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MARKER-ASSISTED DEVELOPMENT OF AN EXTRA-LONG GRAIN AND HIGH YIELDING BASMATI RICE LINE WITH SEMI-ERECT AND DENSE PANICLES

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ABSTRACT

In light of the rapidly growing global population, there is an urgent need to develop high-yielding and climate-resilient rice varieties. The productivity of traditional basmati cultivars is limiting due to region-specific cultivation, and genetic improvements are direly needed to enhance their productivity without compromising quality attributes. This study aimed to develop high yielding basmati advance line with good quality attributes through a marker-assisted selection approach. A cross was attempted between Basmati 515 and IRBB 66, followed by pedigree selection up to the F_5 generation. A uniform recombinant inbred line was identified then backcrossed with PK 1121 Aromatic and again followed by pedigree selection up to BC_3F_5 for desirable genotype development. Genes pyramiding for aroma (*BADEX7-5*), dense and erect panicles (*DEP1*), grain length (*FMGS-7*) and grain width (*GW8-indel*) were achieved using gene-linked markers. The identified line "BLB 18-5001", predominately harbored agronomic and quality characteristics of Basmati 515, panicle architecture, and grain dimension traits of IRBB 66 and PK 1121 Aromatic, respectively. The BLB 18-5001 outperformed in thousand-grain weight (28.5 g), grain yield (4.7 t ha^{-1}), average grain length (9.22 mm), and cooked grain length (19.5 mm) compared to parents. A two-year field evaluation revealed superior agronomic and quality attributes of BLB 18-5001, suggesting an unprecedented genetic potential to meet future varietal demands. This study concludes that the BLB 18-5001 line has overcome the negative correlation between grain quality and yield to some extent and is expected to serve as a valuable breeding source to improve basmati productivity to meet the growing demand for food.

Keywords: Aromatic rice; Gene pyramiding; Food security; Molecular breeding; Rice quality; Varietal development.

INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food for over half of the world's population and provides nearly 20% of the daily caloric intake, particularly in low-income countries (Hussain et al., 2022). This crop is cultivated in almost all continents, except the glaciers area, and Asia being the major contributor (accounting for approximately 90%) of global rice production. Given the rapid growth of the world population, the decline of cultivated land, and the severe impacts of climate change, breeding high-yield and climate-resilient rice varieties is crucial for profitable farming, meeting consumer demand, and ensuring food security (Takeda and Matsuoka, 2008).

Rice genotypes exhibit considerable variations in plant stature, grain yield, and cooking quality traits across the world. People living in tropical and subtropical regions tend to prefer aromatic, slender, medium to long-grain rice with good palatability, while those in temperate regions prefer small, round, and sticky rice (Garris et al., 2005; Wang et al., 2014). Among the various rice ecotypes, basmati rice is considered a premium option due to its aroma, fluffiness, elongation, and good palatability. This has led to an increase in global demand for basmati. However, traditional basmati rice varieties are photoperiod sensitive, low-yielding with short to medium-grain, and prone to lodging, limiting their productivity and cultivation to specific regions. To make basmati cultivation profitable, genetic improvements are needed to enhance agronomic traits without compromising aroma and cooking quality. Over the past few decades, conventional breeding methods have made significant progress in improving plant stature, maturity duration, and grain dimensions of basmati, but these methods are laborious, time-consuming, and highly dependent on environmental conditions. Advanced molecular techniques, such as Marker-assisted selection (MAS) and Genomic-assisted breeding (GAB), in combination with traditional breeding methods have proven to be the most effective approach for introducing target genes into desirable genetic backgrounds (Singh et al., 2012).

Grain yield is a quantitative trait determined by three major components: the number of grains per panicle or panicle density, mean grain weight, and productive tillers per plant (Takeda and Matsuoka, 2008). Grain length, width, and thickness contribute to grain weight and dimension, while the number of grains per panicle, panicle length and primary and secondary branches determine panicle density. These traits are controlled by numerous QTLs/genes that are strongly influenced by the environment. Several QTLs have been identified and most of them have been functionally characterized including *grain number 1a* (*Gn1a)* is responsible for an increase in panicle grains number (Ashikari et al., 2005), and *qPE9-1* is associated with panicle curvature (Yan et al., 2007). Huang et al. (2009) and Wang et al. (2009) reported a panicle architecture gene "*DENSE AND ERECT PANICLE 1 (DEP1)"* which significantly contribute to increasing grain yield. The *DEP1* gene is a gain of function mutation in phosphatidylethanolamine-binding protein (PEBP) that enhance the activity of meristematic tissues. As a result, it reduces the internodal distance and increase the grains per panicle leading to high panicle density and erectness.

Similarly, many grain shape-related genes/QTLs have been mapped throughout the rice genome. A few of them have been fine-mapped and functionally characterized like *grain-shape 3 (GS3), grain-shape 7 (GS7),* and *grain width 8 (GW8)* on chromosome 2, 7 and 8, respectively (Xi et al., 2006; Wang et al., 2012, 2015; Nan et al., 2018). Nan et al. (2018) reported genetic markers to improve rice grain shape through introgression of the *GS3* gene in the elite genotype. The *GS7* gene encodes a TON1 RECRUITING MOTIF-containing protein that regulates microtubules formation in the rice cell. Wang et al. (2015) found a mutation in the promoter region of the *GS7*, which truncated the binding site of SQUAMOSA-promoter binding protein-like transcription factor. As a result, high longitudinal instead of horizontal cell expansion occurred that enhanced grain length and slenderness.

