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Characterization of rice genotypes having indigenous and exotic origin for grain quality traits

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Abstract
450 exotic and indigenous rice germplasm were screened for reproductive stage drought stress under natural field conditions out of which 30 genotypes were selected for further study. The present study was undertaken for characterization of the selected rice genotypes, along with 8 checks, for nine important agro-morphological and grain quality traits. ANOVA revealed significant differences among the genotypes for all the variables observed. In general, all the traits under study were found to be having a decreasing trend under reproductive stage drought. The line Pakistanhmwe and Lwankhan showed highest and lowest grain length (mm), respectively. Dubraj was reported having maximum amylose content of 25.44% while it was very low, only 4.72%, for the genotype ARC 11322.

Keywords: Amylose, drought, grain quality, rice, stability

Introduction
Rice (*Oryza sativa* L.) (2n=2x=24) is one of the oldest cultivated crops and a staple diet for nearly half of the world's population. Globally rice is cultivated over 150 million hectares. It is the most important cereal crop in India (Annual Report, 2019-2020, Ministry of Agriculture and Farmers Welfare, Govt. of India). India is the 2nd largest rice producing country after China and followed by Indonesia. In India, West Bengal is the Bihar holds 6th rank in rice production (Bhandari *et al*., 2020) [24]. There are several natural factors or environmental stresses which are limiting to rice production every year. Among these, drought is the most important. Rice is the most sensitive crop for drought at reproductive stage which affect rice yields drastically (Prasad *et al*., 2013; Singh *et al*., 2014; Khare *et al*., 2014) [25, 26, 27]. Due to moderate to severe drought, failure of the rice crop is a common phenomenon in several Indian states. In this instance, Bihar is in the worst condition possible because rice is simultaneously impacted by both flooding and drought. Moreover, direct seeded rice (DSR) adoption is negatively impacted by the drought conditions.

There may be dry spells at any point during the crop growth cycle. In response to drought stress, rice plants must compromise with photosynthates and go through a number of physiological and biochemical changes that have the potential to impact the quality of the rice that is produced in such conditions (Mishra *et al*., 2013) [28]. Since rice is typically eaten whole, grain quality and consumer preference are intimately correlated. Therefore, research into the grain quality features of genotypes that are drought-tolerant is crucial. Key quality feature of rice grain includes amylose content, grain L/B ratio, hulling and milling percentage, grain appearance, cooking quality, and nutritional elements, etc. Keeping all these things in view, the present study was undertaken to characterize 30 rice genotypes, selected out from screening of 450 indigenous and exotic germplasm under reproductive stage drought stress, along with 8 checks to identify suitable genotype (s).

Materials and Methods
The study was undertaken during Kharif season of 2019 and 2020 at the research farm of the Rice Section, Department of Plant Breeding & Genetics, Bihar Agricultural University, Sabour-813210 (Bhagalpur) Bihar. The research field was located in the Middle Gangetic Plain which belongs to Agro-climatic Zone IIIA of Bihar. The soil was light, sandy loam with a pH range of 7.5 to 7.4. Weather data collected during June to November, 2020 is presented in Table-1. Whole study was done in two parts.

In the first part, a group of 450 exotic and indigenous rice germplasm including checks were evaluated for drought tolerance under field conditions from where 30 genotypes were selected for further evaluation. In second part of the study, selected 30 rice genotypes along with eight checks were evaluated for nine agro-morphological and grain quality traits *viz*. days to 50% flowering, plant height (cm), panicle length (cm), number of tillers plant^{-1}, grain yield plot^{-1} (g), number of filled grain panicle^{-1}, number of unfilled grain panicle^{-1}, total number of grains panicle^{-1} and panicle fertility percentage. The results of the present research article are based on second part of the study.

Length and width of five randomly selected dehusked grains were calculated in millimeters with the help of Vernier Caliper then mean was estimated. Ratio of the length by the breadth of each of the grain was calculated and the average was taken. At the time of maturity number of filled grains and number of unfilled grains per panicle was taken and then total number of grains per panicle was calculating by adding filled and unfilled grains. Fertility Percentage was calculated using the following formula.

$$\text{Fertility Percentage} = \frac{\text{number of filled grains per panicle}}{\text{total no.of grains per panicle}} \text{ x } 100$$

For the Hulling percentage, 100 g of sample was weighed after the removal of other foreign matters from the sample. After hulling, weight of de-hulled grains of the sample was estimated and calculated with the help of formula given below.

$$\text{Hulling \%} = \frac{\text{Weight of the dehusked kernel (g)}}{\text{Weight of paddy (g)}} \text{ x } 100$$

