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Research Paper

Characterization of blast resistant lines in the background of red rice (*Zag variety*) for cooking quality

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ABSTRACT

The landraces are gaining importance because health value and functionality of foods is gaining importance. Red rice "Zag" is one of the important landrace of Kashmir grown in Karnah, a remote belt in North-Western part of India in J&K. The landrace 'zag' is rich in iron and zinc content but it suffers from low grain yield because of the blast disease caused by *Magnoporthae oryzae*. In this study already available backcross progenies generated after crossing Zag variety with blast resistant donor DHMAS70Q-164-1b were evaluated for cooking quality traits like length and breadth of rice grains before and after cooking, hulling and milling percentage, alkali spreading value, gel consistency (GC) and amylose content as these traits are the major contributors with respect to eating and cooking quality. The pyramided lines were similar to that of Tangdhar Zag for most of the traits. The hulling percentage of the parents *viz*: DHMAS and Tangdhar Zag was found to be 72.2 and 73.4 per cent, respectively. The pyramided line found to have hulling percentage close to that of Tangdhar Zag was K16-32-7 (73.6%) followed by K16-32-3 (74.3%). Similarly, the milling per centage of the parents *viz*: DHMAS and Tangdhar Zag was observed to be 66.6 and 64.0 per cent, respectively whileas pyramided line K16-32-24 was found to have similar values for degree of milling (64.0%) as that of Tangdhar Zag followed by K16-32-7 (65.0%). The amylose content of parents Tangdhar Zag and DHMAS was found to be 18.0 and 20.0 per cent, respectively. The pyramided lines K16-32-10, K16-32-3 and K16-32 -7 recorded similar amylose content to that of Tangdhar Zag. The GC of parents Tangdhar Zag and DHMAS was found to be 25 and 23.3 mm, respectively. The pyramided lines that showed similar values as that of Tangdhar Zag were K16-32-24 (25.0 mm), K16-32-7 (24.5 mm) and K16-32-11 (26.6 mm). The outcomes are anticipated to produce a palpable output in the shape of an improved Zag variety, which will help farmers to increase their farm income.

Key words: Blast disease, cooking quality, landraces, pyramided lines, red rice

The world population is growing rapidly; therefore, stable production and healthy food values are major concerns. Nutritionists and food scientists are now paying more attention towards diet and illness risks and whole rice such as red and black rice has far more nutrients in them (Mazumdar *et al*., 2022). So the scientists are looking for quality traits (other than carbohydrates, proteins and fat) in food stuffs like antioxidant properties, glycemic index and mineral content. Rice (*Oryza sativa*L.) is consumed by more than half of the people around the world (Keerthivarman *et al.*, 2019) and constitute around 30-40 per cent of daily dietary calories (Khush, 2005). This depicts the global dependence on rice for calories and also nutrition. Unfortunately, not all the rice varieties are uniformly rich for the proportion of essential nutritive elements.

The landraces represent significantly broader genetic diversity than modern varieties and, therefore, they could contribute to broaden the genetic base of modern cultivars particularly with respect to traits related to nutritive quality. Traditional cultivars of rice have nutritive value higher than hybrid and pureline rice varieties. Red rice “Zag” is one of the important landrace of Kashmir. In Ayurveda, colored rice has been called *shastika* rice and claims that it can restore imbalances in the human body. Pigmented rice is rich in antioxidants and polyphenols and has two to three times higher zinc and iron as compared to white rice (Ramaiah and Rao, 1953), which possess the desirable quality to boost, strengthen, regenerate and energize the body. It is also used as baby food and can replace white rice on special occasion. A number of Red Rice ecotypes are known from Kashmir such as Zag, Tel Zag, Gull Zag, Shel Kew, Kaw Quder, Karnahi zag, Uri zag etc. In recent times, interest in red rice has increased because of the presence of antioxidants. The antioxidant and scavenging activity of red rice is higher than that of white rice (Bhat and Rair, 2015). However, the area under red rice (zag) is shrinking because of its susceptibility to blast disease caused by *Magnoporthe oryzae*. Grain quality is determined by physical, physiological, and biochemical characteristics. Grain shape, degree of milling, grain appearance, milling recovery and kernel shape are important physical qualities. Gel consistency (GC), alkali digestion, and amylose content (AC) are among the physico-chemical features (Chemutai *et al*., 2016). Most of the people in Kashmir prefer rice which has intermediate values for all the cooking traits *viz*., GC, ASV and AC. Therefore, the present study was undertaken to evaluate backcross derived lines for various cooking quality attributes in order to select the lines with better quality.