Similarly, *GW8* has been identified from a segment substitution line (SSL) of Basmati 385 and HJX74 (Xi et al., 2006). It regulates cell elongation by preventing cell proliferation and increasing horizontal cell division, using the transcriptional factor *OsSPL16*. A 10 bp deletion in the promoter region inhibits the binding of *OsSPL16,* resulting in escalating the elongation of cell division leading to fine-type grains. This study also reported that *OsSPL16* reduced plant height and panicle branching and promoted early flowering in rice (Wang et al., 2012).

The presence of 2-acetyl-1-pyrroline (2AP) is crucial in determining the aroma of rice, particularly in basmati varieties, where it is considered a key quality indicator (Wakte et al., 2017). This aromatic compound can be found not only in the grain but also in the aerial parts of the plant. The gene responsible for the accumulation of 2AP in the plant is *betaine aldehyde dehydrogenase 2* (*badh2)*, which is recessive and located on chromosome 8 (Bradbury et al., 2005). Mutations in exons 2 and 7 of *BADH* have been found to modify the function of the BAD enzyme, leading to an increase in 2AP accumulation. While non-aromatic rice also contains 2AP, the concentration is significantly lower, about 10 to 15 times less than that in aromatic rice (Bryant and McClung, 2011).

Genetic introgression of *GS7*, *GW8*, *DEP1* and *badh2* by a combination of conventional and marker-assisted breeding (MAB) could allow rice breeders to develop high-yielding and better-quality improved basmati rice cultivars. Therefore, the present study was carried out to incorporate grain dimension and panicle architecture QTLs/genes from two different genetic backgrounds into the local basmati variety, Basmati 515, using the conventional and marker-assisted backcross-breeding approaches.

MATERIALS AND METHODS

Plant material

This study was carried out at the experimental area and molecular breeding lab of Rice Research Institute (RRI), Kala Shah Kaku (KSK), Pakistan. Plant material consisted of a recombinant inbred line (RIL; PK 9956-14-1-1) developed through hybridization of "Basmati 515" and "IRBB 66". The whole breeding scheme adopted for line development is illustrated in Figure 1. Basmati 515 is an aromatic, long-grain and photoperiod sensitive rice cultivar. It is primarily grown in Punjab, especially in the "Kallar tract" and is famous in the farming community due to high yield and good cooking quality. IRBB 66 is a non-aromatic, short stature, early maturing and small grained breeding line having dense and erect panicles with poor cooking quality. The PK9956-14-1-1 is a photoperiod sensitive, aromatic, and bold grain rice RIL with semi-erect and dense panicles. While PK1121 Aromatic is an extra-long grain, photoperiod sensitive and high yielding variety which is mostly consumed as parboiled rice.

Breeding scheme

Basmati 515 (female parent) was crossed with IRBB 66 (male parent), following the selfing of F_1 plants and subsequently F_2 plants were selected based on phenotypic selection and resemblance with basmati parent. The pedigree method of selection was adopted up to the F_5 generation, where an advanced uniform RIL (PK 9956-14-1-1) was identified and backcrossed with PK 1121 Aromatic for improvement of grain length. The heterozygosity of each F_1 was confirmed using the selected PCR based molecular markers at the seedling stage (Table 1). At BC_3F_1 stage, plants were allowed to self-pollinate, and the pedigree method was utilized for selection up to BC_3F_5, resulting in the identification of a superior uniform line, named "BLB 18-5001".