For milling percentage, the hulled samples were milled and weight of milled grains was estimated. Then milling % was calculated with the help of formula given below.

$$\text{Milling \%} = \frac{\text{Weight of polished kernel (g)}}{\text{Weight of paddy (g)}} \text{ x } 100$$

Amylose is the linear fraction of starch in grains. Amylase content is estimated for calculation of amylose in grains. For estimation of amylase content, 100 mg grinded milled rice flour was taken in test tube and kept overnight for digestion after adding 1 ml 99% ethanol and 9 ml 1N NaOH. This digested product kept for boiling in water bath for 10-15 minutes then shaked vigorously with vortex mixer. After making volume up to 100 ml in standard flask with hot water, 5 ml from this was taken in standard flask. Then 1 ml 1 N acetic acid and 2 ml 0.2% iodine solution was added and it was made up volume up to 100 ml. Then blue color was obtained and read at 620 nm. These colors could be categorized as follows-

Blue: High-amylose rice, which cooks up firm and fluffy
Grayish blue: low amylose rice, which is soft and moist after cooking

Before this, standard solution was made by taking 40 mg amylose standard and follow the same procedure and then concentration gradient are made individually in test tube or flask 1 ml, 2 ml, 3 ml, 4 ml, 5 ml, 6ml, 7ml and blank by adding acetic acid 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1 ml, 1.2ml,1.4ml, and for blank 1 ml respectively with adding 2 ml iodine in each and made-up volume 100 ml with distilled water. Then reading was taken along with samples and amylose content was calculated in percentage by using following formula as suggested by.-

$$\text{Amylose \%} = \frac{(\text{OD}-0.039)}{0.0125} \text{ x } 100$$

Where, OD= absorbance at 620 nm.

Grain types for the genotypes were decided by comparing grain length (mm) to the L/B ratio as mentioned in Table-2. On the basis of visual observations of dehulled grains, grain appearance was decided on the basis of grades as described in the Table-2.

Chalkiness is related to the texture of starch in rice grains. It is defined as the opaque portion present in an endosperm of rice grain which otherwise remains translucent white. Degree of chalkiness was recorded for the milled rice sample with respect to (a) white core (b) white belly and (c) white back.

Correlation among rice quality traits

Results and Discussion

Data for nine grain quality traits namely grain length (mm), grain breadth (mm), grain L/B ratio, chalkiness, hulling percentage, milling percentage, amylose content (%), grain type and grain appearance was recorded on five panicles collected from primary tillers of five randomly selected plants for each genotype. The data obtained was subjected for analysis as per Augmented RCBD design using. ANOVA revealed significant differences among the genotypes for all the grain quality traits under study (Table-1).

Table 1: ANOVA of Augmented RCBD design for grain quality traits in 38 rice genotypes

Source of Variation	Degree of Freedom	Grain Length (mm)	Grain Breath (mm)	Grain L/B Ratio	Chalkiness	Hulling %	Milling %
Block (ignoring Treatments)	1	0.731	0.005	0.121 *	34.783 *	1.857	47.590 **
Treatment (eliminating Blocks)	37	1.565 **	0.082	0.462 **	6.053 *	10.527 *	38.976 **
Checks	7	0.520	0.040	0.125 *	6.714	17.264 **	82.510 **
Checks+Var vs. Var.	30	1.808 **	0.092 *	0.541 **	5.899	8.955 *	28.818 **
Error	7	0.156	0.027	0.021	4.429	2.274	3.204
Block (eliminating Check+Var.)	1	0.031	0.041	0.045	25.000 *	1.195	1.740
Entries (ignoring Blocks)	37	1.584 **	0.082	0.464 **	6.317 *	10.545 *	40.215 **
Checks	7	0.520	0.040	0.125 *	6.714	17.264 **	82.510 **
Varieties	29	1.797 **	0.093 *	0.527 **	5.499	9.243 *	31.207 **
Checks vs. Varieties	1	2.848 **	0.027	1.018 **	27.272 *	1.281	5.394
Error	7	0.156	0.027	0.021	4.429	2.274	3.204