MATERIALS AND METHODS

The present study was carried out at Mountain Research Centre for Field Crops, SKUAST-K Khudwani Anantnag, Kashmir. The available backcross progenies (BC_1F_3) derived from a cross between Tangdhar Zag and blast resistant donor DHMAS70Q-164-1b were evaluated for hulling, milling, kernel length and breadth before and after cooking, gel consistency, alkali spreading value and amylose content. Rice grains were hulled and milled by using a hand huller and an electric miller (Kett grain polisher, Japan). The observations were recorded at five cooked kernels with the help of a vernier caliper for computing the kernel length and breadth before cooking and after cooking. Alkali spreading value was estimated by using Bhattacharya and Sowbhagya (1972) method ollowing the scale as low (1-2), low- intermediate (3), Intermediate (4-5) and High (6-7). Five milled rice kernels without cracks were selected from each pyramided line and placed in plastic transparent box with small separate sections (Fig.1). Then 10 ml of 1.7 % KOH was added to each section. These kernels were evenly placed in separate sections to allow enough space for spreading. The box was then placed for 23 hours at constant temperature of 30°C. Disintegration of endosperm was visually rated as above.

Gel consistency (GC): Rice flour was prepared by using mortar and pestle. GC was worked out using a method given by Cagampang *et al.* (1973) following the scale as hard gel consistency (26-40 mm), medium gel consistency (41-60 mm) and soft gel consistency (61-100 mm). 10 milled grains were grinded using clean mortar and pestle to yield fine flour (100 mesh). Samples of 100 mg flour in quadruplicates was put in culture tubes. Subsequently was added 0.2 ml of ethanol containing 0.25% thymol blue. To this was added 2.0 ml of 0.2 N Potassium Hydroxide. The test tubes were shifted to water bath at 95-100°C for 8 minutes. Cultures tubes were then removed and allowed to cool for 5 minutes. Culture tubes were put in low temperature bath at 0-2°C for minutes. The tubes were then laid horizontally for one hour over graph paper. Length of the blue coloured gel from the inside bottom of the test tube to the gel front was then measured as gel consistency of the sample.

Amylose content (%): Amylose content was determined by the method developed by Julianio. (1971). Fifty milligrams of milled ground rice were weighed and transferred to long test tubes (2 x 19.5). 0.5ml absolute ethanol was added to each sample and was agitated gently for 5 minutes. To each sample, 5ml of 1N NaOH (40g NaOH pellets dissolved in 500 ml double distilled water and the volume made up to 1000 ml) was added and then covered with aluminum foil and allowed to stand at room temperature overnight. The solution was shaked thoroughly and kept for digestion in water bath for 15 minutes. The sample was poured in 100 ml volumetric flask (rinsed with hot water) and then 44.5ml of de-ionized distilled water was added to samples and mixed properly. From each sample, 2.5ml of the extract was taken into a fresh culture tube, to 20ml de-ionized distilled water and three drops of 0.1% phenopthalein in 100 ml distilled ethanol were added and the contents were mixed well (the pink colour appearance indicates the alkaline PH). The contents were neutralized by adding 0.1N HCl drop by drop until the pink colour just disappeared. After the end point was reached, 1ml iodine reagent (one gm iodine and 10gm potassium iodide were dissolved in water and volume was made up to

500ml) was added and volume was made up to 50 ml and the absorbance was read at 620 nm in the spectrophotometer.

Drawing standard curve: 100 mg standard potato amylose (Sigma Chemicals Co.) was dissolved in 10ml of 1N NaOH and volume was made up to 100 ml with de-ionized distilled water (1000ug/ml). The volume of 1ml, 2ml, 3ml, 4ml, and 5ml of the standard amylose solution was pipette out into volumetric flasks in three replicates.