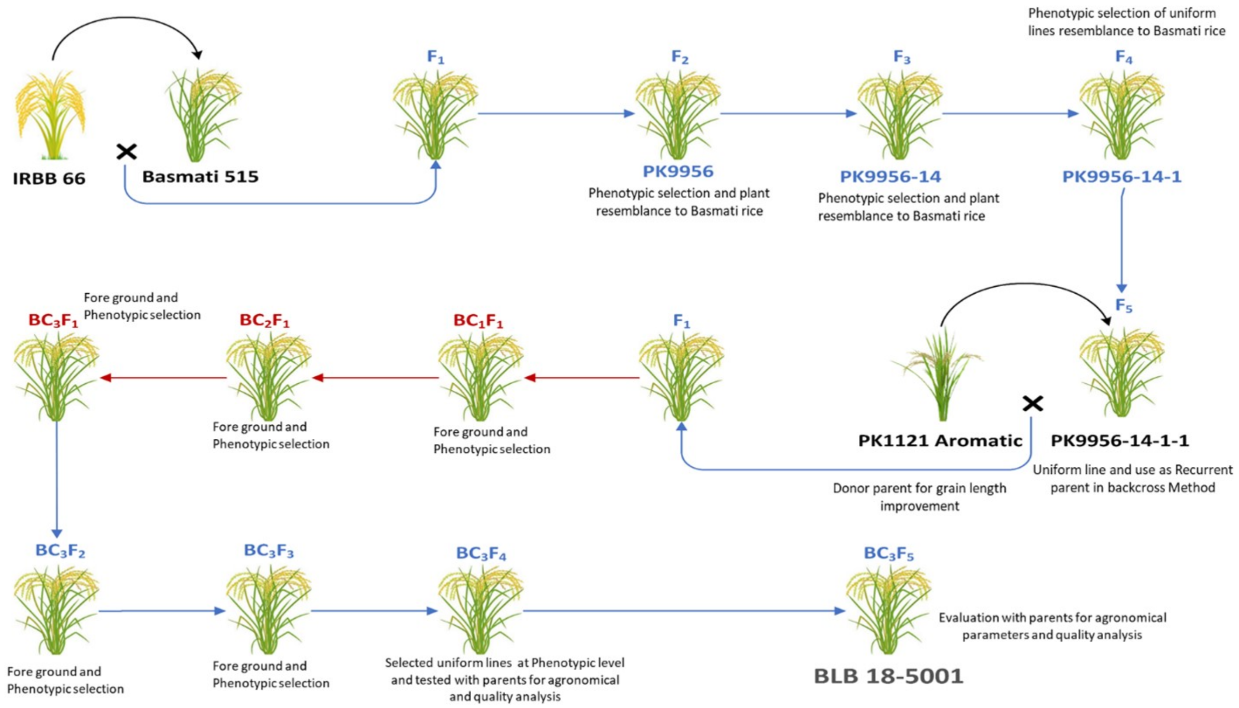

Figure 1. Schematic illustration of the breeding scheme adopted for the development of BLB 18-5001.

Targeted gene amplification

The total genomic DNA (tgDNA) of each selected genotype was extracted from leaf tissues at the seedling stage using a modified CTAB protocol (as described by Doyle and Doyle, 1987). The quantity and quality of tgDNA were determined using a Nanodrop Spectrophotometer (ND 2000, Thermo Scientific, USA) and 0.8% gel electrophoresis in 1X TBE buffer, respectively, following standard protocols.

Table 1. List of molecular markers for marker-assisted development of BLB 18-5001

Sr #	Marker	Trait	Forward (5' – 3')	Reverse (5' – 3')	Tm (°C)	Amplicon size (bp)	Reference
1	BADEX7-5	Aroma	TGTTTTCTGTTAGGTTGCATT	ATCCACAGAAATTTGGAAAC	54	95, 103	Sakthivel et al., 2009
2	DEP1	Panicle density and erectness	TGGACACTTGTTATCTTCTCAT	AACTGGAAGTTTGTAACACTCA	56	500, 200	Ngangkham U et al., 2018
3	GW8-Indel	Grain weight	TTGTGATGGCAATTAGTAAGCAG	GTTCTCCAGCTCGTCGGCTA	61	115, 125	Wang S et al. 2012
4	FMGS-7	Grain length	TGGTCAAATCATGGGCTAAT	TATTATTGTGCCTGCGATCC	51.5	200, 350	Shao et al. 2012

A master mix was prepared for DNA fragment amplification, which consisted of the 10X PCR buffer (50 mM Tris [pH 8.3] and 500 mM KCl), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 unit of Taq polymerase, 0.6 µM of each forward and reverse primer, and approximately 50 ng of tgDNA as a template. The DNA fragment amplification was carried out in a Mastercycler Gradient (Eppendorf, Germany) using the following temperature cycles: 1) denaturation at 94 °C for 3 minutes, followed by 94 °C for 30 seconds; 2) annealing temperature adjusted according to each pair of primers, ranging from 55 to 60 °C for 1 minute; and 3) extension at 72 °C for 60 seconds with a final extension of 5 minutes.

The PCR product was loaded onto 1.5% gel electrophoresis to measure the fragment size using a 50 bp ladder in 1X TBE buffer at 80 V for 40 minutes. The gel was then stained with ethidium bromide in a shaker for 10 minutes and documented under UV light using the NYXTECHNIK electrophoresis documentation and analysis system from the UK. The amplicon size of the aroma (BADEX 7-5) and grain weight (GW8-indel) markers were analyzed using 6% poly-acrylamide gel electrophoresis (PAGE) against a 20 bp ladder as a standard.

Agronomical field evaluation

The method of Sabar et al. (2019) was followed for the nursery raising and transplanting of plant material. Agronomic traits, including maturity days, plant height, tillers per plant, grains per panicle, panicle length, thousand-grain weight, and plot yield, were recorded during the 2018-19 and 2019-20 cropping seasons. The panicle density of each genotype was calculated using the equation provided by Kondo and Futsuhara (1980):

Panicle density = (Number of grains per panicle / Length of panicle × Length of each primary branch)

A representative sample of 1 kg from each genotype was dried to a moisture level of 12% and de-husked using a Satake paddy de-husking machine at the Rice Technology Lab of the Rice Research Institute (RRI) in KSK. The resulting brown rice was polished for 40 seconds with a Grainman 60M-250-50-DT Sheller polishing machine under a pressure of 1 kg, and then sorted to separate the head rice from the broken grains. Physical traits, such as total milled rice percentage (TMR%), brown rice percentage (BR%), head rice recovery (HR%), average grain length (mm), grain thickness (mm), and grain width (mm) were measured using the measuring scale and gauge. After two weeks of incubation, 100 head rice grains from polished samples were taken and cooked in boiling water (100 °C) for 5 to 6 min. Subsequently, the cooked samples were cool down under tap water and data recording of cooked grain length, elongation ratio, and quality with appearance were completed using the standard evaluation system (SES, 2013) of the International Rice Research Institute.

