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MARKER-ASSISTED INTROGRESSION OF BROWN PLANTHOPPER RESISTANCE INTO TELANGANA SONA RICE VARIETY

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ABSTRACT

Increasing outbreaks of brown planthopper threaten the productivity of elite rice cultivars despite significant advances in varietal improvement. Telangana sona, a widely cultivated rice variety valued for its higher yield and grain quality, is susceptible to brown planthopper infestation. The present study aimed to introgress the brown planthopper resistance Quantitative Trait Locus (QTL) *qbph-1-1* from the resistant donor M229 into the genetic background of Telangana Sona using a Marker-Assisted Backcross Breeding (MABB) approach. The breeding and evaluation work was carried out during *kharif* 2021 to *kharif* 2022 at the Regional Sugarcane and Rice Research Station (RS&RRS), Rudrur, Nizamabad, Telangana, with phenotypic screening conducted under controlled conditions at the Entomology glasshouse, ICAR-Indian Institute of Rice Research (ICAR-IIRR), Rajendranagar, Hyderabad. Among 21 BC_1F_1 plants, ten were identified as heterozygous for *qbph-1-1* and advanced through backcrossing to generate the BC_2F_1 population. Foreground screening of 61 BC_2F_1 plants identified 17 individuals carrying the target QTL, of which ten consistently exhibited strong phenotypic resistance with low damage scores, establishing a clear genotype-phenotype association. Selfing of selected BC_2F_1 plants resulted in a BC_2F_2 population, wherein genotyping of 600 plants identified 131 individuals, homozygous for *qbph-1-1*. All marker-positive BC_2F_2 plants expressed resistant to moderately resistant reactions under controlled infestation, confirming stable resistance upon fixation of the target QTL. The integration of molecular foreground selection with rigorous phenotypic screening enabled the rapid development of brown planthopper-resistant lines while retaining the agronomic identity of Telangana Sona.

Key words : Brown planthopper, Insect resistance, Marker-assisted backcross breeding, Modified mass tiller screening, Telangana Sona.

Introduction

Modern rice cultivation increasingly depends on a narrow set of elite varieties that combine high yield potential, superior grain quality, and wide adaptability. While such varieties have substantially enhanced productivity and farmer acceptance, their genetic uniformity has simultaneously increased vulnerability to rapidly evolving insect pests. Among these, the brown planthopper (BPH) (*Nilaparvata lugens* (Stål), Hemiptera: Delphacidae) has emerged as one of the most destructive constraints to rice production. BPH causes direct damage by feeding on phloem sap, leading to the characteristic “hopperburn” symptom, which can result in yield losses of upto 70% or even complete crop failure

under severe infestation (Backus *et al*., 2005; Krishnaiah *et al*., 2008). Besides causing direct feeding damage, it acts as a vector of viral diseases like rice ragged stunt and grassy stunt, compounding its threat to rice productivity (Horgan *et al*., 2018; Zheng *et al*., 2021). The frequent breakdown of resistance and rapid adaptation of BPH populations have therefore made host plant resistance a persistent challenge for rice breeding programmes (Du *et al*., 2020; Horgan, 2021; Yan *et al*., 2023).

Chemical control continues to be the dominant strategy for managing BPH in farmers' fields. However, indiscriminate and repeated insecticide applications have resulted in pest resurgence, development of insecticide resistance, environmental contamination and disruption of natural enemy populations (Horgan *et al*., 2018). These limitations have reinforced the importance of genetic resistance as the most economical and environmentally sustainable option for long-term BPH management (Fujita *et al*., 2019). To date, around 46 BPH resistance genes and more than 70 quantitative trait loci (QTLs) have been identified and mapped across various rice chromosomes (Akanksha *et al*., 2019; Kiswanto *et al*., 2022; Mishra *et al*., 2022; Ishwarya *et al*., 2025). Despite these advances, the effective deployment of resistance loci into farmer-preferred elite cultivars remains limited (Yan *et al*., 2023).

