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KASP genotyping reveals disease resistance and yield enhancement in swarna introgression lines

KOUSIK M B V N[1], PUNNIAKOTTI E[1], REKHA G[1], CHAITRA K[1], HAJIRA S K[1], PRASAD M S[1], BADRI J[1], LAHA G S[1], SUDHAKAR P[2], KRISHNA SATYA A[2], NEERAJA C N[1], FIYAZ R A[1] and SUNDARAM R M[1]*

ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad, Telangana 500 030, India

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ABSTRACT

Deployment of marker-assisted selection (MAS) in the molecular breeding programmes has transformed the crop breeding over the last decade, bringing speed and precision to the breeding programmes. The experiment was conducted during rainy (*kharif)* seasons of 2019, 2020, and 2021 at ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad, Telangana for validation of the Kompetitive Allele Specific PCR (KASP) SNP markers for routine marker-assisted selection through genotyping and phenotyping of a set of breeding lines segregating for grain number, yield and resistance to bacterial blight and blast diseases. Initially, 150 introgression lines of Swarna possessing bacterial leaf blight (*Xa21*), blast (*Pi54*) and yield enhancing gene (*Gn1a*) at ICF_4 generation were genotyped using trait specific KASP markers, and a set of 22 plants was identified to be homozygous for all the three genes, 68 plants were found positive for two genes possessing either *Xa21* + *Gn1a* and *Pi54* + *Gn1a*, rest were having different single gene. Simultaneously, plants which were triple gene positives were also subjected for stringent phenotypic screening for the targeted stresses, viz. bacterial blight and blast disease resistance and further evaluated for grain number and other key agronomic traits. All the three-gene positive plants exhibited high level of resistance to bacterial blight and blast diseases in comparison with positive checks and displayed higher grain number and yield than the recurrent parent, Swarna. The efficiency of SNP marker system provided by Intertek is accurate, cost effective and accurate with phenotype.

Keywords: Bacterial blight, Blast, Grain number, KASP, Rice, SNP

Rice (*Oryza sativa* L.) is a staple crop that feeds the world's rising population. Rice output is threatened by bacterial blight and blast. Thus, rice breeding programmes aim to create disease-resistant cultivars. Microarray, genotyping by sequencing (GBS), high-resolution melting (HRM), and Kompetitive allele-specific PCR (KASP) are genotyping methods. Due to their stability, speed, and ease of use, single nucleotide polymorphism (SNP) markers have become popular among these methods. The KASP assay-based medium-throughput SNP genotyping tool developed by Consortium of International Agricultural Research Centre (CGIAR) and Intertek in Sweden is affordable and accessible to breeders. This platform includes SNP solutions for rice and other crops. Rice breeding has been accelerated using high-throughput SNP-based genotyping technologies (Ishwarya *et al*. 2022). KASP markers have many advantages over other marker types, including less labour, time, cost, dissemination, and adaptability (Arbelaez *et al*. 2019).

Although rice output has increased, more can be done to feed a growing population. Biotic and abiotic stresses severely reduce crop output. *Xanthomonas oryzae* PV. *oryzae* (*Xoo*), a biotic stress (BB), produces one of the worst rice illnesses. This disease reduces yields by 74–81%. *Magnaporthe oryzae* (anamorph *Pyricularia*) causes rice blast disease, threatening rice production. Disease epidemics diminish yield by 70–80% (Khush and Jena 2009). Ashikari *et al*. (2005) found *Gn1a* on chromosome 1 in Habataki indica rice. *Gn1a*-encoded cytokine oxidase protein increases reproductive organs and grains in the inflorescence meristem.

Its adaptability and vast cultivation in India make 'Swarna' rice famous. Marker-assisted backcrossing (MABB) developed and released Swarna variants with improved traits. Submergence-tolerant Swarna sub 1 and blast-resistant DRR Dhan 51 are examples (Neeraja *et al*. 2007, Rambabu *et al*. 2016). Swarna can be improved by adding resistance to key diseases including bacterial blight (BB) and blast and the ability to increase grain output. The current study aims to increase Swarna's grain productivity and resistance to bacterial blight and blast diseases.