Statistical analysis

The recorded agronomic and grain quality data were subjected to Statistix 10.1 for statistical analysis at a 5% significance level, and heritability was calculated using R package "variability" (Popat et al., 2022). Microsoft Office 2019 was used for the graphical presentation of averaged data.

RESULTS

Marker-assisted development of BLB 18-5001

For the development of RILs with erect and dense panicles in the basmati background, Basmati 515 was crossed with IRBB 66. The F_1 plants were allowed to self-pollinate for F_2 population and phenotypic selection was performed on plants with erect and dense panicles that resembled with Basmati 515 (Figure 1). The pedigree method of selection was adopted up to F_5 to advance generations and develop a uniform RIL (PK9956-14-1-1). Although, it exhibited basmati characteristics such as a high density and erect panicle, however, grain dimensions characteristics were parallel to IRBB 66. To improve the grain quality, the RIL was backcrossed with "PK 1121 Aromatic" using foreground selection markers and phenotypic selection. The hybridity of the F_1s was confirmed through genotyping with four gene-linked markers (DEP1, GW8-indel, FMGS-7, and BADEX7-5) (Table 1). The selected F_1s were backcrossed with the recurrent parent (PK9956-14-1-1) up to BC_3 for maximum genome recovery, based on both foreground and phenotypic selections. A set of 40 polymorphic SSR markers were used for background selection, and the results showed that the genome recovery of the recurrent parent ranged from 86.50 to 89.24% in the backcrossed plants (data not shown). The five heterozygous BC_3F_1 plants harboring highest genome recovery were allowed to self-pollinate. At BC_3F_5, a single superior uniform line with desired traits such as dense and erect panicles, aroma, extra-long and bold grains was selected (named as "BLB 18-5001") (Figure 2) and subjected to various agro-morphological and quality analyses along with its parents for two consecutive growing seasons in the field (Table 2).

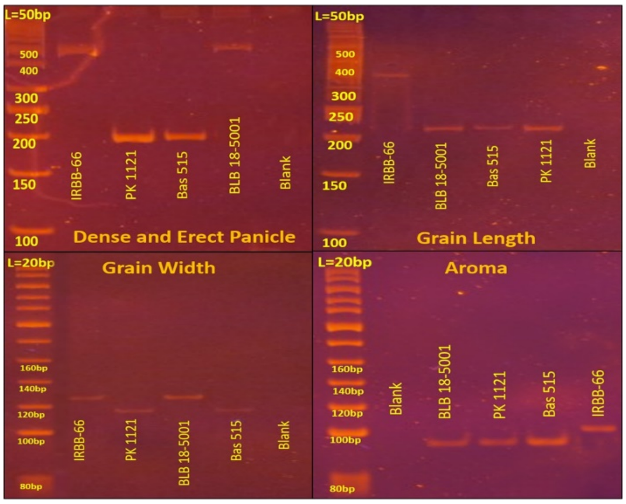

Figure 2. Molecular markers amplification for four targeted traits in BLB 18-5001 and its parental genotypes.

Agro-morphological evaluation of newly developed line

The candidate line "BLB 18-5001" and its parents were evaluated over two-year period (Kharif 2018-19 and 2019-20) under natural field conditions (Figure 3**)**. The mean agronomic performance across two years revealed that all genotypes had significant genetic differences among studied traits, except for tillers per plant (Figure 4a). The BLB 18-5001 outperformed in thousand-grain weight (g) and grain yield (t ha^{-1}) than parental genotypes with average performance in plant height, days to maturity, grains per plant, panicle length and density. Highest '000' GW (28.5 g) and grain yield (4.7 t ha^{-1}) were observed in BLB 18-5001, followed by PK 1121 Aromatic (000 GW = 26 g, grain yield = 4.4 t ha^{-1}), Basmati 515 (000 GW, 23.2 g; yield, 4.2 t ha^{-1}) and IRBB 66 (000 GW,21.6 g; grain yield, 3.9 t ha^{-1}). Compared to Basmati 515, "BLB 18-5001" showed slightly lower, but statistically non-significant, performance in terms of plant height, days to maturity, grains per plant, and panicle length. However, the panicle density of the IRBB 66 parent was significantly higher than "BLB 18-5001" and other parents (Figure 4b). Despite these differences, "BLB 18-5001" was observed to be superior to its parents and demonstrated a high degree of stability in agronomic parameters across growing seasons.

Figure 3. Performance of BLB 18-5001 with its parents (Basmati 515, PK 1121 Aromatic and IRBB 66) in field.

Grain dimension, milling and cooking quality attributes

To assess the performance based on consumer demand, "BLB 18-5001" and its parents were evaluated for grain dimensions, milling and cooking quality attributes. The results showed that "BLB 18-5001" possessed extra-long (AGL, 9.22 mm) and slender grains (width, 1.87 mm; thickness, 1.64 mm) with the highest CGL (19.5 mm) and elongation ratio (2.15) (Figure 5). The milling recovery of "BLB 18-5001" (63%) was significantly affected as compared to Basmati 515 and IRBB 66 (≥70%). Moreover, IRBB 66 had 62% head rice (HR) recovery, followed by Basmati 515 (55%), PK 1121 Aromatic (35%) and BLB 18-5001 (27%) (Figure 5a). The lowest milling recovery in BLB 18-5001 could be explained by lengthy grains which caused their breakage during traditional milling process. Collectively, these results suggested that BLB 18-5001 had superior grain dimension and cooking quality attributes with low milling recovery, which could be improved through the parboiling process.