Elite rice varieties such as Telangana Sona play a major role in regional rice production systems owing to their yield stability, grain quality, and adaptability. However, Telangana Sona is highly susceptible to BPH infestation, making it vulnerable under increasing pest pressure. This susceptibility reflects a common breeding bottleneck, wherein resistance donors often possess inferior agronomic traits and cannot be directly adapted by farmers, while elite cultivars lack adequate pest resistance (Fujita *et al*., 2013). Similar vulnerability of elite rice varieties to planthopper outbreaks has been widely documented across Asian rice-growing regions, underscoring the urgency of incorporating resistance into high-value cultivars (He *et al*., 2020).

Marker-assisted backcross breeding (MABB) provides an effective strategy to address this challenge by enabling precise introgression of resistance loci into elite genetic backgrounds. The use of foreground markers tightly linked to target genes or QTLs allows early and accurate identification of desirable genotypes, thereby reducing population size and breeding duration compared to conventional phenotypic selection (Collard and Mackill, 2008). Marker-assisted backcrossing has been successfully applied to improve several agronomic and resistance traits in rice (Hasan *et al*., 2015). Nevertheless, molecular selection alone is insufficient for insect resistance breeding, as phenotypic expression of resistance can be influenced by pest pressure, genetic background, and environmental conditions, necessitating integration with reliable phenotypic screening methods (Bentur *et al*., 2016)

The Modified Mass Tiller Screening (MMTS) method is widely used for evaluating brown planthopper resistance due to its reproducibility and ability to discriminate resistance levels under controlled infestation (Jairin *et al*., 2007a). Integrating marker-assisted selection with MMTS-based phenotyping has been shown to improve the reliability of resistance introgression and facilitate identification of stable, functionally resistant lines (Fujita *et al*., 2019; Du *et al*., 2020). In this context, the present study aimed to introgress the BPH resistance QTL *qbph-1-1* (Bhargava, 2023) from the donor M229 into the elite rice variety Telangana Sona a through a marker-assisted backcross breeding approach, combining foreground selection with phenotypic validation to ensure stable resistance while preserving the agronomic identity of the recurrent parent.

Materials and Methods

Plant Material

The elite rice variety Telangana Sona (TS) was used as the recurrent parent in the present study. TS is a high-yielding, short-duration (125 days) variety widely cultivated for its superior grain quality. However, it is susceptible to Brown planthopper (BPH) infestation. The donor parent M229 was identified as a reliable source of resistance to BPH biotype 4, exhibiting a low damage score of 3.0 in the Standard Seedbox Screening Test (SSST). Using these two parents, a BC_1F_1 population was subsequently developed (Ishwarya Lakshmi *et al*., 2024).

Development of the Backcross Population

A total of 21 BC_1F_1 plants were raised during *kharif*, 2021, at the Regional Sugarcane and Rice Research Station (RS & RRS), Rudrur, Nizamabad. Foreground selection was employed to identify plant heterozygous for the target QTL *qbph-1-1*, and the confirmed BC_1F_1 plants were backcrossed with the recurrent parent Telangana Sona during the same season to generate BC_2F_1 seeds. During *rabi* 2021–22, BC_2F_1 seeds were germinated in petri dishes and uniform germination was observed within five days. The seedlings were raised on nursery beds, and 28-day-old seedlings were transplanted to the main field at a spacing of 20 × 15 cm, along with the recurrent parent Telangana Sona and the donor parent M229. Foreground selection at the seedling stage was

performed using the tightly linked SSR marker RM11069 to identify heterozygous plants carrying *qbph-1-1*. Marker-positive BC_2F_1 plants were subsequently evaluated for BPH resistance using the Modified Mass Tiller Screening (MMTS) method, and the confirmed resistant plants were selfed to generate BC_2F_2 population. During *kharif* 2022, the BC_2F_2 population obtained from the true BC_2F_1 plants, along with the parents, was raised at RS&RRS, Rudrur. The BC_2F_2 plants were genotyped at the seedling stage using RM11069 and phenotyped for BPH resistance using the MMTS method at ICAR-IIRR to confirm resistant plants. Selected resistant BC_2F_2 plants carrying the target QTL were subsequently selfed to generate the BC_2F_3 generation for further evaluation.