To the 1 ml, 2ml, 3ml, 4ml and 5ml of standard amylose solution, 0.2ml, 0.4ml, 0.6ml, 0.8ml and 1.0ml of acetic acid were added, respectively. 2ml of KI solution was added to each of these volumes. After adding IKI solution, the volume was made to 100ml and the flasks were covered with aluminum foil. After 20 minutes the absorbance was noted in spectrophotometer at 620nm. Assuming that the starch is a mixture of just two pure components, amylose and amylopectin, and also assuming that other starch or flour components do not absorb light at 620nm, the amylose content was approximated by the following regression equation derived from standard curve *i.e* $y= 0.220x+ 0.057$, and classified as per the following scale.

Scale for amylose content (%)

Classification	Amylose Content (%)
Waxy	1-2%
Very low	2.1-9%
Low	9.1-20%
Intermediate	20.1-25%
High	25.1-33%

RESULTS AND DISCUSSION

Rice grain quality traits are attained through the determination of the physicochemical properties of the rice, which provides an indirect key on the cooking quality trait of each genotype. So, evaluation of rice for cooking quality traits is vital given that it controls the manifold uses of rice at both industrial and domestic scale. The evaluation of backcross derived red rice lines carrying blast resistance was carried out for various cooking quality traits. The traits related to cooking and eating quality primarily determine the consumer preference **(**Pokhrel *et al*., 2020)**.**

Hulling and milling percentage: The hulling percentage of the parents *viz*: DHMAS and Tangdhar Zag was found to be 72.2% and 73.4%, respectively. The pyramided line that was found to have hulling percentage close to that of Tangdhar Zag was K16-32-7 (73.6%) followed by K16-32-3 (74.3%). Similarly the milling (%) of the parents *viz*: DHMAS and Tangdhar Zag was found to be 66.6% and 64.0%, respectively. The pyramided line K16-32-24 was found to have similar values for degree of milling (64.0%) as that of Tangdhar Zag followed by K16-32-7 (65.0%). Similarly hulling (%) observed for pyramided lines K16-32-10, K16-32-11, K16-32-4, K16-32-24, K16-32-28, K16-32-7-22, K16-32-9 and K16-32-7-1 were, 71.5, 71.0, 74.6, 76.0, 76.0, 78.0, 66.6 and 68.0 per cent, respectively. The milling percentage for pyramided lines K16-32-10, K16-32-11, K16-32-3, K16-32-4, K16-32-28, K16-32-7-22, K16-32-9 and K16-32-7-1 was recorded as 63 %, 65.6, 70.0, 66.6, 66.6, 70.0, 50.0 and 51.0 per cent, respectively. Rice that has been milled by a scouring process is likely to develop oxidative rancidity; the menace is reduced by the polishing. Of the main factors influencing the milling quality of rice, is the head rice yield (Andrews ***et al.,*** 1992). The milling quality is also determined by the degree of milling which specify the amount of bran remaining on rice kernels after milling.

Kernel length and breadth before and after cooking: The kernel length and breadth before cooking of parent Tangdhar Zag (Table 1) was found to be 5.5 mm and 2.56 mm and that of DHMAS was 7.0 mm and 2.14mm on average basis, respectively. Similarly the kernel length and breadth after cooking of parents Tangdhar Zag was found to be 7.0 mm and 3.5 mm and that of DHMAS was 8.18 mm and 3.16 mm, respectively. The pyramided lines found to have similar kernel length and breadth before and after cooking as that of Tangdhar Zag were K16-32-11, K16-32-7 and K16-32-10.

The grain size and shape were explained by kernel length and breadth before and after cooking. Rice varieties can be classified as round, medium or long shape according to the length to breadth ratio. Amylose content (AC), gel consistency (GC) and Alkali spreading value are the basic parameters that essentially govern the quality of cooking and eating (Wang *et al*., 2017; Thomas *et al*., 2013).