MATERIALS AND METHODS

The experiment was conducted during rainy (*kharif)* seasons of 2019, 2020, and 2021 at ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad, Telangana. A

[1]ICAR-Indian Indian Institute of Rice Research, Rajendra Nagar, Hyderabad, Telangana; [2]Acharya Nagarjuna University, Guntur, Andhra Pradesh. *Corresponding author email: rms_28@rediffmail.com

total of 150 introgression lines (ILs) from the ICF_4 generation onward for trait-based KASP genotyping were selected to study the Kompetitive Allele Specific PCR (KASP) SNP markers for routine marker-assisted selection through genotyping and phenotyping of a set of breeding lines segregating for grain number, yield and resistance to bacterial blight and blast diseases. Two identical Near Isogenic Lines (NILs) were crossed to generate these ILs. ICAR-Indian Institute of Rice Research developed improved Samba Mahsuri (ISM) rice with one NIL, ISM-B, that had the disease resistance genes *Xa21* and *Pi54*. The other YPK 264 NIL, Swarna, from International Rice Research Institute (IRRI), Philippines, had the grain number gene *'Gn1a'*. Leaf samples were collected from each variety at 21 days after planting. The Intertek-Agritech laboratory, Hyderabad provided 96 well microtiter plates, and two 2 mm leaf discs from each genotype were inserted in the tubes. Barcoding was done on the plates as per instructions (https://excellenceinbreeding.org/toolbox/services/high-throughput-genotyping-services-htpg). After sampling, plates were oven dried at 45°C for 12 h before shipping. DNA isolation was done using LGC oKtopureTM automated high-throughput sbeadexTM (surface-coated superparamagnetic beads). AgriTech-Intertek Pvt. Ltd., Hyderabad, extracted and purified DNA per manufacturer's instructions (LGC Bio search technologies 2021b). Steel beads crushed leaf samples in 96-deep-well plates until they were homogeneous, then the sbeadexTM kit's extraction buffer (Bio search technologies 2021b) was used. DNA was purified using sbeadexTM coated super paramagnetic particles, which attract and bind nucleic acids.

KASP genotyping: Genotyping used Kompetitive allele-specific PCR markers (KASP, https://www.lgcgroup.com/) designed for the traits of interest (Fig. 1). The Genotyping Services at ICAR-Indian Rice Research Institute produced these KASP markers for the genes *Xa21, Pi54*, and *Gn1a*. According to Kanyange *et al*. (2019), Kompetitive Allele Specific PCR was used for genotyping.

SNP viewer software: SNP viewer v.4.1.2 (LGC Bio search technologies 2021a) displayed genotype dataset cluster plots. High-throughput genotyping (HTPG) result files (comma-separated value format) were utilised to combine allele calls to identify resistant and susceptible genotypes based on beneficial SNP alleles. Fluorescence distinguishes homozygotes and heterozygotes in the SNP viewer application (Supplementary Fig. 1).

Phenotypic screening for bacterial blight resistance: A virulent isolate of the bacterial blight (BB) pathogen, *Xanthomonas oryzae* pv. *oryzae*, DX-020 (collected from Hyderabad, Telangana State, India; Laha *et al*. 2009), was used to test the ILs alongside susceptible Swarna and resistant improved Samba Mahsuri during *kharif* 2019. The IRRI-SES scale, 2013 (International Rice Research Institute 2013), which measures sick leaf area, was used to evaluate 5 leaves per plant for bacterial blight reactivity 15 days after inoculation.

Phenotypic screening for blast resistance: The ICAR-Indian Institute of Rice Research uniform blast nursery (UBN) used a local virulent isolate of *Magnaporthe oryzae* to screen ILs alongside susceptible (Swarna) and resistant checks (Tetep) during the 2019 rainy season. The IRRI SES scale of 0–9 was utilised to collect data 15 days after inoculation (IRRI 2013).

Evaluation of agro-morphological characters of the improved lines of Swarna: Introgression lines with favourable alleles for all desired attributes and blast and bacterial blight resistance were agro-morphologically evaluated. Two replications of selected ILs and their parents (Swarna, ISM-B, and YPK 264) were grown in 1 m^2 plots with 15 cm × 20 cm spacing at the ICAR-Indian Institute of Rice Research experimental farm during wet seasons 2019–2021 under transplanting conditions. Plant height (PH), days to 50% flowering (DFF), panicle length (PL), number of productive tillers per plant (NPT), grain number per panicle (GNPP), flag leaf length (FLL), 1000-grain weight (TGW) and single plant yield (SPY) were measured.