Phenotypic Screening for BPH Resistance

Brown planthopper (BPH) resistance in the BC_2F_1, and BC_2F_2 populations was assessed using the Modified Mass Tiller Screening (MMTS) method (Jairin *et al*., 2007a). During *rabi* 2021–22, BC_2F_1 plants along with parents, resistant checks (PTB33, Rathu Heenati and RP2068-18-3-5), and susceptible checks (TN1 and BPT 5204) were evaluated at the tillering stage. Three uniform tillers per plant were separated and potted individually. Ten days after establishment, each tiller was infested with ten third or fourth-instar BPH nymphs, and the pots were covered with mylar tubes sealed with moist cloth. The insects were allowed to feed, mate, oviposit, and hatch naturally. Plant damage was scored when the susceptible check (TN1) had completely wilted, following the Standard Evaluation System (SES) for Rice (IRRI, 2013) (Table 1). The same procedure was followed for screening the BC_2F_2 population during *Kharif* 2022.

DNA Extraction and Foreground Marker Analysis

Genomic DNA was isolated from individual plants of the BC_1F_1, BC_2F_1 and BC_2F_2 generations, along with the parental lines, using the CTAB method described by Doyle and Doyle (1987). The quality and quantity of DNA were assessed on 0.8% agarose gel using a standard DNA ladder and DNA concentration and purity were further determined by measuring absorbance at 260 and 280 nm using a NanoDrop ND1000 spectrophotometer. DNA samples were standardized to a working concentration of 50 ng/ìl using 1X TE buffer. Foreground selection for the brown planthopper resistance QTL *qbph-1-1* was carried out in the BC_1F_1, BC_2F_1 and BC_2F_2 populations of the Telangana Sona × M229 cross using the SSR marker RM11069. PCR amplification was performed in an Applied Biosystems (ABI) thermal cycler in a 10 µl reaction volume containing 2 µl template DNA, 0.3 µl each of forward and reverse primers, 4.0 µl Takara PCR master mix, and 3.4 µl sterile distilled water. The PCR programme consisted of an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 45 s, and extension at 72°C for 1 min, with a final extension at 72°C for 10 min. Amplified products were resolved in 3% agarose gels, visualized using a gel documentation system (BIORAD) and scored for further analysis.

Table 1 : Classification of resistance based on damage reaction.

Score	Plant state	Rating
0	No damage	Highly resistant
1	Very slight damage	
3	Lower leaf wilted with two green upper leaves	Resistant
5	Two lower leaves wilted with one green upper leaf	Moderately resistant
7	All three leaves wilted but stem still green	Moderately susceptible
9	All plants dead	Susceptible

Results and Discussion

Foreground selection and Advancement of BC_1F_1 plants

Foreground selection using the tightly linked SSR marker RM11069 enabled precise tracking of the BPH resistance QTL *qbph-1-1* during the early stages of backcross breeding. A total of 21 BC_1F_1 plants derived from the cross between Telangana Sona × M229 were raised during *kharif*, 2021, at the Regional Sugarcane and Rice Research Station (RS & RRS), Rudrur, Nizamabad. Among these, ten (BC_1F_1-2, BC_1F_1-4, BC_1F_1-7, BC_1F_1-9, BC_1F_1-14, BC_1F_1-16, BC_1F_1-18, BC_1F_1-19, BC_1F_1-20 and BC_1F_1-21) individuals were confirmed to be heterozygous for the target QTL, as revealed by marker profiling (Fig. 1). The recovery of nearly half of the BC_1F_1 plants with the donor allele indicates effective transmission of *qbph-1-1* and validates the suitability of RM11069 as a reliable diagnostic marker. Similar application of tightly linked SSR markers for early-generation foreground selection in BPH resistance breeding was demonstrated by Jairin *et al*. (2007a) and Korinsak *et al*. (2016).