Heritability

The heritability of morphological and quality traits of the candidate line and its parents was estimated using the 'variability' package in R. The results showed that plant height had the highest heritability (95%) among the morphological traits, followed by days to maturity (90%), panicle density (83%), and yield (75%) (Figure 6). On the other hand, panicle length, grains per panicle, tillers per plant, and grain weight had observed lower heritability (60%, 64%, 65%, and 67%, respectively) due to their strong dependence on environmental conditions. The heritability of grain quality traits, such as grain width and thickness, CGL, AGL, and E/R, was found to be high, ranging from 93% to 100% based on two years studies. In contrast, the heritability of grain milling attributes, such as TMR (%), BR (%) and HR (%), was near to 85% (Figure 6), suggesting that they are more influenced by environmental conditions.

Table 2. Phenotypic comparison among the parents and selected progeny.

Sr #	Phenotypic trait	IRBB 66	Basmati 515	PK 1121 Aromatic	BLB 18-5001
1	Maturity duration (Days; seed to seed)	110	140	132	135
2	Photosensitivity	Insensitive	Sensitive	Partial sensitive	Sensitive
3	Grain type	Coarse	Fine	Fine	Fine
4	Plant height (cm)	100	140	125	133
5	Stem attitude	Semi-erect	Erect	Erect	Erect
6	Stem thickness	Medium	Medium	Medium	Medium
7	Tillers per plant	12-14	14-16	13-15	15-17
8	Stem color	Green	Green	Green	Green
9	Flag leaf attitude	Erect	Semi-erect	Semierect	Semierect
10	Flag leaf length	Medium	Long	Long	Long
11	Panicle exertion	Full exerted	Full exerted	Full exerted	Full exerted
12	Panicle type	Compact	Semi-compact	Semi-compact	Semi-compact
13	Stigma color	White	White	White	White
14	Awns	Absent	Rare	Absent	Absent
15	Threshability	Easy	Moderate	Moderate	Moderate
16	Paddy length	Medium	Long	Extra-long	Extra-long
17	Paddy width	Medium	Medium	Medium	Medium
18	Kernel length (mm)	~6.80	~7.50	~8.20	~9.20
19	Kernel width (mm)	~1.90	~1.72	~ 1.84	~1.86
20	Kernel thickness (mm)	~1.76	~1.62	~1.72	~1.64
21	000 grain weight (g)	~21.5	~23.0	~26.0	~28.10
22	Kernel chalkiness	Translucent	Transcalent	Central chalkiness	Translucent
23	Kernel quality	Medium	Excellent	Excellent	Excellent
24	Kernel aroma	Absent	Strong	Mild	Strong
25	Head rice recovery (%)	~60.2	~55.0	~35.0	~27.0
26	Cooking quality	Good	Excellent	Excellent	Excellent

DISCUSSION

The use of marker-assisted selection in conventional breeding is a highly efficient and precise approach to introgress desired genes in chosen genetic background. Molecular markers offer high degree of accuracy compared to phenotypic selection, as they eliminate the influence of the environment, and can also aid in the phenotypic selection of desirable plants that resemble the recipient parent (Sabar et al. 2019). Additionally, they allow for the monitoring of the introgressed genomic region(s) or gene(s) during early stages of growth and development for resistance or tolerance against biotic and abiotic stresses (Gopalakrishnan et al., 2008; Singh et al., 2013, 2018; Ellur et al., 2016; Babu et al., 2017).