Statistical analysis: The data were analysed using Freeman *et al*. (1978) method. Microsoft Excel generated least significance difference (LSD) values at 5% significance and coefficient of variation (CV) using standard errors of mean (S Em±). Statistics were performed using SAS Version 9.2 (SAS Institute Inc. Cary, NC, USA). For Swarna's high-yielding ILs, SAS PROC GLM was used for ANOVA. KASP genotypic data from the Intertek SNP genotyping platform were compared to phenotypic data on bacterial blight, blast, grain number, and other critical agro morphological parameters associated with yield.

RESULTS AND DISCUSSION

Swarna, a popular mega rice variety, was developed and released in 1982 by the Andhra Pradesh Rice Research Institute (APRRI) in Maruteru, Andhra Pradesh. Because of the mega-variety's high yield, attractive plant type, adaptability, improved tillering, higher nitrogen response, tolerance to low soil P, and grain quality, over 8 Mha of it were planted in India (Swamy *et al*. 2020). Despite these benefits, Swarna is susceptible to bacterial blight and blast, which are endemic in numerous sections of the country, including coastal areas where it is frequently grown (Mohapatra *et al*. 2020), and produces less than many other rice types. In Habataki, a high-yielding Indica rice variety with a sturdy stem and increased grain number, the gene *Gn1a* and candidate gene Ckkx2 were favourable (Ashikari *et al*. 2005). A near-isogenic line (NIL) of improved Samba Mahsuri with the *Xa21* and *Pi54* genes that is resistant to bacterial blast and blight was developed by Rekha *et al*. (2018).

A total of three SNP markers reported to be specific for *Xa21*, *Pi54* and *Gn1a* genes were used for validating the ILs for targeted stresses and it was observed that 22 ILs were homozygous for all the targeted genes, viz. *Xa21 + Pi54+Gn1a* (Table 1). A total of 68 ILs were observed to be positive for two genes, i.e. 40 for *Xa21 + Gn1a* and 28 for *Pi54 + Gn1a.* Results from the three KASP assays combined for genotyping revealed an average SNP call rate of 98.38%, exceeding the required SNP call rate of 95%

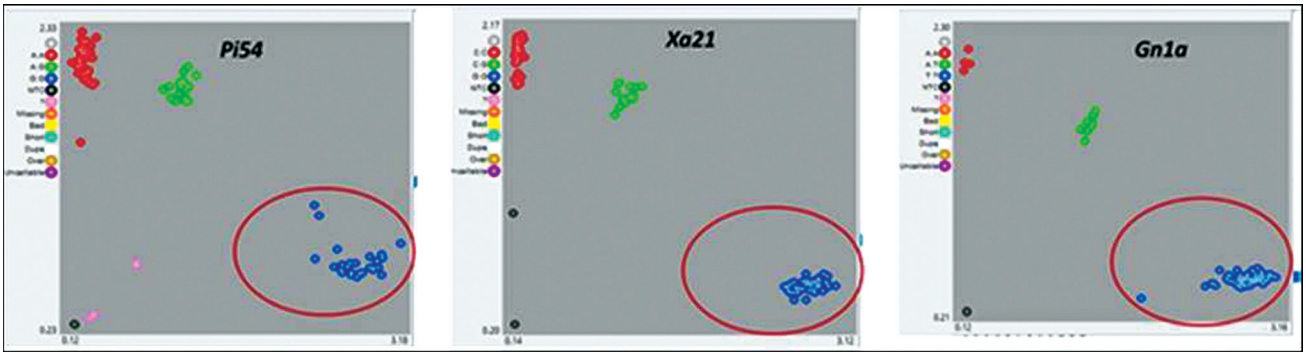

Fig. 1 Graphical representation of SNPs as genotyping cluster plots using SNP viewer.
Favourable alleles for *Xa21*-C; *Pi54* - G; *Gn1a* - T, while unfavourable alleles for *Xa21*-G; *Pi54* - A; *Gn1a* - A.
Favourable alleles are indicated in blue colour; unfavourable in red and hetero in green colour, Clear distinction was observed between the allelic states, homozygotes, and heterozygotes.