Early identification of heterozygous plants allowed efficient elimination of non-target genotypes and facilitated rapid advancement of only those individuals carrying the resistant locus. This clearly highlights the advantage of marker-assisted selection over conventional phenotypic screening, particularly for insect resistance

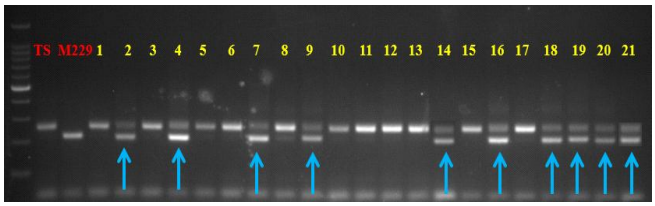

Fig. 1 : Confirmed BC_1F_1 plants of TS//TS/M229 using RM11069 marker specific to *qbph-1-1*, TS-Telangana Sona (Recurrent parent) and M229 (Donor parent).

traits that are strongly influenced by environmental variation and fluctuating pest pressure, as emphasized by Collard and Mackill (2008) and Horgan *et al*. (2018).

Molecular Confirmation of BC_2F_1 Plants and Transmission of Resistance

The confirmed BC_1F_1 plants were backcrossed with the recurrent parent Telangana Sona during *Kharif*, 2021, at RS&RRS, Rudrur, to generate BC_2F_1 seed. The BC_2F_1 population was subsequently grown during *rabi*, 2021-22, and a total of 61 BC_2F_1 plants that survived and established in the field were subjected to molecular screening. Foreground selection identified seventeen (BC_2F_1 -15, BC_2F_1 -17, BC_2F_1 -24, BC_2F_1 -25, BC_2F_1 -27, BC_2F_1 -30, BC_2F_1 -31, BC_2F_1 -33, BC_2F_1 -36, BC_2F_1 -37, BC_2F_1 -38, BC_2F_1 -41, BC_2F_1 -42, BC_2F_1 -51, BC_2F_1 -54, BC_2F_1 -59 and BC_2F_1 -61) plants positive for *qbph-1-1*, confirming continued transmission of resistant locus in the second backcross generation (Fig. 2). The segregation pattern observed in the BC_2F_1 population was consistent with expectations for a single major QTL under backcross conditions, further confirming the genetic stability of *qbph-1-1*. Similar segregation behavior of major BPH resistant loci under backcross breeding has been reported by Jairin *et al*. (2007a), Korinsak *et al*. (2016), Xiao *et al*. (2016), and Thulasinathan *et al*. (2020).

The successful recovery of marker-positive plants in BC_2F_1 generation indicates that the resistant QTL can be effectively retained while progressively restoring the genetic background of elite recurrent parent. This balance between resistance introgression and varietal integrity is a central objective of marker-assisted backcross breeding, as discussed by Hospital (2009) and Hasan *et al*. (2015).

Phenotypic Validation and Genotype-Phenotype association in BC_2F_1

Phenotypic screening of BC_2F_1 plants for BPH resistance was carried out during *Rabi* 2021-22 at the Entomology glass house, ICAR-Indian Institute of Rice Research (IIRR), Rajendranagar, Hyderabad, using the Modified Mass Tiller Screening (MMTS) method. Screening revealed substantial variation in BPH response,

