft Excel.

Analysis of variance (ANOVA) for the traits was carried out using the augmented RCB procedure. Genetic parameters like phenotypic coefficient of variation (PCV), the genotypic coefficient of variation (GCV), broad sense heritability (h_{bs}^2), genetic advance (GA) and genetic advance as a percentage of the mean (GAM) were calculated using the following formulae. From the ANOVA, the phenotypic variance (V_p) was obtained from the genotype mean squares (GMS) directly. The error mean square (EMS) was taken as the environmental variance (V_e). From this, the genotypic variance (V_g) was calculated as $V_g = V_p -$

V_e . GCV was estimated from the expression $\frac{\sqrt{V_g}}{\bar{X}} \times 100$, where \bar{X} , was the genotype mean. Similarly, PCV

was estimated from $\frac{\sqrt{V_p}}{\bar{X}} \times 100$. The heritability in broad

sense was calculated as $h_{bs}^2 \frac{\sqrt{V_g}}{\sqrt{V_p}} \times 100$, the genetic

advance as $GA = k \cdot h_{bs}^2 \sqrt{V}$, where k is the selection differential at 5 per cent selection pressure *i.e.* 2.06 and genetic advance as a percentage of mean,

$$GAM(\%) = \frac{GA}{\bar{X}} \times 100.$$

Estimation of genetic diversity

Analysis of principal components (PC) encompassing total variation for grain physicochemical traits and nutritional traits were derived separately. The correlation structure of the component traits was decomposed to components, which accounted for maximum variation progressively on a reducing scale. The components having eigenvalues exceeding one were identified as significant PCs. The factor coordinates of the genotypes were computed for the derived PCs. The factor-variable correlations (factor loadings) were used to compute the contribution of variables to individual PCs. The most influential traits were identified from their relative contribution to the first PC followed by second PC and so on. The contributions of genotypes to PCs were used to scatter them for identifying the genotypes associated with those variables determining the total variability in the data.

The factor coordinates for the genotypes were used for cluster analysis. Only PCs accounting up to 99% of the cumulative variation were used for the clustering process. Clustering was done using K-means clustering procedure, an unsupervised algorithm using a set of *a priori* K values and Euclidean distances. The optimum number of clusters was determined based on elbow method by plotting the inter-cluster deviation against the k value and determining the lowest k value at which the inter-cluster deviation is minimized. For the K sets, the mean intra-cluster distance, D(k) of the genotypes from the respective cluster centroid was worked out. D(k) will drop to zero as the number of clusters equals to the number of genotypes. By plotting the deviations of D(k) between adjacent K [D'(k)], the K at which D'(k) showed the flattening trend (the elbow) was taken as the optimal K. Cluster statistics of the clusters identified, were worked out.

Results

Estimation of variance components and genetic parameters

The coefficient of variation (CV) for grain nutritional traits varied from 15.11 % for GPC to 51.89 % for Fe in polished rice (Fe-PR) and from 9.33 % for KWBC to 40.09 % for ER among physico-chemical traits (Table 1). The GPC ranged from 3.43 % (CN 1268-7) to 10.84 % (Uphar). The trait Fe in brown rice (Fe-BR) varied from 6.5 to 23.1 mg g⁻¹ with a CV of 25.84 % while Fe-PR exhibited a maximum variance of 51.89 % with a range of 0.80-12.30 mg g⁻¹. The genotype Shah Pasand recorded highest Fe-BR concentration of 23.10 mg g⁻¹ but in polished rice it was only 3.50 mg g⁻¹. In contrast, the genotype IC 2127 exhibited as high as 12.30 mg g⁻¹ for Fe-PR while its Fe-BR was 16.20 mg g⁻¹, showing Fe retention of 75.9 % in the endosperm. The Fe retention on polishing varied from 5.40 % (Aziz Beoul) to 96.30 % (Kudrat 3). Zn concentration among genotypes ranged from 13.00-46.20 mg g⁻¹ in brown rice and 8.20-40.90 mg g⁻¹ in polished rice. The genotype Karupunnel exhibited the highest concentration of Zn both in brown rice (46.20 mg g⁻¹) and polished rice (40.90 mg g⁻¹) with a retention of 88.5 %, while the accession Sagar Damba exhibited the lowest concentration of Zn in brown rice (Zn-BR) (13.00 mg g⁻¹) and Zn in polished rice (Zn-PR) (8.20 mg g⁻¹). The per cent retention after polishing for grain Zn was much higher than the grain Fe content and it ranged from 57.3 % (Kanak) to 96.8 % (VOH-PCR-3113).

Among the physico-chemical traits, the KLBC ranged between 3.33 mm (C1268-7-10) and 8.00 mm (Pusa 1301) with an average of 5.33 mm and CV of 15.61 %. The KLAC ranged from 5.23 mm to 17.22 mm with an average of 8.0 mm and CV of 14.54 %. The longest cooked grain length was recorded in Pusa Basmati 1121 (17.22 mm), which also has recorded the maximum LW ratio (5.1). The CV for KWBC was 9.33 % while for KWAC was 13.48 %, with an average grain width of 1.73 mm and 2.44 mm before and after cooking, respectively. The average LW ratio was 3.11 with a CV of 16.62 %. The ER ranged from 1.02 in Pusa 1447 to 2.33 in Pusa Basmati 1121 with an average of 1.51 and very high CV (40.09 %). GC showed wide variation among the genotypes, with minimum of 21.00 mm (CR 246-16 and UPRI 2003-45) and a maximum of 122.50 mm (Khuch), and a CV of 18.54 %.

The PCV for the grain nutritional traits ranged from 15.19 % to 88.02 % while the GCV ranged from 15.16 % to 71.57 % (Table 2). Three of the five nutritional traits viz., Fe-BR, Fe-PR and Zn-BR recorded significantly higher PCV than GCV indicating

the role of environment. These traits showed moderately high level of broad sense heritability. The traits like Zn-PR and GPC, showed high heritability (<80%) with very close correspondence between PCV and GCV. Genetic advance as a percentage of the mean (GAM) ranged from 31.15 % (GPC) to 119.87 % (Fe-PR) and showed high values for all the nutritional traits.

Similar to the grain nutritional traits, the grain physico-chemical traits exhibited varying degree of genetic variance. The PCV ranged from 12.92 % (KWAC) to 41.62 % (GC) and the GCV ranged from 10.82 % (KWBC) to 40.99 % (GC). Corresponding heritability range was between 42.12 % (KWBC) and 97.60 % (KWAC). Among the seven-grain appearance and cooking quality traits, four (KLBC, LWR, KLAC and GC) were in the category of high PCV (> 20 %) while the rest showed medium level (10-20 %) of variation. In case of GCV, the traits LWR, KLAC and GC had high range, while the rest were in the category of medium GCV. Broad sense heritability ranged from 42.12 % (KWBC) to 97.60 % (KWAC) while GAM ranged from 14.47 % (KWBC) to 83.16 % (GC). All

Table 2. Parameters of genetic variance for the grain nutritional and physicochemical quality traits

Variable	V_g	V_e	V_p	PCV%	GCV%	h^2_{bs} %	GA	GAM
Nutritional traits								
Fe-BR	25.03	7.73	32.76	42.22	36.90	76.41	9.01	66.45
Fe-PR	9.32	4.78	14.11	88.02	71.57	66.11	5.11	119.87
Zn-BR	38.32	16.92	55.24	31.62	26.34	69.37	10.62	45.19
Zn-PR	26.23	0.38	26.60	29.23	29.02	98.58	10.47	59.36
GPC	1.31	0.01	1.31	15.19	15.16	99.51	2.35	31.15
Physicochemical traits								
KLBC	1.03	0.55	1.59	23.48	18.96	65.23	1.69	31.55
KWBC	0.04	0.05	0.08	16.68	10.82	42.12	0.25	14.47
LW Ratio	0.61	0.11	0.72	27.23	25.01	84.37	1.48	47.32
KLAC	4.24	4.54	8.78	35.70	24.80	48.26	2.95	35.49
KWAC	0.12	0.00	0.12	12.92	12.77	97.60	0.70	25.99
ER	0.05	0.03	0.08	17.84	14.54	66.45	0.38	24.42
GC	471.89	14.67	486.56	41.62	40.99	96.99	44.07	83.16