Grain length was found ranging between 4.60 mm to 7.91 mm with average value of 6.25 mm. The maximum grain length of 7.91 mm was recorded by Pakistanhmw which was statistically at par with the check variety Sabour Deep (LC-2) (7.06 mm) having maximum grain length while minimum grain length was recorded by the genotype Lwankhan (4.60 mm) which was also at par with the check Sabour Shree (LC-1) (5.70 mm) having lowest grain length. Range of grain breadth was reported from 1.40 mm to 2.95 mm with average value of 2.17 mm. Genotype namely CAC-75 (2.95 mm) recorded maximum grain breadth which was statistically at par with the check IR-64 (2.34 mm) having maximum grain breath while the genotype Keeripala Chill Paddy (1.40 mm) recorded minimum and significantly lower grain length than the check DRR Dhan-44 (2.03 mm) having minimum grain breath. Grain L/B ratio was recorded to be ranging from 2.06 to 4.49 with average value of 3.275. The maximum grain L/B ratio was recorded by Nyauli (4.49) which was statistically superior to the check having highest grain L/B ratio i.e. DRR Dhan 44 (3.34) while minimum grain L/B ratio was reported for Lwankhan (2.06) which was also significantly lower than the check having lowest L/B ratio i.e. MTU1010 (2.68).

Grain chalkiness is an important trait pertaining to rice quality. It is undesirable and have negative effect on the grain appearance and milling quality. Therefore, chalkiness is directly linked to the consumer preference and market acceptability as rice having chalkiness is not preferred. It is a complex trait and found to be governed by multigenes. In the present study, chalkiness was found to be varying from 1 to 9 score among the genotypes. Three genotypes namely ARC 13934, Keeripala Chill Paddy and Nyauli showed maximum grain chalkiness of score 9 (more than 75% of chalkiness) and grain chalkiness was minimum (absent) (score 1) in four genotypes namely Chandina, Pakistanhmwe, Mharaka and DRR Dhan-44. Among all selected 30 genotypes hulling percentage was ranged from 67.13% to 79.50% with the mean value of 73.314% under the control condition. The maximum hulling percentage was shown by genotype Muta Ganje (79.50%) which was higher than the best check Sahbhagi Dhan (77.59%) while minimum hulling percentage was shown by Lwankhan (67.13%) which was lower than Sukhadhan-5 (69.78%) lowest among the checks. Among genotypes milling percentage was ranged from 45.80% to 76.60% to with the mean value of 61.2%. The maximum milling percentage was shown by the genotype Ih Pen Shim Ming (76.60%) which was at par to the best check Sahbhagi Dhan (74.26%) and minimum hulling percentage was shown by Chhote Dhan (45.80%) which was significantly lower than Sabour Deep (53.56%), the lowest among checks. Amylose percentage was categorized as Very low (30%). Among all the selected genotype Amylose percentage was varied between 25.44% (medium) to 4.72% (very low) with mean values was 15.08% (low) (Fig.-1 and 2). The maximum amylose content was found to be present in Dubraj (25.44%) which was higher than the check Sabour Deep which was having medium (24.4%) but highest amount of amylose content among while minimum amylose content was found to be present in ARC 11322 (very low, 4.72%) which was significantly lower than the check DRR Dhan 44 was low (11.92%) which was lowest among the check genotypes.

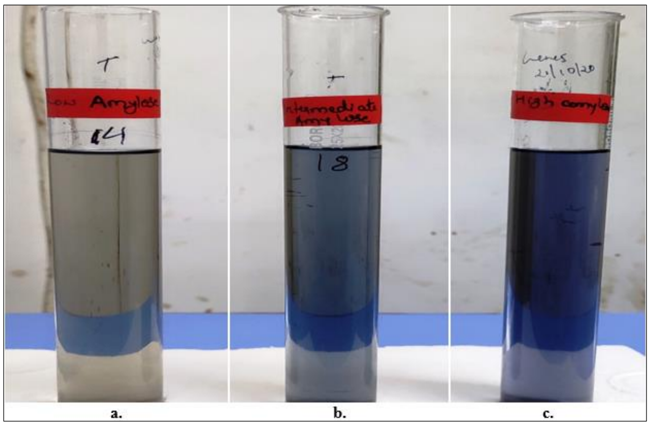

Fig 1: Test tubes showing estimation of Amylose content: a. Low amylose, b. intermediate amylose, c. high amylose