Alkali Spreading Value (ASV): The ASV of parents, Tangdhar Zag and DHMAS was found to be 3 and 4.5, respectively (Table 1). The lines K16-32-7-22, K16-32-7, K16-32-11 and K16-32-24 recorded similar ASV as that of Tangdhar Zag (Fig.1). The values of ASV for other lines are given in Table 1. The ASV, which signifies higher alkali spreading and thus a lower Gelatinization temperature (GT) while a lower ASV indicates a higher GT, was used to determine the GT indirectly. Chemutai *et al.* (2016) reached the same conclusions about alkali digestion and discovered that rice genotypes can be divided into three categories based on their alkali digestion values: low, middle, and high. They also observed that Red Afaa was the genotype with the lowest alkali digestion value, whereas IR 2793, BS 217, BS 370, and IR 64 were the genotypes with the highest alkali digestion values.

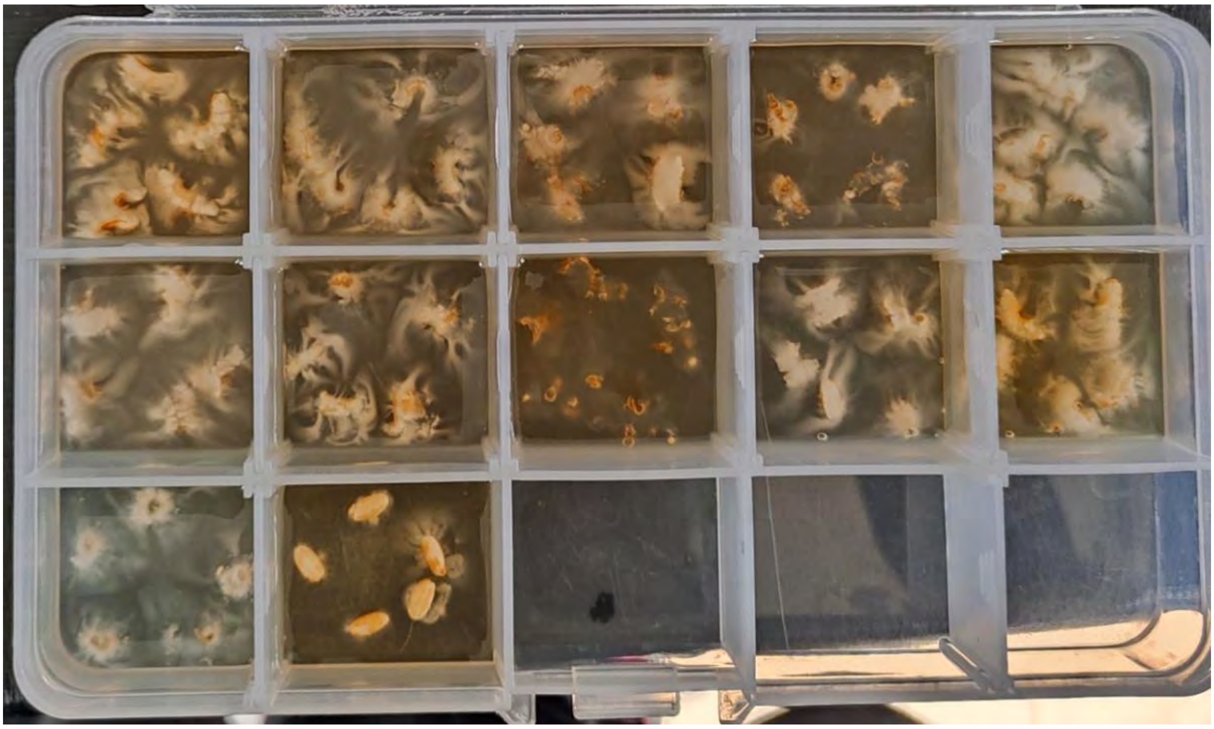
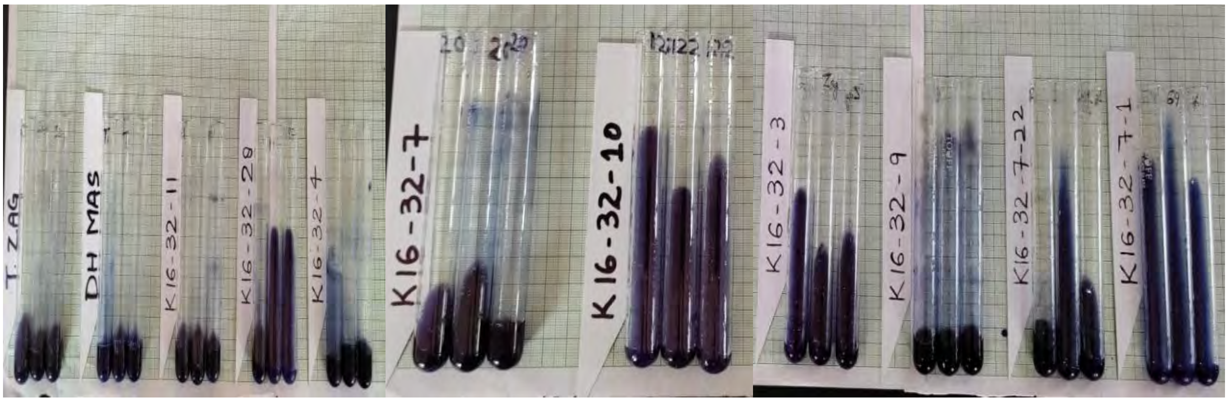