Fe-BR = Fe content of brown rice in $\mu\text{g/g}$; Fe-PR = Fe content of polished rice in $\mu\text{g/g}$; Zn-BR = Zn content of brown rice in $\mu\text{g/g}$; Zn-PR = Zn content of polished rice in $\mu\text{g/g}$; GPC = grain protein content in %; KLBC = kernel length before cooking in mm; KWBC = kernel width before cooking in mm; LWR = length-width ratio; KLAC = kernel length after cooking in mm; KWAC = kernel width after cooking in mm; ER = elongation ratio; GC = gel consistency in mm; V_g = genotypic variance; V_e = environmental variance; V_p = phenotypic variance; PCV = phenotypic coefficient of variation in %; GCV = genotypic coefficient of variation in %; h^2_{bs} = heritability in broad sense in %; GA = genetic advance; GAM = genetic advance as percentage of mean

PCV and GCV categories: 0-10% low; 10-20% medium; >20% high (Sivasubramanian and Menon, 1973)

Heritability categories: 0-30% low; 30-60% moderate; >60% high (Robinson et al. 1949)

GAM categories: 0-10% low; 10-20% moderate; >20% high (Johnson et al. 1955)

the physicochemical traits had high heritability, except for KWBC and KLAC, which showed moderate values. Similarly, except for KWBC which had moderate GAM, remaining traits were in the high GAM category.

Association among the traits

The direction and magnitude of correlation among the traits studied is depicted in Fig. 1. Among the grain nutritional parameters, Fe-BR showed a significant positive correlation with Zn-BR (0.74) and Zn-PR (0.71). While Zn-BR and Zn-PR were also strongly correlated (0.95), Fe-BR showed very minimal correlation with Fe-PR (0.16). The GPC did not show any association with both the mineral nutrients. Among the grain quality traits, KLBC had a significant positive correlation with KLAC (0.63) and LW ratio (0.81), while it showed a significant negative correlation towards ER (-0.33) and poor but negative correlation with KWAC. KWBC was positively correlated with KWAC (0.41) and negatively correlated with LW ratio (-0.53). KLAC was positively correlated to LW ratio (0.52) and ER (0.44), while KWAC was negatively correlated to LW ratio (-0.33). ER showed a negative association with LW ratio (-0.40).

Principal component analysis

Principal component analysis was used to understand how grain quality and nutritional parameters contributed to the total variability for these traits amongst the 190 rice accessions. The proportion of total variance explained by each PC, arranged in decreasing order of importance is presented in Table 3. For the two groups of traits, two PCs each having eigenvalues more than one were chosen as the most significant components. These two PCs explained almost 73.15 % of the total phenotypic variation among the accessions for five grain nutritional traits, while cumulative variation explained for the grain physico-chemical traits was 62.82 %. The first PC explained 52.85 % and 34.92 % of phenotypic variance for nutritional and physico-chemical traits, respectively while the respective variances explained by the second PC were 20.3 % and 27.9 %. Partitioning of eigenvalues of the significant PCs, the factor-variable correlations (factor loadings) indicated that all the nutritional traits had positive influence on PC1, while Fe-BR and GPC had negative influence towards PC2. Similar positive trend was observed for physico-chemical traits also except KWAC for PC1 and KLBC for PC2 which had influence on the negative direction.

Eigenvectors, the coefficient of orthogonal transformation and the degree of influence of the

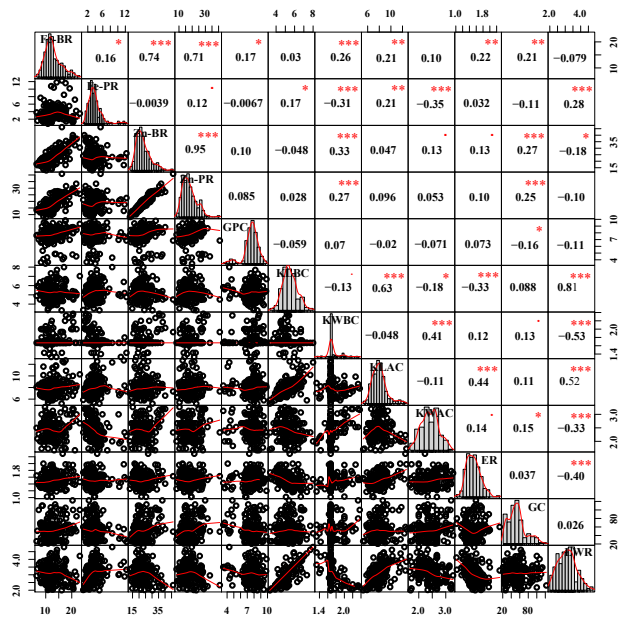


Fig. 1. Correlogram of grain physico-chemical and nutritional parameters from the rice germplasm set used in the study. The upper diagonal shows the correlation coefficients. The diagonal cells show the histogram of the traits. Physico-chemical traits are kernel length before cooking in mm (KLBC), kernel width before cooking in mm (KWBC), kernel length after cooking in mm (KLAC), kernel width after cooking in mm (KWAC), elongation ratio (ER), gel consistency in mm (GC) and length-width ratio (LWR). The nutritional parameters are Fe content of brown rice in µg/g (Fe-BR), Fe content of polished rice in µg/g (Fe-PR), Zn content of brown rice in µg/g (Zn-BR), Zn content of polished rice in µg/g (Zn-PR) and grain protein content in % (GPC). *, **, *, correlation coefficients are significant at p<0.05, 0.01 and 0.001 respectively**

variables towards the factors are given in Table 4. Vectors of variable contributions to the physico-chemical and nutritional quality parameters towards major PCs are given in Fig. 2. The contributions are the squared eigenvectors scaled 100 times to bring into % scale. Variable contributions for nutritional traits show high influence of Zn-PR, Zn-BR and Fe-BR towards PC1 with values of 34.4 %, 34.1 % and 29 % respectively. Fe-PR was the most contributing trait in PC2 explaining as high as 75.5 % of the variation encompassed by the PC2, while GPC contributed towards 23.25 % of variation to the PC2. Among the physico-chemical variables, KLBC (46.58 %) and KLAC (46.3 %) remained the most contributing traits to PC1. Total variation of the PC2 is explained by

Table 3. Principal components (PCs) extracted for the grain quality traits based on the correlation structure and the factor-variable correlations (factor loadings)

Parameters	Principal components				
	PC1	PC2	PC3	PC4	PC5
Nutritional quality traits					
Standard deviation	1.63	1.01	0.99	0.57	0.21
Proportion of variance	0.53	0.20	0.19	0.07	0.01
Cumulative proportion (%)	52.85	73.15	92.63	99.14	100.0
Eigenvalue	2.64	1.02	0.97	0.33	0.04
Factor loadings					
Fe-BR	0.876**	-0.042	0.056	-0.477**	-0.017
Fe-PR	0.156*	-0.875**	0.451**	0.072	0.022
Zn-BR	0.954**	0.102	-0.174	0.165*	0.151*
Zn-PR	0.950**	-0.021	-0.128	0.249**	-0.139*
GPC	0.198**	0.486**	0.849**	0.065	0.000
Physicochemical quality traits					
Standard deviation	1.32	1.18	0.96	0.79	0.57
Proportion of Variance	0.35	0.28	0.18	0.12	0.07
Cumulative Proportion	34.92	62.82	81.07	93.50	100.00
Eigenvalues	1.75	1.40	0.91	0.621	0.33
Factor loadings					
KLBC	0.902**	-0.070	0.059	0.121	0.405**
KWBC	0.152*	0.740**	0.436**	-0.489**	0.021
KLAC	0.899**	0.040	0.090	0.149*	-0.400**
KWAC	-0.238**	0.783**	0.099	0.564**	0.037
GC	0.211**	0.477**	-0.837**	-0.163*	0.001

Fe-BR = Fe content of brown rice in µg/g; Fe-PR = Fe content of polished rice in µg/g; Zn-BR = Zn content of brown rice in µg/g; Zn-PR = Zn content of polished rice in µg/g; GPC = grain protein content in %; KLBC = kernel length before cooking in mm; KWBC = kernel width before cooking in mm; KLAC = kernel length after cooking in mm; KWAC = kernel width after cooking in mm; GC = gel consistency in mm. Eigenvalues in boldface indicate most significant principal components.