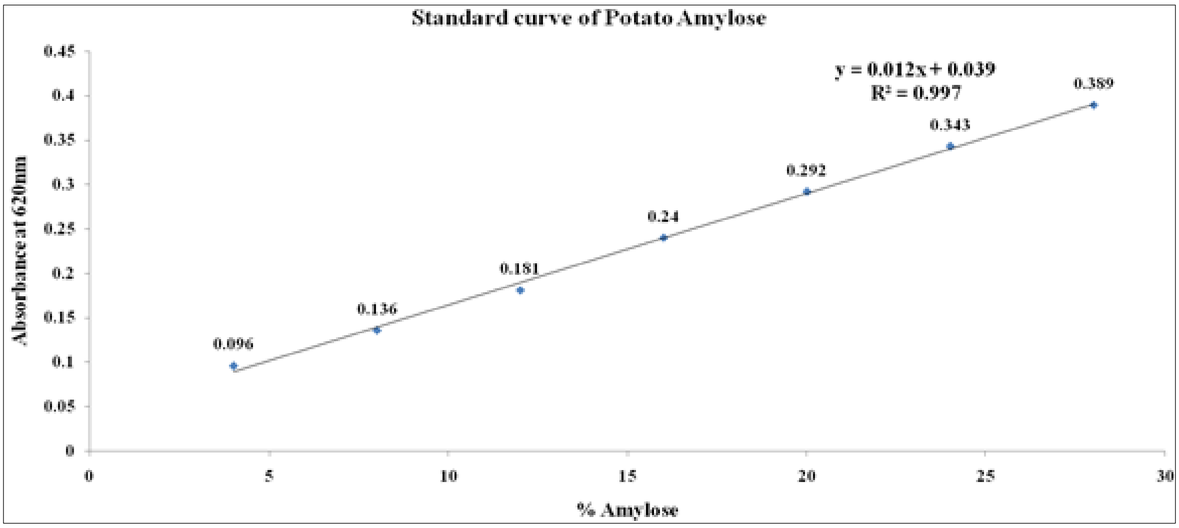

Fig 2: Graph showing standard curve of amylose used for estimation of amylose content in 38 rice genotypes

Phenotypic correlation among quality traits

Result of phenotypic correlation based on nine grain quality traits has been presented in Table-2. Data analysis for correlation revealed highly significant and positive correlation between grain length (mm) and grain breath (mm); grain length (mm) and grain L/B ratio while negative and significant correlation was observed between chalkiness and hulling%; and chalkiness and milling %.

Table 2: Phenotypic correlation for grain quality traits in 38 rice genotypes

Trait	Grain Length (mm)	Grain Breath (mm)	Grain L/B Ratio	Chalkiness	Hulling %	Milling %
Grain Length (mm)						
Grain Breath (mm)	0.1019*					
Grain L/B Ratio	0.8442**	-0.1204				
Chalkiness	-0.3549*	-0.2325	-0.2168			
Hulling %	-0.2498*	-0.0139	-0.1685	-0.4639*		
Milling %	-0.3809*	-0.0268	-0.1803	-0.3224*	0.2272	

We selected 30 out of 450 test genotypes on the basis of their performance for grain yield plot-1 in control as well as drought condition. These 30 genotypes along with 8 checks were subjected to analysis for 9 important grain quality traits. Grain length (mm) is one of the important grain quality traits which fetches high commercial value because it decides the non-stickiness and fluffy of rice grain. Indica varieties of Asian rice are long grain and usually grown in hot climates and japonica varieties of Asian rice are short- grain. It is measured by standard digital vernier caliper after removing the husk of the paddy grain. In the present study, the line Pakistanhmwe and Lwankhan showed highest and lowest grain length (mm), respectively. While studying grain quality traits and marker-trait association in rice, Suman *et al*. revealed high level marker trait association among diverse rice cultivars. Multivariate Analysis could be successfully used for grain quality traits. Similar kinds of results were also reported in the work of Supriya *et al*. The characters contributing maximum to the divergence need greater emphasis for deciding on the clusters for purpose of further selection and choice of parents for hybridization [8]. Grain breadth (mm) was measured by standard digital vernier caliper. This study finds the maximum grain breadth for CAC 75 while minimum for Keeripala Chill Paddy it may be helpful in deciding parents in further breeding programme. Vennila *et al*. [9] in their study also worked on number of filled grains per panicle, plant height, and grain breadth and concluded that these could be given due importance for selection of genotypes for further improvement.

Higher elongation ratio of cooked rice is preferred [10]. Kernel length and L/B ratio were used to categorize the rice into different grain types. It is calculated by dividing the grain length by its breadth. In this study the genotype Nyauli and Lwankhan shows maximum and minimum grain L/B ratio, respectively. These may be further utilized in breeding programme. Similar study was conducted by Singh *et al*. [11] where they reported germplasm accession HUBR 40 and Adamchini having good grain quality and cooking properties, indicating their potential for consumer preferences. Grain type is an important trait which is relevant with the market point of view especially in case of rice and acts as main factor of consumer preference [12]. Different grain types of rice are used for different purposes for example long slender and basmati type are preferred for pulao and short slender or short bold are used for making kheer in India. Grain types are decided by comparing length of kernel (mm) with the L: B ratio (mm). For that five decorticated rice milled grains were taken randomly and their average length and breadth were taken with help of vernier caliper and finally L: B ratio was calculated. Grain types of the decorticated grain are categorized as Short Slender (SS), Short Bold (SB), Medium Slender (MS), Long Slender (LS), Long Bold (LB), Extra Long Slender (ELS) and basmati type.