Fig 1: Alkali spreading value test of some blast resistant lines

Gel Consistency (GC): The GC of parents Tangdhar Zag and DHMAS was found to be 25.0 and 23.3 mm, respectively. The pyramided line that showed similar values as that of Tangdhar Zag was K16-32-24 (25.0 mm) followed by K16-32-7 (24.5 mm) and K16-32-11 (26.6 mm) (Fig. 2). Similarly for other lines GC values are given in Table 1. Yi *et al.* (2009) measured GC by the length of grain starch slurry in a culture tube of cold gel; the length of the gel, the distance from the bottom of the tube to the front of the gel migration, was measured in millimetres after one hour later. It is believed that the longer gel is softer than the shorter gel. GT serves as a gauge for the amount of time needed for cooking. Oko *et al*. (2012) found that when rice is cooked, varieties with a hard gel consistency harden more quickly than those with a soft gel consistency. Soft gel-like rice cooks more delicately and keeps its softness even after cooling.

Table 1: Grain and cooking quality traits of parents along with pyramided lines

Family	Hulling %	Milling %	KLBC	KBBC	KLAC	KBAC	GC (mm)	ASV	Amylose %
DHMAS	72.2	66.6	7	2.14	8.18	3.16	23.3	4.5	20
Tangdhar Zag	73.4	64.0	5.5	2.65	7	3.5	25	3	18
K16-32-7	73.6	65.0	5.42	2.2	7.28	3.42	24.5	3.5	16.4
K16-32-10	71.5	63.0	6.86	2.48	8.32	3.25	70	4.5	17
K16-32-11	71.0	65.6	5.2	2.8	7.2	3.6	26.6	5	19.5
K16-32-3	74.3	70	5.78	2.16	7.84	3.24	40	3.5	20
K16-32-4	74.6	66.6	4.96	2.36	6.98	3.14	20	4	17.3
K16-32-24	76.0	64.0	5.95	2.8	8.2	3.44	25	3.5	16
K16-32-28	76.0	66.6	5.04	2.6	7.12	3.42	80	5	14.1
K16-32-7-22	78.0	70.0	6.04	2.5	7.35	3.22	40	3.5	15.3
K16-32-9	66.6	50.0	5.78	2.48	7.52	3.36	20	6	7
K16-32-7-1	68.0	51.0	5.9	2.83	7.78	3.45	90	4	14.5



Amylose Content (%): The amylose Content of parents Tangdhar Zag and DHMAS was found to be 18.0 and 20.0 per cent, respectively. The pyramided lines K16-32-10, K16-32-3 and K16-32 -7 recorded similar amylose content to that of Tangdhar zag (Table 1; Fig. 3). The amylose content for other lines is given in Table 1.

Amylose content is considered as one of the most important factor to govern the cooking behaviour of rice which has a direct impact on the grain hardness and, in turn, the stickiness of cooked rice (Xie *et al*., 2007). Pereira *et al*. (2009) observed that the red rice has a lower amylose content thus sticky grains than white rice and needs more time to cook effectively, which supports our low amylose level in backcross lines of red rice (Tangdhar Zag).