to declare a genotyping project as valid. Fluorochrome dye intensity values, which are frequently used to gauge the allelic discriminatory power of each SNP assay, were plotted to create Cartesian cluster plots for each KASP assay (Fig. 1). SNP markers used in this study showed high-quality allelic discrimination of Kompetitive allele-specific PCR Cartesian plots for the loci *Xa21*, *Pi54*, and *Gn1a*. Triple gene positives were subjected for stringent phenotypic screening for the targeted biotic stresses, viz. bacterial blight and blast diseases and evaluated for grain number and other key agronomic traits.

To improve Swarna, we added *Xa21, Pi54*, and *Gn1a*, which resist bacterial blight and blast and increase in grain number. This study developed Swarna ILs with better yield, and resistant to bacterial blight and blast. MABB is one of the most practical ways to improve rice varieties and hybrids (Hari *et al*. 2013, Abhilash 2017, Balachiranjeevi 2018). Similar to the present study, Janaki *et al*. (2021) reported simultaneous introgression of resistance/tolerance to multiple stresses in Swarna and Naveen cultivar backgrounds with *xa5* + *xa13* + *Xa21* + *Gm4* + *gm8* + *qDTY1.1* + *qDTY3.1 in IL-6 and Pi9* + *Xa21* + *Gm8* + *qDTY2.2* + *qDTY4.1* in MAFB 1 for BB, blast, gall midge, and drought Singh *et al*. (2021) and Badri *et al*. (2022) in Lalat and Krishna Hamsa, while Swamy *et al*. (2020) and Rekha *et al*. (2022), respectively, reported sequential introgression of low P tolerance and salinity tolerance with incorporation of *PUP1* and *Saltol* QTLs in the background of ISM with inherent *xa5* + *xa13* + *Xa21*, now released as DRR Dhan 60 and DRR Dhan 58.

Introgression lines exhibited resistance to BB and blast. HR12 and Swarna, the recurrent parent, were highly vulnerable to blast (Score 9), while Tetep and donor parent ISM-B were highly resistant (Score 1-2) (Table 2). Screening for bacterial blight resistance revealed that the donor parent (ISM-B) had a lesion length of <1 cm (score 1), while the recurrent parent (Swarna) was very vulnerable with a lesion length of 16.5 cm (score 7). All 22 ILs were blast and bacterial blight resistant. Five ILs-BPK-159-1-3-9, BPK-163-2-1-7, BPK-168-1-7-9, BPK-173-2-1-7, and BPK-173-2-9-1 were highly resistant to bacterial blight and blast resistance (Fig. 2).

Significant differences were observed between the ILs and the parents with moderate to high heritability for the studied agro-morphological traits (Table 2). The donor parent, ISM-B was observed to flower earlier with a mean DFF of 102.7 ± 0.3 while the recurrent parent, Swarna has a mean DFF of 124.0 ± 0.6 and all the improved lines were significantly earlier by 10–15 days as compared to the recurrent parent. It was also observed that improved lines

Table 1 SNP marker allelic pattern of 3-gene confirmed lines of Swarna (at ICF_4 Generation)

Code	snpOS00061 (*Xa21*)	snpOS00499 (*Pi54*)	snpOS00396 (*Gn1a*)
Swarna	G:G	A:A	A: A
ISM-B	C:C	G:G	T:T
YPK-264	G:G	A:A	T:T
Tetep	NA	G:G	NA
HR12	NA	A:A	NA
BPK-19-6-3-10	C:C	G:G	T:T
BPK-22-4-6-5	C:C	G:G	T:T
BPK-25-4-6-9	C:C	G:G	T:T
BPK-86-2-4-8	C:C	G:G	T:T
BPK-93-3-6-9	C:C	G:G	T:T
BPK-133-1-14-2	C:C	G:G	T:T
BPK-159-1-3-9	C:C	G:G	T:T
BPK-161-1-8-7	C:C	G:G	T:T
BPK-162-1-2-6	C:C	G:G	T:T
BPK-163-2-1-7	C:C	G:G	T:T
BPK-166-2-6-8	C:C	G:G	T:T
BPK-168-1-7-9	C:C	G: G	T:T
BPK-170-1-9-4	C:C	G:G	T:T
BPK-173-1-3-9	C:C	G:G	T:T
BPK-173-2-1-7	C:C	G:G	T:T
BPK-173-2-9-1	C:C	G:G	T:T
BPK-175-1-4-8	C:C	G:G	T:T
BPK-175-2-5-10	C:C	G:G	T:T
BPK-177-2-1-3	C:C	G:G	T:T
BPK-183-2-2-6	C:C	G:G	T:T
BPK-184-1-4-8	C:C	G:G	T:T
BPK-189-2-8-16	C:C	G:G	T:T