After dehulling various grades of colors are obtained which are also important features of rice grains to determine their market value. These are also supposed to be linked with the cooking quality and nutritional values. In the present study different appearances of grain color were observed which were denoted as white (W), light brown (LB), variegated brown (VB), dark brown (DB), light red (LR), red (R), variegated purple (VP), purple (P), dark purple (DP). Among the most undesirable traits, grain chalkiness is one related to the rice grain quality. It is one of the determining factors of quality and price of rice. Chalkiness is seen as an opaque area (chalk) in the rice grain which may situated as white belly and white core which develops due to malformation of starch granules with air spaces between them and cooked differently from translucent areas [13, 14]. Chalk index was determined by taking 10 de-husked rice grains which were placed on light box and visually identified the grain with more than 50% of chalkiness, and percentage of chalkiness was calculated. Results of the present studies revealed that there were three genotypes namely ARC 13934::IRGC 41325-2, Keeripala Chill Paddy::IRGC 49790-1 and Nyauli::IRGC 88628-1 which showed higher degree of chalkiness (>75) that is fully chalky which was denoted by score 9 and four genotypes namely Chandina::IRGC 36420-1, Pakistanhmwe::IRGC 70763-1, Mharaka::IRGC 70612-1 and DRR Dhan 44 showed minimum grain chalkiness or absence of chalkiness which were denoted by score 1. These results were in the vicinity of the studies done by Shimoyanagi *et al*. [15] where they indicated increases in grain chalky areas due to high temperature during grain filling and increments were also recorded for grain mineral contents.

Hulling and milling percentage are another important parameter for rice grain quality which are directly related with the economic value of a cultivar. For calculating hulling percentage, 100 g of paddy seeds were dehulled using a standard Satake de-husker and average whole-grain yield was calculated. After hulling, milling was done using standard Satake sample miller and that milled rice was used to determine Head Rice Recovery (HRR). Milled rice means removal of all or part of bran and germ from husked rice. For calculation of milling percentage, the dehusked sample of selected line was taken and milling was done by standard Satake miller for 1.29 min. Then milling percentage was calculated by dividing weight of milled kernels by weight of grains (paddy) and multiplied by 100. Highest hulling percentage was recorded for the genotype namely Muta Ganje (79.50%) while highest milling percentage was recorded for Ih Pen Shim Ming (76.60%) while minimum hulling and milling percentage were recorded by the genotypes namely Lwankhan (67.13%) and Chhote Dhan (45.80%), respectively. Gimhavanekar *et al*. [16] in their studied on quality traits of aromatic and non-aromatic rice cultivars and found hulling percentage ranging from 70.60% (Dhanaprasad) to 78.35% (HMT Sona) while milling percentage from 60.80% (Girga) to 67.88% (Karjat-2). Ponnappan *et al*. [17]

also found similar kind of results while they studied milling and physical quality traits in pigmented rice varieties.

Amylose content in rice grain is another parameter on which is directly related to the consumer preferences. A variety could not be successful even if giving very good yield in the field but having very high or very low content of amylase in its grains. According to the Rani *et al*. [18], amylose content in rice genotypes is divided into five groups that is very low (30%). There are several methods to calculate amylase content in rice grain [19]. Cooking and processing quality of rice depends on its amylose content. If high level of amylose is present in rice it absorbs more water and consequently expands more during cooking, and grain tend to be too cook dry, fluffy and separate. While if amylose content present is in low amount, it causes too cohesiveness, tenderness, and glossiness of the cooked rice. In India, medium amount of amylose is preferred which leads to the cooked rice neither too fluffy nor glossy or sticky [20]. In the present study, high level of amylose content was reported for the genotype Dubraj (25.44%) and very low amylose content was reported for ARC 11322 (4.72%). Bhuvana *et al*. (2015) [29] also estimated the amylose content in the same way and reached at the conclusion that using of conical flask gives better performance than test tubes. The stability of amylase content in rice varieties is another important concern [21]. Verma *et al*. [22] and Kaur *et al*. [23] also conducted studies on association analysis and evaluation of different grain quality traits including amylase content based on phenotypical and molecular markers.

Conclusion

For all the quality traits under study, genotypes having significantly superior parameters to the best performing checks were identified. Furthermore, these genotypes may be used for parent materials and for selection in grain quality breeding programme.

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