The characteristics of starch primarily decide how well rice grains cook and taste when consumed (Champagne *etal*., 2004). Amylose content (AC), gelatinization temperature (GT), and gel consistency (GC) are three key characteristics of starch that affect how rice cooks and tastes. Only a little proportion of starch is made up of the amylose fraction, which is effectively the linear polymer of glucose. Amylose is an essential rice quality feature that is thought to be an indirect predictor of cooking and sensory quality (Li et *al*., 2016).

Fig. 2: Gel Consistency test of some blast resistant rice lines

The sensory quality of cooked rice is often correlated with AC, yet some varieties have the same AC but have different levels of cooked rice hardness (Perez and Juliano, 1979). Gel consistency (GC) is often employed to quantify the length travelled by rice flour gel before it sets in order to account for such variations (Cagampang *etal*., 1973). GC is a type of colloidal characteristic of rice starch and is an essential predictor of rice quality. Rice with a hard GC is rough and fluffy, and it cools down to become hard and dry. In contrast, rice with a soft GC is elastic and soft, and it stays that way. Generally, rice with a higher GC has a greater flavour, is stickier, and is softer (Wang *etal*., 2007).

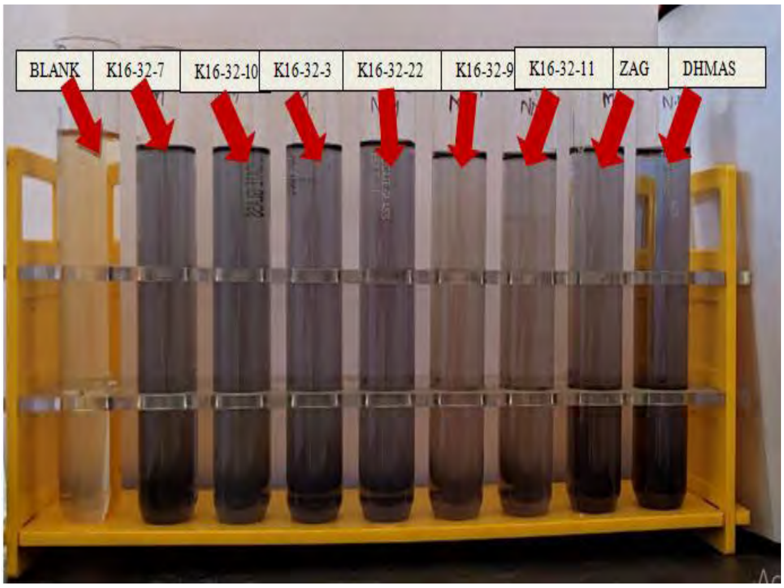


Fig 3: Amylose content test of some blast resistant rice lines

The gelatinization temperature classes (the temperatures at which the crystalline forms of starch irreversibly melt) are identified using the alkali spreading values (ASV). Rice varieties are divided into three categories according to this temperature: high, moderate, and low GT. Nevertheless, there are four GT groups based on alkali spreading: high (2), intermediate-high (3), moderate (4-5), and low (6-7) (Graham, 2002). According to various researches, there are only two GT classifications: high and low (Cuevas *et al*., 2010). The low-GT class group may be a prerequisite for market and public acceptance, and those involved in the rice trade as well as rice breeders whose goals are in the intermediate GT levels may have issues due to the lack of clarity in the delineation of the GT classes. In the present study the promising blast resistant lines showed resemblance with respect to almost all quality parameters qualifying as that of recurrent parent zag.

CONCLUSION

The economic worth and customer acceptance or preference of rice varieties are influenced by the quality of the rice grains. Rice quality is derived from a polygenic collection of attributes that are influenced by crop management, environmental conditions, and their interactions. Physical appearance, milling quality, cooking, sensory, and nutritional content are all examples of quality attributes that play a significant role in determining market value. Depending on regional consumer preferences, market demand, and intended functional purpose, several qualities may be given more weight than others. Consequently, having straight forward, reliable, high throughput methodologies for evaluating numerous quality attributes that might represent customer desire is necessary to overcome the problems faced by rice development programmes. In the present study four blast resistant pyramided lines *viz*., K16-32-3, K16-32-7, K16-32-10 and K16-32-11 with cooking quality attributes similar to that of parent zag were identified. These lines need further validation for other quality traits as well and can be recommended as varieties *viz-a viz* may serve as donors in various rice improvement programmes.

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