Favourable alleles for *Xa21*-C; *Pi54* - G; *Gn1a* - T, while unfavourable alleles for *Xa21*-G; *Pi54* - A; *Gn1a* - A.

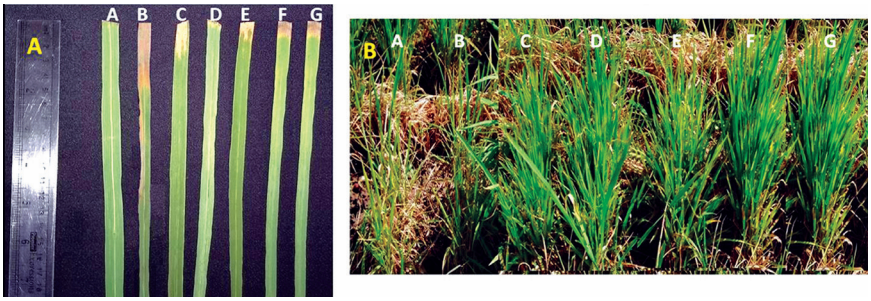

Fig. 2 Screening of the three-gene pyramided ICF_4 plants under a uniform nursery for blast disease.
A. A, ISM; B, Swarna; C, IR123309-1-9-7; D, ISM-B; E, BPK-159-1-3-9; F, BPK-163-2-1-7; G, BPK-168-1-7-9.
B. A, Swarna; B, IR123309-1-9-7; C, Tetep; D, ISM-B; E, BPK-159-1-3-9; F, BPK-163-2-1-7; G, BPK-168-1-7-9.

were taller (15–20 cm) than Swarna, with mean values ranging from 93.3 ± 0.3 to 118.0 ± 0.6 cm in comparison to the values recorded for the recurrent parent (82.7 ± 1.5 cm). The mean number of productive tillers per plant varied between 9.3 ± 0.3 to 17.7 ± 0.3 in the improved lines as compared to Swarna, which recorded a value of 9.3 ± 0.3. Panicle length and panicle weight of Swarna was observed to be 22.2 ± 0.6 cm and 2.4 ± 0.0 g, respectively, while the improved lines possessed significantly higher mean values ranging from 23.5 ± 1.2 to 26.3 ± 0.2 cm and 3.8 ± 0.0 to 6.4 ± 0.1 g, respectively. With respect to grain number per panicle, we observed significant difference in the grain number between the recurrent parent, Swarna (137.7 ± 1.5) and the improved lines (228.7 ± 1.5 to 355.0 ± 5.8). Thousand grain weight of the improved lines was on par with Swarna (17.7 ± 0.1 g), with mean values ranging from 17.2 ± 0.7 to 17.9 ± 1.4 g. Significant difference was observed in the improved lines and the recurrent parent with respect to grain yield, with the backcross derived lines showing mean values ranging from 26.8 ± 0.9 to 42.6 ± 0.4 g, as compared to Swarna, which recorded a mean value of 0.1 ± 0.2 g (Table 2). The results indicated that the panicle weight has the highest phenotypic coefficient of variance (PCV) and genotypic coefficient of variance (GCV) of 20.30 and 19.14%, while DFF has lowest PCV and GCV of 3.93 and 3.85%, respectively. Panicle length and 1000-grain weight showed marginal differences between PCV and GCV. However, with respect to broad sense heritability, plant height was observed with highest heritability (99.13%), while 1000-grain weight (82.03%) and panicle length (58.88%) had moderate heritability (Table 2). Among the 22 ILs; 5 Ils, viz. BPK-159-1-3-9, BPK-163-2-1-7, BPK-168-1-7-9, BPK-173-2-1-7, and BPK-173-2-9-1 were observed to be highly resistant to both bacterial blight and blast resistance also showed high grain number and corresponding high single plant yield than remaining ILs.