*,** significant at $p < 0.05$ and $p < 0.01$ respectively

KWAC (43.98 %), KWBC (39.24 %).

Similarly, the influence of genotypes on the PCs was determined from their PC scores, which was used to disperse them (Fig. 3). In case of nutritional traits, Karuppunel (GP44) showed maximum influence on PC1, while IC2127 (GP151) showed greater influence on PC2. There were a distinct set of seven genotypes that showed significant effect on PC2. Pusa Basmati 1121 (GP125) was distinctly separated on PC1 in case of grain physicochemical traits, followed by Pusa1301 (GP133), PRR121 (GP86), PRR115 (GP83) and on the PC2 axis, Khuch (GP62) followed by Mehvan (Purple) (GP182) were the most influential genotypes.

In this case also, distinct grouping of genotypes that has differential influence on the major axes could be identified.

Cluster analysis and diversity

Cluster statistics and diversity parameters obtained are given in Table 5. The elbow points based on the inter-cluster deviation grouped 190 genotypes into three and two clusters on the basis of grain nutritional traits and physicochemical traits respectively (Fig. 4). Basing nutrition quality (Fig. 5a), Cluster 2 was the largest with 115 genotypes (60.5%) followed by cluster 1 with 49 genotypes (25.8%) and cluster 3 with 26 genotypes

Table 4. Eigenvectors of the quality variables and their contributions towards the significant principal components

Particulars	Eigenvectors		Contribution (%)	
	PC1	PC2	PC1	PC2
Nutritional quality traits				
Fe-BR	0.539	-0.042	29.05	0.17
Fe-PR	0.096	-0.869	0.93	75.50
Zn-BR	0.587	0.102	34.41	1.03
Zn-PR	0.584	-0.021	34.13	0.04
GPC	0.122	0.482	1.48	23.25
Physicochemical quality traits				
KLBC	0.682	-0.059	46.58	0.35
KWBC	0.115	0.626	1.32	39.24
KLAC	0.680	0.034	46.30	0.12
KWAC	-0.180	0.663	3.24	43.98
GC	0.160	0.404	2.56	16.30

Fe-BR = Fe content of brown rice in µg/g; Fe-PR = Fe content of polished rice in µg/g; Zn-BR = Zn content of brown rice in µg/g; Zn-PR = Zn content of polished rice in µg/g; GPC = grain protein content in %; KLBC = kernel length before cooking in mm; KWBC = kernel width before cooking in mm; KLAC = kernel length after cooking in mm; KWAC = kernel width after cooking in mm; GC = gel consistency in mm. Contribution % = Eigenvector² x 100

(13.7%). Cluster 3 exhibited the highest average inter-cluster distance from cluster 2 (1.33). Comparing the cluster means, Cluster 3 exhibited the highest mean for Zn-BR (33.39) followed by Zn-PR (27.37) and Fe-BR (18.42) while the highest mean value for Fe-PR was observed in cluster 1 (5.5). The contribution of GPC to all the three clusters was almost similar. For the grain physico-chemical traits (Fig. 5b), Cluster 2 was the largest with 127 genotypes (66.8 %) while cluster 1 consisted of 63 genotypes (33.2 %). Cluster 1 exhibited highest mean values for KLAC (9.37 mm), KLBC (6.16 mm), LWR (3.59) and GC (59.26 mm) while KWAC recorded highest mean in cluster 2 (2.52 mm).

Patterning of diversity of germplasm set for the traits, the distribution of diverse genotypes in the clusters was at a level of 0.92 evenness for nutritional traits and 0.84 for the physico-chemical quality traits. However, Shannon-Weiner diversity index of the grain nutritional traits was 0.63 and for physico-chemical was 0.93.

Discussion

Understanding the genetic parameters of the complex grain quality traits is a prerequisite for the implementation of the plant breeding programmes

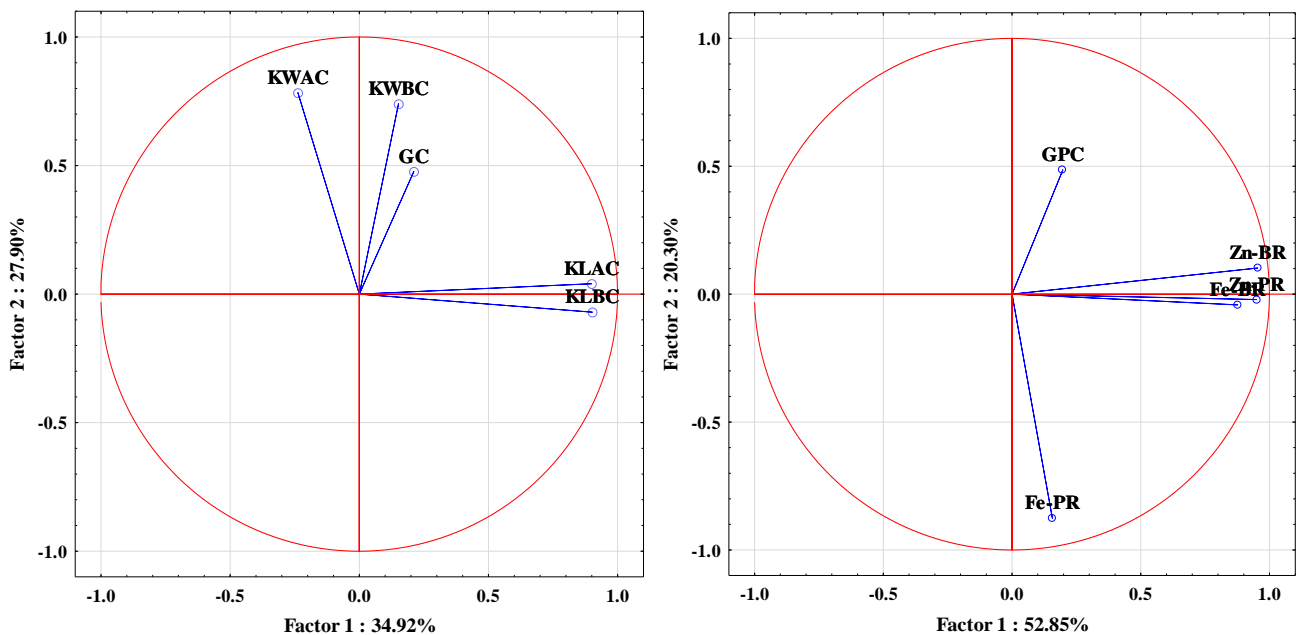


Fig. 2. Vectors of variable contributions towards physicochemical and nutritional quality parameters towards major principal components (PCs). The directions of the variable coordinates show the direction of their influence on PCs

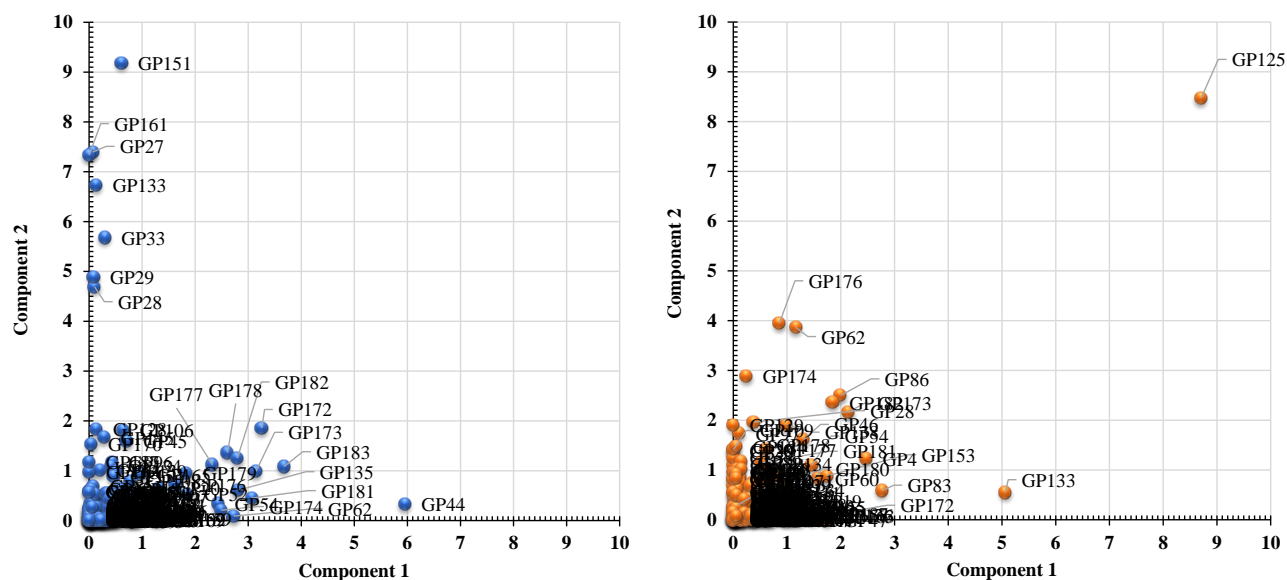


Fig. 3. Dispersion of genotypes based on their contribution towards major principal components (PCs) for (a) grain nutritional traits and (b) physicochemical traits. The most divergent genotypes had extreme phenotypes for the most contributing traits towards the respective PCs. Genotypes codes are provided in Supplementary Table 1