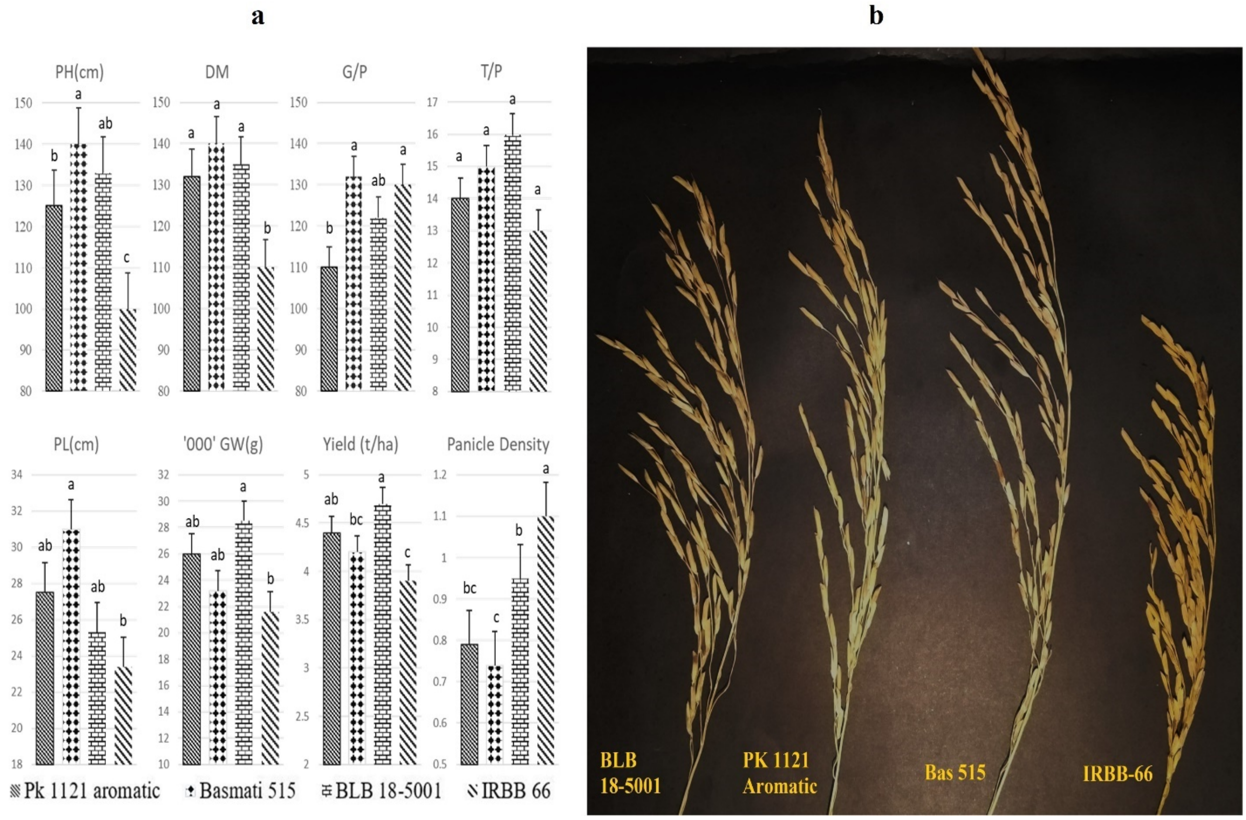

Figure 4. Agro-morphological attributes of BLB 18-5001 and its parental genotypes. (**a**) Mean agronomical performance across two growing seasons. Bars sharing the same alphabets are not statistically significant from each other (HSD, p>0.05). (**b**) Comparison of representative panicles. Abbreviations; PH (Plant height), DM (Days to maturity), G/P (Grains per panicle) T/P (Tillers per panicle), PL (Panicle length), '000' GW (Thousand-grain weight).

Basmati rice is a unique among cultivated rice ecotypes and is primarily grown in a specific ecological zone known locally as the "Kallar tract" in Punjab, as well as in a few spots in Sindh and Baluchistan province of Pakistan, and in Indian Punjab. This special type of rice is crucial in terms of food security and is a valuable cash crop, generating more than 2 billion USD annually due to its premium grain quality and aromatic attributes (Akhter and Haider, 2020). Locally developed varieties, such as Super Basmati and Basmati 515, hold a prominent status and are widely cultivated in Pakistan. These varieties are consumed as white milled or polished rice and command a premium price on the international market. Similarly, PK 1121 Aromatic, an extra-long grain fine rice variety, is becoming increasingly popular among rice growers and consumers, although it is primarily grown in non-Kallar tract areas and consumed in parboiled form. The development of a high-yielding, extra-long grain basmati rice variety is, therefore, an urgent need and could provide a potential alternative to commercially available basmati rice cultivars, ensuring food security, and economic stability. In this study, we developed an advanced basmati rice advance line using MAS techniques and conventional breeding (Figure 1). The newly developed line predominantly exhibits the agronomic and quality characteristics of Basmati 515, with the panicle architecture and grain length attributes of IRBB 66 and PK 1121 Aromatic, respectively. Field evaluation showed that BLB 18-5001 exhibits superior agronomic and quality attributes (Figures 3 and 4, Table 2), indicating its exceptional genetic potential to meet future varietal demands.

The *Dense and Erect Panicle 1 (DEP1)* locus is a crucial factor in determining the architecture of a rice panicle. As demonstrated by Huang et al. (2009), the *DEP1* locus has a range of pleiotropic effects, including the number of grains per panicle, grain length, panicle density, and nitrogen uptake and metabolism. In the study by Huang et al. (2009), the wild-type allele, *dep1*, reduced grain length with high panicle density, erectness, and grains per panicle. Whereas *DEP1* caused the reduction in grains per panicle with increase in grain length and thickness as well as semi-curvature panicle shape. The *DEP1* encodes a phosphatidylethanolamine-binding protein-like transcription factor sharing some homology with *GS3*, a negative regulator of grain size*,* that participate to decrease grain length. Huang et al*.* (2009) reported that the wild type allele (*dep1*) improved panicle density by increasing primary and secondary branches and grain numbers with small grain size. In this research, we selected plants that had wild type allele for improvement in panicle architecture and grains per panicle. The wild type and mutant allele showed amplicon sizes of 500 bp and 200 bp, respectively (Figure 2). IRBB 66 and BLB 18-5001 harbored wild type allele with high panicle density as compared to Basmati 515 and PK 1121 Aromatic (Figure 4b). Previous studies reported that *dep1* was involved to strengthen the sclerenchyma cell wall, part of plant's vascular system, and played an integral role in transporting nutrients within the plant body (Huang et al., 2009; Taguchi-Shiobara et al., 2011; Xu et al., 2016). These finding indicated that *dep1* allele is pivotal for indirect selection to develop stiff stem, lodging resistant and high yielding rice cultivars.