Single plant yield and panicle weight have substantial positive correlations with all variables except DFF, demonstrating that yield genes increase yield. TGW and PW, SPY and GNPP, and GNPP with PL and NPT were positively correlated. Fig. 3 shows a considerable negative correlation between DFF and yield attributes.

For conventional breeding, PCR-based marker-assisted selection (MAS) has significant drawbacks. Accurate prediction-based genotyping with high throughput at a reasonable cost is necessary for fast-tracking marker-assisted breeding programmes. The discovery and validation of competitive allele-specific PCR markers (KASP) allowed for the efficient, high-throughput screening of huge populations at moderate expenses. These markers aided in the development of blast resistance and marker-assisted bacterial blight (Kanyange *et al*. 2019). We employed KASP markers to find plants with favourable alleles for *Xa21* and *Pi54*, which resist bacterial blight and blast, and *Gn1a*, which enhances grain number and yield. 22 plants have all target genes which were in homozygous condition. KASP genotyping showed that ILs carried *Xa21* even though not all plants possessed the allele or were homozygous. 28 plants carried *Pi54*. These results corroborated with the findings of Kanyange *et al*. (2019).

Twenty two, homozygous positive ILs had favourable alleles for all three targeted genes, *Xa21, Pi54*, and *Gn1a*. Five ILs with beneficial alleles showed strong resistance to BB and blast, improved grain number, and grain production. In this study, *Gn1a* increases grain number and yield in ILs by 25–30%. Using 'Nipponbare' (japonica) as the recipient and CSSLs from 9311 (indica) as the trait donor, (Xu *et al*. 2019) shown a 13.34 to 14.46% increase in grain yield in NIL-qGN1c9311. According to Ashikari *et al*. (2005), Habataki *Gn1a* caused a 23% increase in grain number in Koshihikari NILs. All yield-attributing traits, such as grain number, tiller number, and 1000-grain weight, had a

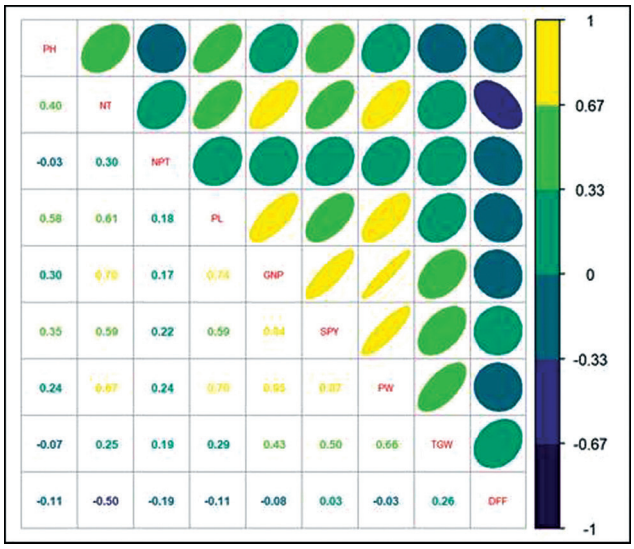

Fig. 3 Correlation analyses of improved lines of Swarna for the agro morphological traits and yield attributes.

Table 2 Phenotypic evaluation of the introgression lines with favourable alleles of Xa21, Pi54, and Gn1a for disease reaction against bacterial blight, blast and agro-morphological traits