Table 5. Cluster statistics and diversity indices for grain quality traits based on principal component scores of genotypes

Particulars	Nutritional traits			Physicochemical traits		
	Cluster 1	Cluster 2	Cluster 3	Cluster 1	Cluster 2	
No. of genotypes	49.0	115.0	26.0	63.0	127.0	
Proportion %	25.8	60.5	13.7	33.2	66.8	
Dissimilarity	$d_{(1,2)} = 1.10$	$d_{(1,3)} = 1.22$	$d_{(2,3)} = 1.33$	$d_{(1,2)} = 1.00$		
Evenness	0.92			0.84	-	
Shannon-Weiner DI	0.63			0.93	-	
Cluster means	Fe-BR	14.19	10.89	18.42	-	-
	Fe-PR	5.50	3.10	2.98	-	-
	Zn-BR	25.06	19.53	33.39	-	-
	Zn-PR	20.65	14.70	27.37	-	-
	GPC	7.83	7.39	7.86	-	-
	KLBC	-	-	-	6.16	4.91
	KWBC	-	-	-	1.74	1.73
	KLAC	-	-	-	9.37	7.37
	KWAC	-	-	-	2.28	2.52
	ER	-	-	-	1.53	1.51
	LW Ratio	-	-	-	3.59	2.88
	GC	-	-	-	59.26	52.20

Fe-BR = Fe content of brown rice in $\mu\text{g/g}$; Fe-PR = Fe content of polished rice in $\mu\text{g/g}$; Zn-BR = Zn content of brown rice in $\mu\text{g/g}$; Zn-PR = Zn content of polished rice in $\mu\text{g/g}$; GPC = grain protein content in %; KLBC = kernel length before cooking in mm; KWBC = kernel width before cooking in mm; LWR = length-width ratio; KLAC = kernel length after cooking in mm; KWAC = kernel width after cooking in mm; ER = elongation ratio; GC = gel consistency in mm; DI = diversity index

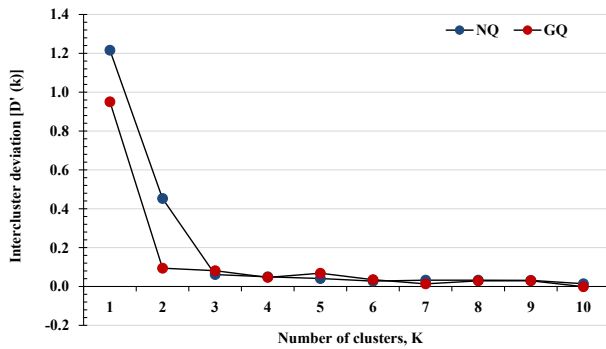


Fig. 4. Determination of optimum clusters by K-means clustering using the elbow method

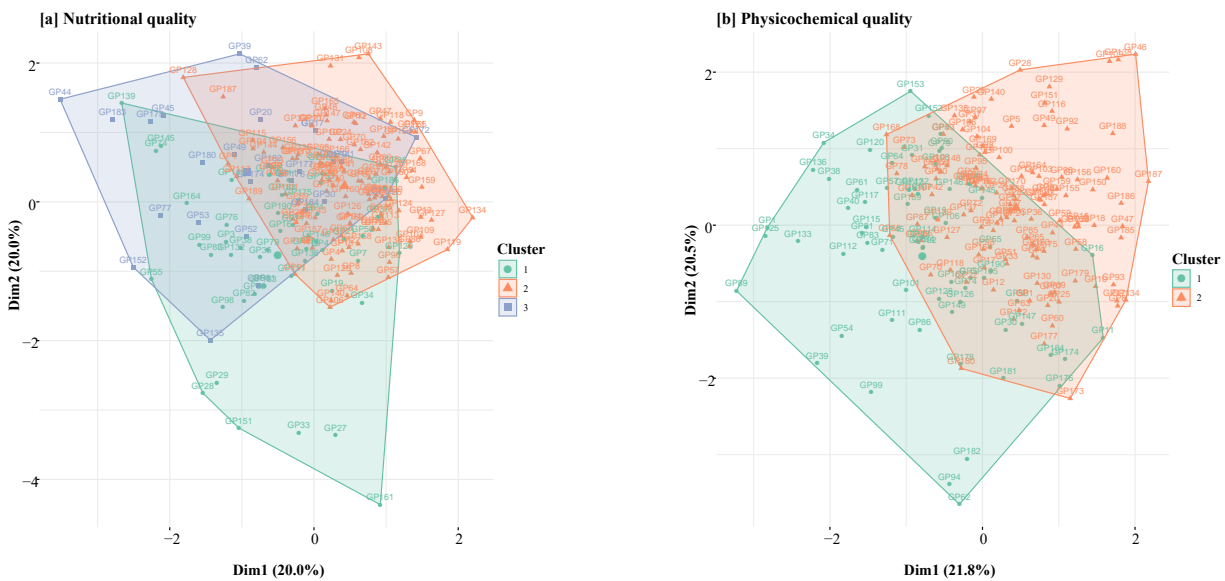


Fig. 5. Cluster-wise distribution of genotypes for [a] nutritional quality and [b] physicochemical quality traits based on principal component analysis

targeting quality improvement. The present study revealed the existence of moderate to high PCV and GCV for all the traits analysed. As per the Deshmukh et al. (1986) classification of PCV and GCV, Fe-BR, Fe-PR, Zn-BR and Zn-PR were in the category of high PCV and GCV (>20 %) while GPC was in the medium category (10-20 %). Similar results were reported by earlier studies on quality traits in Basmati rice (Singh et al. 2017), physico-chemical and cooking characteristics (Rani et al. 2019; Umadevi et al. 2010), for grain Fe and Zn in brown rice (Patil et al. 2015) and yield and its related traits (Osman et al. 2012; Khatun et al. 2015). The environmental effect on a trait is indicated by the magnitude of the difference between PCV and GCV. Of the 12 traits assessed in the study four traits viz., GPC, Zn-PR, GC and KWAC exhibited close correspondence between PCV and GCV depicting minimum influence of the environment on

the expression of these traits. This indicated that much of the total variation is contributed by the genetic factors and hence selection for these traits would be effective. Rest of the traits showed moderate influence of environments, which may require precision evaluation across the environments to make the selections effective. The distribution of the variability was in distinct groups for the grain nutritional traits, which had high richness than the grain physicochemical traits, and therefore, showed more categorical diversity and low Shannon-Weiner diversity index. Moreover, the Fe and Zn content in the bran and endosperm had

distinct pattern which might have formed diverse combinations in the germplasm set for these two traits. Alternatively, the physicochemical quality was almost singly determined by the grain length in the present germplasm assembly. Since, GC was distributed evenly across the clusters, it had very little role in categorising the genotypes. Therefore, the distinct characterisation was based on the extra-long slender grains of Basmati type which was emphasised by the high elongation ratio on cooking. However, since proportion of such genotypes was low in the assembly, Shannon-Weiner diversity index was high in the case of physico-chemical quality traits in the germplasm assembly.