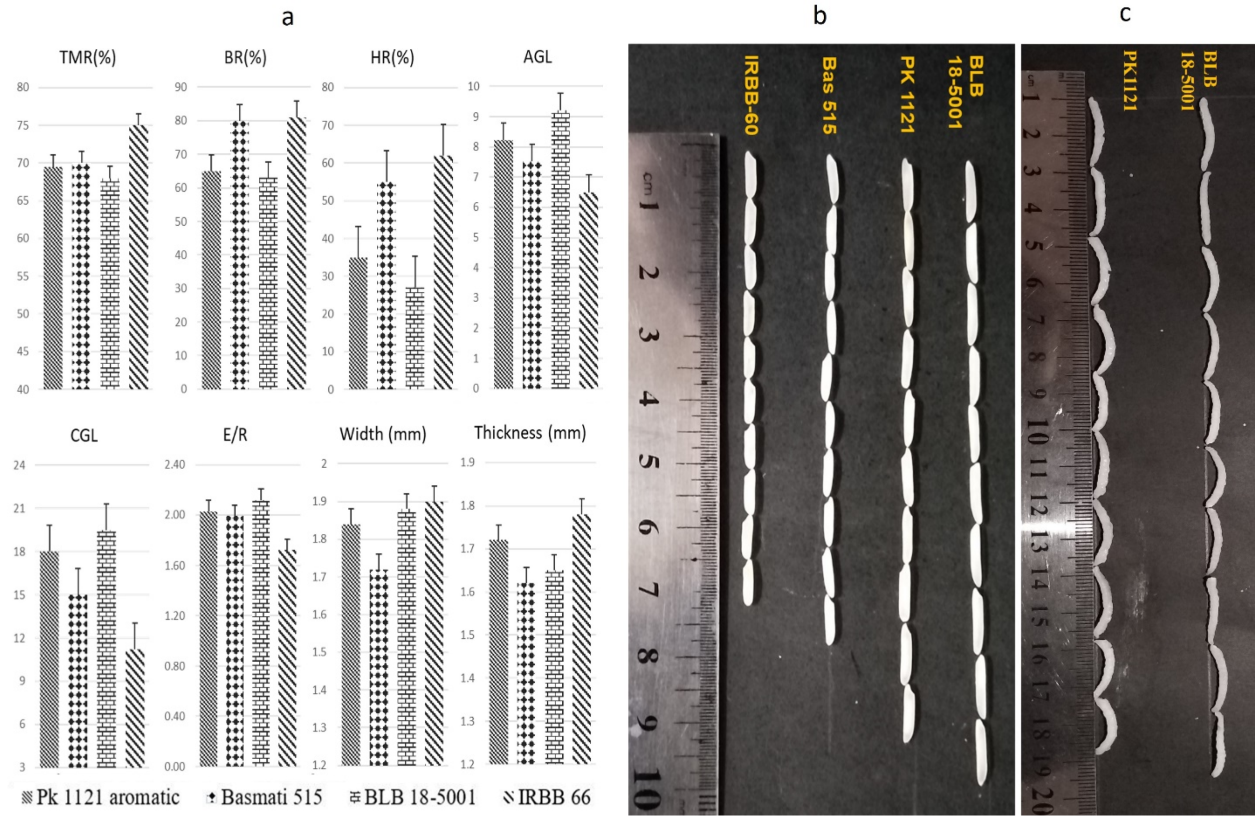

Figure 5. Quality attributes of BLB 18-5001 and its parental genotypes. (**a**) Averaged data of all studied traits across two years. Error bars are standard errors. (**b**) Comparison of AGL among representative samples of all genotypes. Abbreviations; TMR (total milled rice), BR (brown rice), HR (head rice), AGL (average grain length), CGL (cooked grain length) and E/R (elongation ratio). (**c**) Comparison of cooked grain length between BLB 18-5001 and PK 1121 Aromatic.

Grain shape and quality are important traits for all stakeholders of the rice industry. Several grain length QTLs/genes have been functionally characterized and molecular markers were developed for their possible exploitation using molecular rice breeding. Shao et al. (2012) reported that GS*7* controls seed shape by cell proliferation and enhance grain length in combination with other favorable alleles. Their study also reported a *GS7* linked InDel marker '*FMGS-7*' that distinguishes rice genotypes on basis of grain length with 350 bp and 200 bp amplicon sizes. In this study, genotypes with 200 bp allele had significantly longer grains, whereas 350 bp allele containing genotypes had smaller grains as like IRBB 66 (Figures 2 and 5). These results are in accordance with Shao et al. (2012) study, indicated that *GS7* in combination with *GS3* significantly improve grain length, grain width and thousand-grain weight via microtubules expansion in latitudinal and longitudinal cells. Similarly, Ngangkham et al. (2018) also reported that rice genotypes with a 350 bp allele of *GS7* had smaller grain length compared with the 200 bp allele.

During seed development, the length, width, and thickness are determined by longitudinal and latitudinal cell division, proliferation, and expansion of seed cells. The *"GW8"* QTL plays a role in regulating grain width and thickness by influencing latitudinal cell proliferation, leading to an increase in thousand-grain weight and grain yield (Wang et al., 2012). Lee et al. (2015) developed a *GW8-indel* marker that can distinguish rice genotypes based on grain width and thickness. In this study, genotypes with higher grain width and thickness, such as IRBB 66 and BLB 18-5001, were found to have amplicon sizes of 115 bp, while slender and thin grain genotypes, like PK 1121 Aromatic and Basmati 515, had amplicon sizes of 125 bp (Figures 2 and 5). These results were further validated by morphometric data, as IRBB 66 and BLB 18-5001 were found to have bold and thicker grains. However, it is worth noting that the grain shape of BLB 18-5001 is slender compared to other genotypes, due to its longer grain length.