Lines	DFF	PH	NT	NPT	PL	PW	GNP	TGW	SPY	FLA	Bacterial blight Score (DXI20)	Blast score (SPI-40)
Swarna	124.0 ± 0.6	82.0 ± 0.6	10.3 ± 0.3	9.3 ± 0.3	22.2 ± 0.6	2.4 ± 0.0	137.7 ± 1.5	17.7 ± 0.1	20.1 ± 0.2	E	7	9
ISM-B	102.7 ± 0.3	93.0 ± 0.6	19.7 ± 0.3	17.7 ± 0.3	23.0 ± 0.6	3.1 ± 0.1	188.0 ± 1.5	16.8 ± 0.0	23.5 ± 0.6	SE	3	3
YPK-264	120.0 ± 0.6	101.7 ± 0.3	20.7 ± 0.3	18.3 ± 0.3	24.4 ± 0.6	4.4 ± 0.1	237.7 ± 1.5	19.2 ± 0.2	30.5 ± 0.2	SE	7	7
BPK-19-6-3-10	114.0 ± 0.6	103.3 ± 0.3	20.3 ± 0.3	17.0 ± 0.6	24.2 ± 0.3	4.5 ± 0.1	238.0 ± 1.7	18.6 ± 0.2	38.2 ± 0.2	E	3	2
BPK-22-4-6-5	114.7 ± 0.3	93.3 ± 0.3	21.0± 0.6	11.7 ± 0.3	24.5 ± 0.3	4.5 ± 0.1	231.3 ± 2.0	19.4 ± 0.3	27.2 ± 1.3	E	3	2
BPK-25-4-6-9	111.3 ± 0.3	100.3 ± 0.3	19.0 ± 0.6	12.3 ± 0.3	24.9 ± 0.3	4.2 ± 0.1	233.3 ± 1.5	18.3 ± 0.1	28.1 ± 0.2	SE	1	2
BPK-86-2-4-8	109.7 ± 0.3	99.0 ± 0.6	21.3 ± 0.9	11.3 ± 0.3	24.5 ± 0.4	4.1 ± 0.2	228.7 ± 1.5	18.1 ± 0.2	28.9 ± 0.2	SE	3	2
BPK-93-3-6-9	110.7 ± 0.7	110.7 ± 0.3	21.0 ± 0.6	9.3 ± 0.3	23.8 ± 0.0	3.8 ± 0.0	234.3 ± 2.9	17.1 ± 0.2	27.2 ± 0.4	E	3	1
BPK-133-1-14-2	104.0 ± 0.6	105.3 ± 0.3	20.3 ± 0.3	12.3 ± 0.3	25.0 ± 0.1	4.0 ± 0.1	231.0 ±2.6	17.9 ± 0.3	26.9 ± 0.3	SE	3	2
BPK-159-1-3-9	105.7 ± 0.3	101.3 ± 0.3	21.3 ± 0.3	15.7 ± 0.3	24.9 ± 0.7	3.9 ± 0.1	231.3 ± 3.3	17.4 ± 0.3	27.1 ± 0.1	SE	3	3
BPK-161-1-8-7	110.3 ± 0.3	102.3 ± 0.3	20.7 ± 0.3	11.3 ± 0.6	24.1 ± 0.2	3.9 ± 0.2	230.0 ± 3.5	18.2 ± 0.1	27.6 ± 0.6	Erect	3	2
BPK-162-1-2-6	110.7 ± 0.3	102.0 ± 0.3	21.3 ± 0.3	14.0 ± 0.0	23.6 ± 0.2	4.5 ± 0.2	239.3 ± 0.9	19.1 ± 0.2	35.9 ± 0.5	SE	3	1
BPK-163-2-1-7	111.0 ± 0.6	100.3 ± 0.3	21.3 ± 0.3	12.7 ± 0.3	24.2 ± 0.2	5.5 ± 0.5	310.0 ± 0.6	18.8 ± 0.5	40.4 ± 0.5	SE	1	1
BPK-166-2-6-8	112.3 ± 0.3	120.3 ± 0.3	19.3 ± 0.3	13.3 ± 0.3	25.1 ± 0.6	4.5 ± 0.1	231.7 ± 0.7	18.9 ± 0.3	31.5 ± 0.6	E	3	3
BPK-168-1-7-9	112.7 ± 0.3	112.3 ± 0.3	20.7 ± 0.7	12.3 ± 0.3	25.4 ± 0.6	4.9 ± 0.1	253.3 ± 4.4	19.4 ± 0.3	37.8 ± 0.3	SE	2	2
BPK-170-1-9-4	110.0 ± 0.6	100.0 ± 0.6	21.0 ± 0.6	10.7 ± 0.3	24.5 ± 0.6	4.9± 0.3	260.3 ± 3.2	18.8 ± 0.3	38.1 ± 0.2	SE	1	1
BPK-173-1-3-9	112.0 ± 0.6	104.7 ± 0.3	22.7 ± 0.3	14.7 ± 0.3	26.1 ± 0.4	6.4 ± 0.1	355.0 ± 5.8	19.3 ± 0.2	42.6 ± 0.4	SE	1	1
BPK-173-2-1-7	112.3 ± 0.3	100.0 ± 0.6	21.7 ± 0.3	14.3 ± 0.9	26.0 ± 0.2	6.0 ± 0.1	336.0 ± 4.4	19.1 ± 0.2	40.8 ± 0.8	SE	1	1
BPK-173-2-9-1	112.0 ± 0.6	105.0 ± 0.6	20.0 ± 0.6	12.7 ± 0.3	23.5 ± 1.2	4.6 ± 0.1	253.0 ± 1.5	18.2 ± 0.3	34.5 ± 0.5	E	3	2