The study depicted wide variation in grain size and shape which is a reflection on the effect of various allelic combinations of the genes governing grain

appearance in rice. In recent years several QTLs governing grain size were identified and few of them including GS3 (Fan et al., 2006; Mao et al. 2010), GS5 (Li et al. 2011), GW2 (Xian-Jun et al. 2007), qSW5/GW5 (Shomura et al. 2008; Weng et al. 2008), GW8 (Wang et al. 2012), qGL3/qGL3.1 (Qi et al. 2012; Zhang et al. 2012), GL7/GW7 (Wang et al. 2015; Wang et al. 2015), TGW6 (Ishimaru et al. 2013) and OsSPL14 (Jiao et al. 2010; Miura et al. 2010), OsSPL16 (Wang et al. 2012) were cloned and characterized. These studies deciphered the complex nature of grain appearance trait involving multiple signalling pathways like G-protein signalling pathways, ubiquitination followed by proteasomal degradation and phytohormones. Nevertheless, a comprehensive molecular mechanism underlying grain appearance still remains elusive. The vivid variations in physicochemical traits observed in the accessions used in the study indicated that the germplasm set constituted valuable genetic resources for allele mining studies to identify various allelic variants at a locus and determine the interactions among the alleles of different loci. It is pertinent here to emphasise that the genotypic constituents in the present assembly contained several landraces that are seldom cultivated on commercial scale.

Several studies have established that Fe is mostly localized in embryo and aleurone layer and very less Fe is distributed in the endosperm (Kaur et al. 2019; Kyriacou et al. 2014; Choi et al. 2007; Promu-thai et al. 2003). Conversely, a significant concentration of Zn is localized in endosperm and retained even after polishing. The wide variation for the quantum loss of these metal elements upon polishing among the 190 accessions shows the opportunity for selecting genotypes with reduced loss of Fe and Zn upon polishing, and also for their potential use in development of biofortified varieties. Harvestplus challenge programme working on biofortification of staple foods has set targets of 9-11 % of protein, 13 mg g⁻¹ of Fe and 28 mg g⁻¹ of Zn in polished rice to meet approximately 30% of Estimated Average Requirement (EAR) (HarvestPlus 2005; Andersson et al. 2017). Eight genotypes viz., Karuppunel, Budgi, Mehvan (green), Khuch, Mehvan (purple), Begum, Bala Kaun and PRR 109 recorded Zn concentrations above 28 mg g⁻¹ in polished rice. For grain Fe concentration, none of the accessions recorded >13 mg g⁻¹ of Fe in polished rice except for IC 2127 which has a value almost close to the HarvestPlus target. About eleven accessions had GPC in the target range. These accessions can be utilized as donors in rice

biofortification programs for enhancing the micronutrient status of rice endosperm. The landrace 'Karuppunel' was grouped in cluster 3 with the highest concentration of grain Zn in both brown (46.2 mg g⁻¹) and polished rice (40.9 mg g⁻¹) had recorded maximum distance of 1.88 from the cluster centre. Such varieties can be crossed with the genotypes from cluster 1 to combine high Zn content with high grain Fe in PR. Mapping populations are being developed using Karuppunel as a high Zn parent and efforts are underway to map the genomic regions governing high Zn accumulation in Karuppunel.

In conclusion, the study analysed the extent of genetic variability and diversity for grain nutritional and quality parameters in the primary gene pool of rice and identified promising donors for enhancing the nutritional status of rice endosperm *vis-a-vis* maintaining the superior quality and yield.

Authors contribution

Research idea (AKS, HB), experimental design (HB, PKB), research material contributions (AKS, HB, PKB, GKS), field and laboratory execution (HB, SR), data collection (HB, PKB, RKE, NM), analysis and interpretation of data (HB, KKV, AKS), script preparation (HB, AKS, KKV).

Declaration

The authors declare no conflict of interest.

Acknowledgements

The research work was carried out under the support of the project "Consortium Research Platform-Biofortification" funded by Indian Council of Agricultural Research (ICAR). We thankfully acknowledge the support of the funding agency.

References

- Bao J., Shen S., Sun M. and Corke H. 2006 Analysis of genotypic diversity in the starch physicochemical properties of nonwaxy rice: Apparent amylose content, pasting viscosity and gel texture. *Starch-Stärke*, **58**(6): 259-67.
- Bollinedi H., Krishnan S. G., Sundaram R. M., Sudhakar D., Prabhu K. V., Singh N. K., Pal M., Mishra S., Khurana J. P. and Singh A. K. 2014. Marker assisted biofortification of rice with pro-vitamin A using transgenic Golden Rice[®] lines: progress and prospects. *Indian J. Gen. PlantBreed. (The)*, **74**(4s): 624-630.
- Cagampang B. G., Perez C. M. and Juliano B. O. 1973. A gel consistency test for eating quality rice. *J. Sci. Food Agric.*, **24**: 1589-1594.

- Chinese Bureau of Standardization. 1982. Method for determination of crude protein in cereals and bean seeds (semi-micro-Kjeldahl method). GB 2905-32, UDC 633.1/8.
- Choi E. Y., Graham R. and Stangoulis J. 2007. Semi-quantitative analysis for selecting Fe- and Zn-dense genotypes of staple food crops. *J. Food Comp Anal.*, **20**: 496-505.
- Dela Cruz N. M. and Khush G. S. 2000. Rice Grain Quality Evaluation Procedures. In: Singh RK, Singh US, Khush GS, editors. *Aromatic Rices*. New Delhi, India: Mohan Pramlani, pp. 15-28.
- Fan C., Xing Y., Mao H., Lu T., Han B. et al. 2006. GS3, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. *Theor. Appl. Genet.* **112**: 1164-1171.
- FAO. 2013. *The State of Food and Agriculture, Food and Agriculture Organisation*. Rome.
- Federer W. T. 1956. Augmented (or hoonuiaku) designs. *Hawaii Plant Rec.*, **55**: 191-208.
- HarvestPlus. 2005. *Breeding crops for better nutrition*. Washington, DC: International Food Policy Research Institute. <http://www.harvestplus.org/>.
- IIPS. 2016. *India Fact Sheet - NFHS-4 (2015-16)*. Mumbai: International Institute for Population Sciences. Available online at: <http://rchiips.org/NFHS/pdf/NFHS4/India.pdf>.
- Ishimaru K., Hirotsu N., Madoka Y., Murakami N., Hara N., et al. 2013. Loss of function of the IAA-glucose hydrolase gene TGW6 enhances rice grain weight and increases yield. *Nat. Genet.*, **45**: 707-711.
- Jiao Y., Wang Y., Xue D., Wang J., Yan M. et al. 2010. Regulation of OsSPL14 by OsmiR156 defines ideal plant architecture in rice. *Nat. Genet.*, **42**: 541-544.
- Johson H. W., Robinson J. F. and Comstock R. E. 1955. Estimates of genetic and environmental variability in soybeans. *Agron. J.*, **47**: 314-318.
- Juliano B. O. 1979. The chemical basis of rice quality. In *Proe. of the Workshop on Chemical Aspects of Rice Grain Quality*. International Rice Research Institute, P. O. Box 933, Manila, Philippines, pp. 69-90.
- Juliano B. O. 1985. Criteria and tests for rice grain qualities. In: Juliano BO (ed) *Rice chemistry and technology*, 2nd edn. American Association of Cereal Chemists, Inc, MN, pp 43-524.
- Kaur K., Sohu V. S., Sharma A., Srivastava P. et al. 2019. Biofortification strategies to increase wheat nutrition and sustaining yield simultaneously. *Indian J. Genet.*, **79**: 15-24, doi: 10.31742/IJGPB.79.1.3.
- Kyriacou B., Moore K. L., Paterson D., de Jonge M. D., Howard D. L. et al. 2014. Localization of iron in rice grain using synchrotron X-ray fluorescence microscopy and high resolution secondary ion mass spectrometry. *J. Cereal Sci.* **59**(2): 173-180.
- Li Y., Fan C., Xing Y., Jiang Y., Luo L. et al. 2011. Natural variation in GS5 plays an important role in regulating grain size and yield in rice. *Nat. Genet.*, **43**: 1266-1269. doi:10.1038/ng.977.
- Mao H., Sun S., Yao J., Wang C., Yu S. et al. 2010. Linking differential domain functions of the GS3 protein to natural variation of grain size in rice. *PNAS*, **107**: 19579-19584 doi: 10.1073/pnas.1014419107.
- Miura K., Ikeda M., Matsubara A., Song X. J., Ito M. et al. 2010. OsSPL14 promotes panicle branching and higher grain productivity in rice. *Nat. Genet.*, **42**: 545-549.
- Osman K. A., Mustafa A. M., Ali F., Yonglain Z. and Fazhan Q. 2012. Genetic variability for yield and related attributes of upland rice genotypes in semi arid zone (Sudan). *Afr. J. Agric. Res.*, **7**(33): 4613-4619.
- Patil R. 2015. Genetic Diversity of Brown Rice for Iron and Zinc Content. *EJPB*, **6**: 196-203.
- Prom-u-thai C., Dell B., Thomson G. and Rerkasem B. 2003. Easy and rapid detection of iron in rice grain. *Science Asia*, **29**: 203-207.
- Qi P., Lin Y. S., Song X. J., Shen J. B., Huang W., et al. 2012. The novel quantitative trait locus *GL3.1* controls rice grain size and yield by regulating *Cyclin-T1*; 3. *Cell Res* **22**(12): 1666-80.
- Rao D., Madhu Babu P., Swarnalatha P., Kota S. and Bhadana V. P. 2014. Assessment of grain zinc and iron variability in rice germplasm using energy dispersive X-ray Fluorescence Spectrophotometer (ED-XRF). *Journal of Rice Research*, **7**: 1-2.
- Ritchie H., Reay D. S. and Higgins P. 2018. Quantifying, Projecting, and Addressing India's Hidden Hunger Front. *Sustain. Food Syst.*, <https://doi.org/10.3389/fsufs.2018.00011>.
- Robinson H. F., Comstock R. E. and Harvey P. H. 1949. Estimates of heritability and the degree of dominance in corn. *Agron. J.*, **41**: 353-359.
- Rani W., Noren S. K., Rai M., Tyagi W. and Khanna V. K. 2019. Physico-chemical characteristics and nutritional quality analysis of aromatic rice (*Oryza sativa* L.) genotypes. *Indian J. Genet.*, **79**: 641-650, doi: 10.31742/IJGPB.79.4.1.
- SES (Standard Evaluation System for Rice). 2014. IRRI, Manila, Philippines.
- Shomura A., Izawa T., Ebana K., Ebitani T. and Kanegae H. 2008. Deletion in a gene associated with grain size increased yields during rice domestication. *Nat. Genet.*, **40**: 1023-1028. doi:10.1038/ng.169.