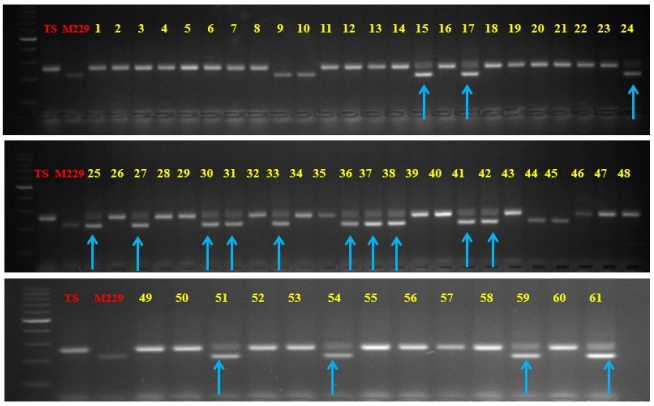

Fig. 2 : Foreground selection in BC_2F_1 plants of TS///TS//TS/M229 using *qbph-1-1* specific RM11069 marker, TS-Telangana Sona (Recurrent parent) and M229 (Donor parent).

ranging from resistant to susceptible reactions. Out of the 61 plants evaluated, 19 exhibited resistant reactions, 13 were moderately resistant, 10 were moderately susceptible, and 19 were susceptible, as summarized in Table 2. Among the resistant plants, ten (BC_2F_1-15, BC_2F_1-24, BC_2F_1-25, BC_2F_1-31, BC_2F_1-33, BC_2F_1-36, BC_2F_1-42, BC_2F_1-54, BC_2F_1-59 and BC_2F_1-61) individuals were also confirmed to carry *qbph-1-1* through molecular analysis and consistently recorded low damage scores, establishing a strong genotype-phenotype association. The reliability of MMTS for validating BPH resistance has been clearly demonstrated by Jairin *et al*. (2007a) and later supported by Bentur *et al*. (2016).

Interestingly, Nine BC_2F_1 plants (BC_2F_1-5, BC_2F_1-9, BC_2F_1-10, BC_2F_1-22, BC_2F_1-44, BC_2F_1-45, BC_2F_1-49, BC_2F_1-53 and BC_2F_1-56) displayed phenotypic resistance despite lacking the donor allele for *qbph-1-1*. This observation suggests the involvement of additional minor genes, background resistance factors, or epistatic interactions contributing to BPH tolerance. Such genotype-phenotype incongruence has been reported earlier by Fujita *et al*. (2013) and Du *et al*. (2020), highlighting the complex genetic architecture of BPH resistance. Nevertheless, the consistent performance of *qbph-1-1* positive plants confirms the major and stable contribution of this QTL to resistance.

Fixation and Stabilization of Resistance in BC_2F_2 Generation

Selfing of confirmed resistant BC_2F_1 plants during *rabi* 2021-22 resulted in the development of a large BC_2F_2 population, which was grown during *kharif* 2022. Foreground genotyping of 600 BC_2F_2 plants identified 131 individuals homozygous for *qbph-1-1*. These plants exhibited strong phenotypic resemblance to Telangana Sona, indicating substantial recovery of the recurrent

Table 2 : Phenotypic reaction of BC_2F_1 plants to BPH.