The aroma of basmati rice is attributed to the presence of 2-acetyl-1-pyroline (2AP). A recessive missense mutation in the *Betaine Aldehydes De-Hydrogenase* (*BADH*) gene impairs its normal function, resulting in increased accumulation of 2AP in aerial plant parts (Sakthivel et al., 2009). The *BADEX7-5* marker is linked to the seventh exon of the *BADH* gene and distinguishes aromatic genotypes from non-aromatic ones. In this study, we selected plants from segregating generations that carried an 8 bp deletion in the seventh exon of the *Badh* gene. The aroma content of all these plants was found to be higher than IRBB 66, suggesting that the BADEX7-5 marker is a useful tool for the development of basmati/aromatic rice (Figure 2).

The determination of heritability is crucial for the improvement of desirable traits. Traits with high heritability can be easily improved with low selection intensity, whereas those with low heritability require more intense selection. The heritability of traits can be influenced by both genetic and environmental factors. In this study, all agronomic and morphological grain quality and milling traits showed high heritability, ranging from 64 to 100% (Figure 6). This indicates that these traits are predominantly controlled by genetics and are less susceptible to environmental influences, making phenotypic selection a viable method of improvement. This conclusion is consistent with previous studies by Immanuel et al. (2011) and Roy et al. (2020) who also found high heritability for various agronomic and quality traits in both uniform and segregating populations.

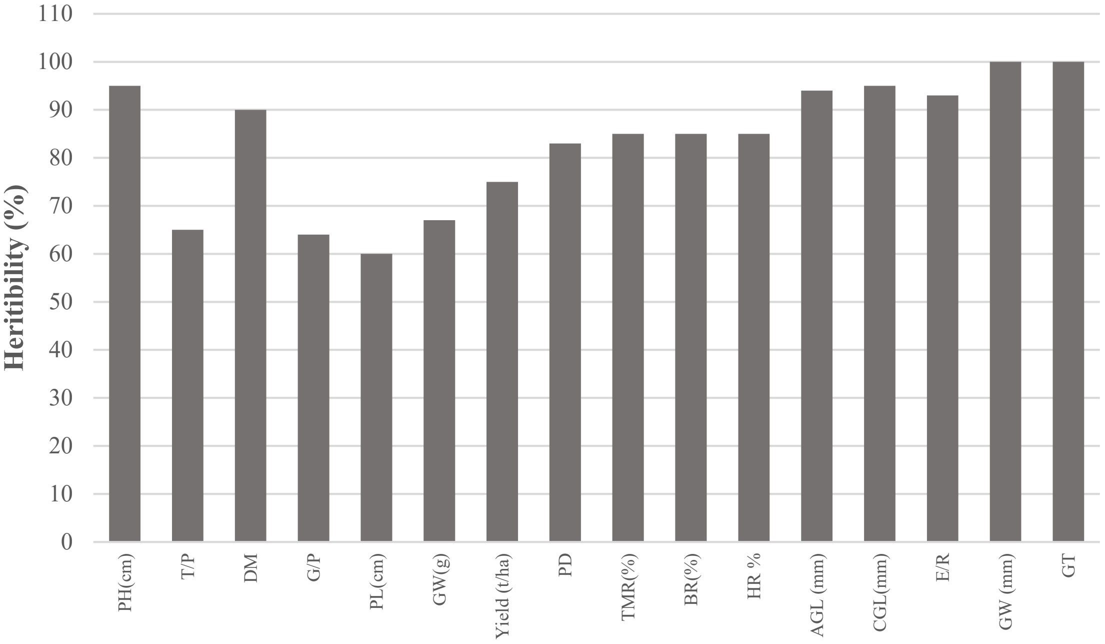

Figure 6. Heritability of different agro-morphological and quality traits in BLB 18-5001. Plant height (PH), Tillers/plant (T/P), Days of maturity (DM), Grain per panicle (G/P), Panicle length (PL), Grain weight (‘000’ GW (g)), Panicle Density(PD), Total Milled Rice (TMR), Brown Rice (BR), Head Rice (HR), Average grain length (AGL), Cooked Grain length (CGL), Elongation ration (E/R), Grain Width (GW), and Grain Thickness (GT).

In conclusion, the developed BLB 18-5001 basmati rice line showed good performance to other genotypes in regard to tillers per plant, thousand-grain weight, grain yield, average and cooked grain length, and elongation ratio, as well as superior agronomic and quality parameters. This was achieved through the diligent phenotypic selection of plants and their backcrossing with the recurrent parent to incorporate desirable agronomic and grain quality attributes in each segregating generation. This approach of phenotypic selection along with MAS has been proven effective in previous studies for the introgression of desired traits and the recovery of the genome of the recurrent parent through molecular markers (Babu et al., 2017; Sabar et al., 2019). However, the BLB 18-5001 line showed lower white milled rice parameters (TMR, BR, and HR) than all parental genotypes, which was largely due to its extraordinary grain length and the milling apparatus being more suitable for long and medium grain rice. To improve milling recovery, parboiling or other appropriate processing technologies could be implemented.

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