- Singh M. K., Singh S., Nautiyal M. K., Pandey I. D. and Gaur A. K. 2017. Variability, heritability and correlation among grain quality traits in basmati rice (*Oryza sativa* L.). *IJCS*, **5**(5): 309-312.
- Sivasubramanian S. and Madhava Menon P. 1973. Genotypic and phenotypic variability in rice. *Madras agric. J.*, **60**.
- Umadevi M., Veerabathiran P., Manonmani S. and Shanmugasundaram P. 2010. Physico-chemical and cooking characteristics of rice genotypes. *EJPB*, **1**(2): 114-123.
- Wang S., Li S., Liu Q., Wu K., Zhang J., et al. 2015. The OsSPL16-GW7 regulatory module determines grain shape and simultaneously improves rice yield and grain quality. *Nat. Genet.*, **47**: 949-954.
- Wang S., Wu K., Yuan Q., Liu X., Liu Z. et al. 2012. Control of grain size, shape and quality by OsSPL16 in rice. *Nat. Genet.*, **44**: 950-954. doi:10.1038/ng.2327.
- Wang Y., Xiong G., Hu J., Jiang L., Yu H., et al. 2015. Copy number variation at the GL7 locus contributes to grain size diversity in rice. *Nat. Genet.*, **47**: 944-948.
- Welch R M. and Graham R. D. 2004. Breeding for micronutrients in staple food crops from a human nutrition perspective. *J Exp. Bot.*, **55**(396): 353-364 <https://doi.org/10.1093/jxb/erh064>.
- Weng J., Gu S., Wan X., Gao H., Guo T., et al. 2008. Isolation and initial characterization of GW5, a major QTL associated with rice grain width and weight. *Cell Res.*, **18**: 1199-1209.
- Zhang X., Wang J., Huang J., Lan H., Wang C., et al. 2012. Rare allele of OsPPKL1 associated with grain length causes extra-large grain and a significant yield increase in rice. *Proc. Natl. Acad. Sci.*, **109**: 21534-21539.

Supplementary Table 1. Details of germplasm used in the study

GP#	Genotype	Parentage	Year	Duration	Ecosystem	Adaptation area
GP 1	CR 2461-9	-	-	-	IRL	OD
GP 2	UPRI 2003-45	IR00A102/IR66452-179-2-6-4-1	-	-	-	-
GP 3	PNR 381	Tainan 3 mutant/ Basmati370	1992	85-105	RfU	WB
GP 4	Punjab Mehak 1	IR 70423-170-2-3 / IR 70446-85-3-2 // IR 70423-170-2-3	2009	125	-	PB
GP 6	Jayati	Rajeswari/ T 141	-	135	-	OD
GP 7	CT 10006-7-2M-5-1 P3-M	-	-	-	-	Columbia
GP 8	Chandratasini	Abhaya/ Phalguna	2007	120-125	RfU	CH
GP 9	HRP 2143	HRP 9020-2-2-2 1-1-1/ Phul Patas/ HUP 741	2006	125-135	IRL	HP
GP 10	MAS 946-1	IR64/ Azucena	-	120	RfU	KA
GP 11	PRR 126	-	-	-	IRL	PB
GP 12	Ajaya (RR 8585)	IET4141/ CR 98-7216	1992	130-135	IRM	AS, GO, PY
GP 13	Early Samba (RNRM 7)	Mutant of BPT5204	-	130-135	-	AP
GP 14	Phalguna	IR-8 / Siam-29	1978	140-145	CoIr	KA, AP
GP 15	VLT 6	-	-	-	-	-
GP 16	RIL 10	-	-	-	-	-
GP 17	Pant Dhan 19	BG 132/ UPRI 95-141	2007	130	IRL	PB, HR, GJ, MH
GP 18	NDR 8015-1	IR 72014-8 / NDR 1-1-1 B 53	-	-	-	UP
GP 19	Pusa Sugandh 3	Pusa 1238-1/ Pusa 1238-81-6	2002	125-130	IRL	PB, HR, DL, UK, UP
GP 20	Bala	N22/ TN1	1970	105	RfU	OD
GP 21	Pant Sankar Dhan 3	UPRI 9517A/ UARI 93287R	2006	126	IRL	UK
GP 22	Samanta	T90/ IR8/ Vikram/ Siam/// Mahsuri	1997	140	IRM	OD
GP 23	Tapaswini	Jagannath/ Mahsuri	-	135	-	HP
GP 24	HRP 2083	Landrace	-	-	-	HP
GP 25	Birupa	ADT 27/ IR 8 // Annapura	1992	-	RfU	OD
GP 26	Bhubana	-	1988	-	IRM	OD
GP 27	Pant Dhan 10	IR 32 // Mahsuri / IR 28	1993	125	IRM	UP
GP 28	OYR 128	-	-	-	-	KA
GP 29	OYC 183	-	-	-	-	-
GP 30	BJ 1 (Red Kl, Purple awns)	-	-	-	-	Bangladesh
GP 31	Chandana	-	1989	145	Ir	AP
GP 32	Urvashi	Sona/Manoharasali	-	135	-	-
GP 33	Pant Dhan 16	BG380/BG367-4	2001	-	-	-
GP 35	CSR 27	Nona Bokra/ IR565-33-2	1998	125	IRL	AI
GP 36	China 988	Introduction	1956	147-150	HIR	JK
GP 37	DV 85	AC 26903	-	-	-	Bangladesh
GP 38	IRAT 240 (IREM950)	Mutant of IAC25	1980	-	-	Guyana
GP 39	CR 2363-26	-	-	-	IRL	OD

Aro, aromatic; IRL, irrigated lowland; RfU, rainfed upland; IR, irrigated; IRM, irrigated medium; IRU, irrigated upland; IRSA, irrigated saline alkaline; SwL, swampy lands; CoS, coastal saline; - information not available; AI, all India; AP, Andhra Pradesh; AS, Assam; BH, Bihar; CH, Chattisgarh; DL, Delhi; GJ, Gujarat; HP, Himachal Pradesh; KA, Karnataka; KL, Kerala; OD, Odisha; MN, Manipur; MH, Maharashtra; MP, Madhya Pradesh; PB, Punjab; JK, Jammu & Kashmir; HR, Haryana; WB, West Bengal; CH, Chhattisgarh; PY, Pondicherry; TG, Telangana; UK, Uttarakhand; UP, Uttar Pradesh; -, no information available.