BPK-175-1-4-8	113.0 ± 0.6	119.7 ± 0.3	20.3 ± 0.3	12.7 ± 0.7	25.7 ± 0.3	4.8 ± 0.1	250.0 ± 2.9	18.3 ± 0.3	32.9 ± 0.7	E	3	3
BPK-175-2-5-10	114.0 ± 0.6	116.0 ± 0.6	19.3 ± 0.3	11.3 ± 0.3	24.9 ± 0.3	3.8 ± 0.2	253.0 ± 2.0	16.7 ± 0.4	26.8 ± 0.9	E	3	3
BPK-177-2-1-3	115.0 ± 0.6	103.3 ± 0.9	22.3 ± 0.3	14.7 ± 0.3	26.3 ± 0.2	6.0 ± 0.1	327.7 ± 1.3	18.8 ± 0.2	40.4 ± 0.4	SE	1	1
BPK-183-2-2-6	109.3 ± 0.3	103.3 ± 0.3	20.3 ± 0.3	17.7 ± 0.3	25.3 ± 0.4	4.3 ± 0.5	241.0 ± 2.0	18.0 ± 0.1	34.0 ± 1.1	SE	3	3
BPK-184-1-4-8	112.0 ± 0.3	105.0 ± 0.6	20.3 ± 0.9	12.7 ± 0.3	24.6 ± 0.5	4.0± 0.1	241.3 ± 1.9	16.9 ± 0.2	33.3 ± 0.6	SE	3	3
BPK-189-2-8-16	113.7 ± 0.3	118.0 ± 0.6	21.0± 0.6	10.7± 0.3	25.1 ± 0.8	3.9 ± 0.2	244.3 ± 3.5	16.6 ± 0.4	37.0 ± 0.6	E	3	3
PCV	3.93	8.26	11.55	19.58	5.30	20.30	18.04	5.25	18.73			
GCV	3.85	8.23	10.86	18.74	4.07	19.14	17.95	4.75	18.49			
CV (%)	3.88	8.24	11.09	19.02	3.94	19.53	17.98	4.92	18.57			
Heritability (H^2)	96.33	99.13	88.38	91.62	58.88	88.93	98.98	82.03	97.46			
F	79.84	342.65	23.81	33.78	5.29	25.10	292.58	14.68	116.05			
P value (<0.01)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001			

DFF, Days to 50% flowering; PH, Plant height; NT, No. of tillers; NPT, No. of productive tillers; PL, Panicle length; PW, Panicle weight; GNP, Grain no./panicle, TGW, 1000 grain weight; FLA, Flag leaf angle; E, Erect; SE, Semi erect; SPY, Single plant yield.

significant positive correlation with single plant yield, while DFF and plant height had a strong negative association with all the key yield attributes, demonstrating the individual traits' contribution to yield enhancement as reported earlier in several studies.

The current study identified introgression lines in mega cultivar Swarna with bacterial blight and blast resistance and improved grain number and yield through phenotyping and validated the presence of favourable alleles of *Xa21, Pi54, and Gn1a* through SNP genotyping using Intertek Platform. A low-cost high-throughput SNP genotyping tool like Intertek will accelerate molecular breeding efforts to generate superior cultivars with value-added features. Genomic/SNP marker-assisted breeding can combine effective resistance genes/QTLs for multiple biotic stresses with yield-enhancing genes to develop climate-resilient, high-yielding cultivars and hybrids.

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