Details of germplasm used in the study (continued)

GP#	Genotype	Parentage	Year	Duration	Ecosystem	Adaptation area
GP 40	Selected Sabarmati	Landrace	-	-	-	-
GP 41	Cotton Dora Sannalu (MTU 1010)	Krishnaveni/ IR-64	-	120-125	IM	AP
GP 42	Kataktara	Landrace	-	-	RfU	-
GP 43	VOH-PCR-3110	-	-	-	-	-
GP 44	Karuppunel	Landrace	-	-	-	TN
GP 45	Kasturi	Basmati 370/ CR88-17-1-5	2007	120	-	BH
GP 46	CN 1268-7-10	Jaya/ Pusa Basmati 1	-	-	-	WB
GP 47	BJ 1	-	-	-	Ir	Bangladesh
GP 48	VL 88-97-1-7	-	-	-	-	-
GP 49	Kamlesh	-	-	-	IL	-
GP 50	IR 78908	-	-	116-120	RfU	Philippines
GP 51	B 6144-MR-6-0-0	Landrace	-	-	HIR	West Africa
GP 52	Seond Basmati	Landrace	-	-	Aro	HP
GP 53	HPR 2104	-	-	-	-	HP
GP 54	Shah Pasand	Landrace	-	-	Aro	-
GP 55	Chimbalate Basmati	Landrace	-	-	-	JK
GP 56	PMK 1	CO 25/ ADT 31	1982	120-125	RfL	TN
GP 57	Sitwa Dhan	Landrace	-	-	-	-
GP 58	Gouri	MO 4 (Bhadra) (Cul, 2533)	2006	115-120	IL	KL
GP 59	Ananga	Kumar (T 90/ IR 8) // CR 57-49	1989	120	IL	AI
GP 60	Pant Dhan 4	IR 262/ Remadja	1983	128-130	IL	UP, UK
GP 61	OYR 69	-	-	-	-	KA
GP 62	Khuch	Landrace	-	-	-	JK
GP 63	CR 2499	BG 90-2/IR 67962-84-2-2-2	-	-	IL	OD
GP 64	Pusa 1460	Pusa Basmati 1//Pusa Basmati 1//IRBB 55	2007	-	Aro	-
GP 66	UPRI 2003-24	-	-	-	-	-
GP 67	Poorima	Poorva/ IR-8608-298	1997	100-105	RfU	MP
GP 68	UPRI 2003-18	-	-	-	-	-
GP 69	Narendra Usar Dhan III	Lung YAI 148/ IR 9125-209-2-2-2-1 // IR 1872-27-3-1	2000	125-140	IrSA	UP
GP 70	NDR 359	BG-90-2-4/ 08677	-	-	Ir	UP
GP 71	NDR 97	N22/ Ratna	1991	100	RfU	UP
GP 72	Bhadrakali	Phalguna/ IR 36	1994	130-135	IM	AP
GP 73	Shiva	Phalguna/ IR-50	1997	130-135	RFL	AP
GP 74	IR 77384-12-35-3-6-7-2-B	-	-	-	-	-
GP 75	Narendra Usar Dhan II	IR1814/IR1366-120-3-1//IR1539-37-3-1	1997	130	IrSA	UP
GP 76	SKAU 220	-	-	-	-	JK

Aro, aromatic; IL, irrigated lowland; RfU, rainfed upland; HIR, hill rice; Ir, irrigated; IM, irrigated medium; IRU, irrigated upland; RfL, rainfed lowland; IrSA, irrigated saline alkaline; SwL, swampy lands; CoS, coastal saline; - information not available; AI, all India; AP, Andhra Pradesh; AS, Assam; BH, Bihar; CH, Chattisgarh; DL, Delhi; GJ, Gujarat; HP, Himachal Pradesh; KA, Karnataka; KL, Kerala; OD, Odisha; MN, Manipur; MH, Maharashtra; MP, Madhya Pradesh; PB, Punjab; JK, Jammu & Kashmir; HR, Haryana; WB, West Bengal; CH, Chhattisgarh; PY, Pondicherry; TG, Telangana; UK, Uttarakhand; UP, Uttar Pradesh; -, no information available.

Details of germplasm used in the study (continued)

GP#	Genotype	Parentage	Year	Duration	Ecosystem	Adaptation area
GP 77	PRR 105	-	-	-	-	-
GP 78	PRR 117	-	-	-	-	-
GP 79	PRR 103	-	-	-	-	-
GP 80	PRR 123	-	-	-	IrL	PB
GP 81	PRR 114	-	-	-	-	-
GP 82	PRR 120	-	-	-	-	-
GP 83	PRR 115	-	-	-	-	-
GP 84	PRR 104	-	-	-	-	-
GP 85	PRR 108	-	-	-	-	-
GP 86	PRR 121	-	-	-	IrL	PB
GP 87	Pant Dhan 18	IR 25394-3-57// RD 23// IR 27316-96// SPRLR 77205-3-2// SPRLR 79234-51-2 Pedigree- SPR 85163- 5-1-2-4 IR 56/ OR 142-99	2007	105-135	IrL	BH, WB, OD, CH, AP, KA, TN, KL
GP 88	Indravati	Pusa Basmati 1 /UPRM 500	2006	150	IrL	OD
GP 89	Pant Sugandh Dhan 17	-	-	135-140	IrL	UK
GP 90	HUR 200-57-1	-	-	-	-	-
GP 92	JGL 11727	JGL 420/ Vijetha	2012	135-140	IrL	AP
GP 93	Mahanadi	IR 19661-131/ Savitri	-	150	IrL	OD
GP 94	Pant Sugandh Dhan 15	Basmati 370/ Sudari// Behra// Muskan 41	2002	145	IrL	UP
GP 95	Bhuman San	Landrace	-	-	-	-
GP 96	JR 75	IR20/ L14//BSJ205	-	80-85	RfU	MP
GP 97	CO 37	TN 1/ CO 29	1978	115	IrL	TN, PY
GP 98	Sumati	Chandan / Pakistan Basmati	2002	140	-	-
GP 99	SAF 1221-83	-	-	-	-	-
GP 100	Pusa 1447	P1324/ Ajay	-	-	-	-
GP 101	Pusa 1447-00-5-1	P1324/ Ajay	-	-	-	-
GP 102	HUR105	Mutant of MPR7-2	-	-	-	-
GP 103	Manaswini	Swarna / Lalat	2008	130-135	Ir	EI
GP 105	CSR 23	IR64//IR4630-22-2-5-1-3//IR9764-45-2-2	2004	125-132	IrL, Rf	OD
GP 106	Kudrat 3	Selection from landrace	-	130-135	IRSA	MH, GJ, KE, TN, WB
GP 107	RAU 3061	Kasturi / Sugandha	-	120-135	-	UP
GP 108	NDR 625	Mutant of Badshah Pasand	-	-	-	BH
GP 109	Sagar Samba	IR 8 / Siam 29 // IR 8 / PTB 21	1993	150	Ir	UP
GP 110	JGL 3828	Samba Mahsuri/ Aganni	2009	135-150	RfL	AP
GP 112	Muskan	Landrace	-	-	IrL	TG
GP 113	Pusa 1342	P1154-2/ P1201-92-11	-	-	-	JK
GP 114	MR 219	MR 137/MR 151	-	-	Aro	-
GP 115	UPRI 2003-15	IR00A102/IR66452-179-2-6-4-1	-	-	-	Malaysia

Aro, aromatic; IrL, irrigated lowland; RfU, rainfed upland; Ir, irrigated; IrM, irrigated medium; IrU, irrigated upland; RfL, rainfed lowland; IRSA, irrigated saline alkaline; SwL, swampy lands; CoS, coastal saline; - information not available; AI, all India; AP, Andhra Pradesh; AS, Assam; BH, Bihar; CH, Chattisgarh; DL, Delhi; GJ, Gujarat; HP, Himachal Pradesh; KA, Karnataka; KL, Kerala; OD, Odisha; MN, Manipur; MH, Maharashtra; MP, Madhya Pradesh; PB, Punjab; JK, Jammu & Kashmir; HR, Haryana; WB, West Bengal; CH, Chhattisgarh; PY, Pondicherry; TG, Telangana; UK, Uttarakhand; UP, Uttar Pradesh; -, no information available.

Details of germplasm used in the study (continued)

GP#	Genotype	Parentage	Year	Duration	Ecosystem	Adaptation area
GP 117	Sharbati	Landrace	-	-	-	-
GP 118	HKR 200-57-1	-	-	-	-	HR
GP 119	WGL 23985	Kavya / AC20	2009	Early	IRM	AP
GP 120	Rajendra Basmati	-	-	-	-	-
GP 121	MR 220	MR 137/ MR 151	-	-	-	Malaysia
GP 122	Raja Vaellu	-	-	-	-	-
GP 123	CR 246-16	-	-	-	IRL	OD
GP 124	Phunchi	Landrace	-	-	-	JK
GP 125	Pusa Basmati 1121	Pusa 614-1-2/ Pusa 614-2-4-3	2003	140-145	-	PB, HR, UP, UK
GP 126	Improved Sabarmati	T(N)1/ Basmati 370/ Basmati 370	1972	-	-	PB, HR, West UP
GP 127	Tompha Khau	Landrace	-	-	-	MN
GP 128	CN 1268-7	-	-	-	IRL	WB
GP 129	WGL 14	BPT 5204 / ARC 5984 // BPT 3291	2005	135-140	IRM	AP
GP 130	Hema	T141/ IR8	1975	130-135	-	OD
GP 131	UPRVS 8-26	-	-	-	-	-
GP 132	Super Basmati	Basmati 320/ IR 661	2004	145	IRL	PB
GP 133	Pusa 1301	Improved Sabarmati/ Khalsa 7	-	135	Aro	-
GP 134	Kanak	Swarna/ IR 36// Mohan/ Khatish	2012	145	IRL	WB, BH, OD, MH, AP, KA
GP 135	PRR 109	-	-	-	-	-
GP 136	PRR 110	-	-	-	-	-
GP 137	PRR 127	-	-	-	IRL	PB
GP 138	PRR 122	-	-	-	-	PB
GP 139	PRR 118	-	-	-	-	-
GP 140	HUR 36	-	-	135	RfU	UP
GP 141	RR 166-645	-	-	130-135	IRL	OD
GP 142	Nirri	Mahsuri	1992	-	-	PB
GP 143	C 22	C22/CR 289-1208	-	-	-	Philippines
GP 144	DHMAS-70G-164-29	Tjerinas/BPI 76/Palawan/Azucena	-	130	RfU	-
GP 145	Ranbir Basmati	HPU741/ Tetep	-	-	IRL	-
GP 146	T23	Selection from Basmati 370- 90-95	1996	120-125	Aro	JK
GP 147	Lang Phou	Sel from Kala Sukhdas	-	-	-	HP
GP 148	ASD 19	Lang Phou	-	100	-	MN
GP 149	Basmati 370	Lalnakanda/ IR 30	1997	120-132	IRL	TN
GP 150	BPT 5204	Selection from Dehraduni Basmati	1973	150	IRL	HR
GP 151	IC 2127	GEB 24/ T(N) 1// Mahsuri	1979	-	IRL	AP
GP 152	VOH-PCR-3113	-	-	-	-	-
GP 153	Type 3	Landrace	-	-	-	UP

Aro, aromatic; IRL, irrigated lowland; RfU, rainfed upland; HR, hill rice; Ir, irrigated; IRM, irrigated medium; IRL, rainfed lowland; IRSA, irrigated saline alkaline; SwL, swampy lands; CoS, coastal saline; - information not available; AI, all India; AP, Andhra Pradesh; AS, Assam; BH, Bihar; CH, Chattisgarh; DL, Delhi; GJ, Gujarat; HP, Himachal Pradesh; KA, Karnataka; KL, Kerala; OD, Odisha; MN, Manipur; MH, Maharashtra; MP, Madhya Pradesh; PB, Punjab; JK, Jammu & Kashmir; HR, Haryana; WB, West Bengal; CH, Chhattisgarh; PY, Pondichery; TG, Telangana; UK, Uttarakhand; UP, Uttar Pradesh; -, no information available.

Details of germplasm used in the study (continued)

GP#	Genotype	Parentage	Year	Duration	Ecosystem	Adaptation area
GP 154	Nagina 12	Selection from landrace	-	-	-	UP
GP 155	Sonasa	Landrace	-	-	-	-
GP 156	Khara Munga	Landrace	-	-	-	-
GP 157	IR 70	IR 25604/ IR 9828	1988	-	-	Philippines
GP 158	ADT 39	IR 8/ IR 20	1989	119-125	IrL	TN
GP 159	Haidimuri	Landrace	-	-	-	-
GP 160	Swarna (MTU 7029)	Vasista/ Mahsuri	1987	150	IrL	OD, AP
GP 161	PDKV Chinnor 2	-	-	-	-	-
GP 162	Swarna Sub1	Swarna 3/ IR 49830-7-1-2-3	2009	140	-	AI
GP 163	Tilak Chandan	Landrace	2009	-	-	UK
GP 164	Pusa 1490-03	Heibao/ P1302-3-3-1-10-02-1	-	-	Aro	-
GP 165	Pratikshya	Swarna/ IR 64	2006	142	IrL, RfL	OD
GP 166	Jhulhat	Landrace	-	-	-	-
GP 167	NDR 9830144	IR 60185-B-25-2-2 / IR 57519-PMI-5-3-2-2 // IR 55008-10-3-3 - 3-3	2008	140-145	IrL	UP
GP 168	MTU 1001 (Vijetha)	MTU 5249/ MTU 7014	1995	120-125	IrM	AP
GP 169	Bameshwari	RP 2154-40-1/ IR 9828-23 2	2002	130-135	IrM	MP, CH, UP
GP 170	Ramachandi	IR 17494-32-2-2-1 / Jagannath	1998	160	RfL	OD
GP 171	Apo	UPL RI 5/ IR 12979-24-1	2012	120	RfU	OD, CH
GP 172	Aziz Beoul	Landrace	-	-	HIR	JK
GP 173	Begum	Landrace	-	-	HIR	JK
GP 174	Bala Koun	Landrace	-	-	HIR	JK
GP 175	Buta Baber	Landrace	-	-	HIR	JK
GP 176	Baber	Landrace	-	-	HIR	JK
GP 177	Baber Safed	Landrace	-	-	HIR	JK
GP 178	Budgi	Landrace	-	-	HIR	JK
GP 179	Gull Baber	Landrace	-	-	-	JK
GP 180	Kaw Qudder	Landrace	-	-	-	JK
GP 181	Kew	Landrace	-	-	-	JK
GP 182	Mehvan (purple)	Landrace	-	-	-	JK
GP 183	Mehvan (green)	Landrace	-	-	-	JK
GP 184	Mir Zag	Landrace	-	-	-	JK
GP 185	CO 50	CO 43 / ADT 38	2010	130-135	IrM	TN
GP 186	CO 51	ADT 43/ RR 272 – 1745	2005	105-110	IrL	TN
GP 187	Arupathaam Kuruvai	Landrace	-	60	IrL	TN
GP 188	Improved Samba Mahsuri	Samba Mahsuri*4/SS1113	208	140-145	IrL	AP, TN, GH
GP 189	Sabour Surbhit (RAU 3036)	Mutant of Rajendra Suhasini	2017	92-95	IrM	BH
GP 190	North Andaman 2	-	-	-	-	AN

Aro, aromatic; IrL, irrigated lowland; RfU, rainfed upland; HIR, hill rice; Ir, irrigated; IrM, irrigated medium; IrU, irrigated upland; IrSA, irrigated saline alkaline; SwL, swampy lands; CoS, coastal saline; - information not available; AI, all India; AP, Andhra Pradesh; AS, Assam; BH, Bihar; CH, Chattisgarh; DL, Delhi; GJ, Gujarat; HP, Himachal Pradesh; KA, Karnataka; KL, Kerala; OD, Odisha; MN, Manipur; MH, Maharashtra; MP, Madhya Pradesh; PB, Punjab; JK, Jammu & Kashmir; HR, Haryana; WB, West Bengal; CH, Chhattisgarh; PY, Pondicherry; TG, Telangana; UK, Uttarakhand; UP, Uttar Pradesh; - no information available.