S. no.	Entries	Score	Phenotypic reaction	S. No.	Entries	Score	Phenotypic reaction
1	BC_2F_1(b)-1	6.3	MS	32	BC_2F_1(b)-32	9.0	S
2	BC_2F_1(b)-2	9.0	S	33	BC_2F_1(b)-33	2.3	R
3	BC_2F_1(b)-3	6.3	MS	34	BC_2F_1(b)-34	5.7	MS
4	BC_2F_1(b)-4	9.0	S	35	BC_2F_1(b)-35	3.7	MR
5	BC_2F_1(b)-5	2.9	R	36	BC_2F_1(b)-36	2.0	R
6	BC_2F_1(b)-6	7.7	S	37	BC_2F_1(b)-37	4.3	MR
7	BC_2F_1(b)-7	9.0	S	38	BC_2F_1(b)-38	3.7	MR
8	BC_2F_1(b)-8	9.0	S	39	BC_2F_1(b)-39	9.0	S
9	BC_2F_1(b)-9	2.7	R	40	BC_2F_1(b)-40	9.0	S
10	BC_2F_1(b)-10	3.0	R	41	BC_2F_1(b)-41	3.7	MR
11	BC_2F_1(b)-11	6.3	MS	42	BC_2F_1(b)-42	3.0	R
12	BC_2F_1(b)-12	9.0	S	43	BC_2F_1(b)-43	8.3	S
13	BC_2F_1(b)-13	9.0	S	44	BC_2F_1(b)-44	1.7	R
14	BC_2F_1(b)-14	9.0	S	45	BC_2F_1(b)-45	2.7	R
15	BC_2F_1(b)-15	2.3	R	46	BC_2F_1(b)-46	6.3	MS
16	BC_2F_1(b)-16	9.0	S	47	BC_2F_1(b)-47	9.0	S
17	BC_2F_1(b)-17	3.3	MR	48	BC_2F_1(b)-48	5.7	MS
18	BC_2F_1(b)-18	9.0	S	49	BC_2F_1(b)-49	3.0	R
19	BC_2F_1(b)-19	6.3	MS	50	BC_2F_1(b)-50	4.3	MR
20	BC_2F_1(b)-20	9.0	S	51	BC_2F_1(b)-51	3.7	MR
21	BC_2F_1(b)-21	9.0	S	52	BC_2F_1(b)-52	6.3	MS
22	BC_2F_1(b)-22	3.0	R	53	BC_2F_1(b)-53	3.0	R
23	BC_2F_1(b)-23	5.0	MR	54	BC_2F_1(b)-54	2.3	R
24	BC_2F_1(b)-24	2.3	R	55	BC_2F_1(b)-55	5.7	MS
25	BC_2F_1(b)-25	2.3	R	56	BC_2F_1(b)-56	2.3	R
26	BC_2F_1(b)-26	5.7	MS	57	BC_2F_1(b)-57	3.7	MR
27	BC_2F_1(b)-27	4.3	MR	58	BC_2F_1(b)-58	3.7	MR
28	BC_2F_1(b)-28	7.7	S	59	BC_2F_1(b)-59	2.6	R
29	BC_2F_1(b)-29	8.3	S	60	BC_2F_1(b)-60	3.7	MR
30	BC_2F_1(b)-30	3.7	MR	61	BC_2F_1(b)-61	3.0	R
31	BC_2F_1(b)-31	3.0	R				

R- Resistant, MR- Moderately Resistant, MS- Moderately Susceptible, and S- Susceptible.

parent genome despite the absence of explicit background selection, a trend also observed in marker-assisted introgression studies by Huang *et al*. (1997) and Neeraja *et al*. (2017). Representative genotypic profiles and phenotypic uniformity of BC_2F_2 plants are shown in Fig. 3.

Phenotypic evaluation of homozygous BC_2F_2 plants was conducted during *kharif* 2022 at the Entomology glass house, ICAR-Indian Institute of Rice Research (ICAR-IIRR), Rajendranagar, Hyderabad, using the MMTS method. All marker-positive BC_2F_2 plants exhibited resistant to moderately resistant reactions with consistently low damage scores and were advanced to develop BC_2F_3 generation for further screening and evaluation. The complete concordance between homozygous marker genotype and resistant phenotype confirms the stability of *qbph-1-1* and demonstrates its effectiveness when fixed in the elite genetic background. Similar stability of resistance upon fixation of major BPH resistance loci has been reported by Jairin *et al*. (2007b) and Xiao *et al*. (2016).

The integration of molecular foreground selection with rigorous phenotypic screening across multiple seasons and locations enabled rapid development of BPH-resistant lines without compromising agronomic identity. The effectiveness of this approach in reducing breeding cycles compared to conventional methods has been emphasized by Collard and Mackill (2008) and Xu and Crouch (2008).

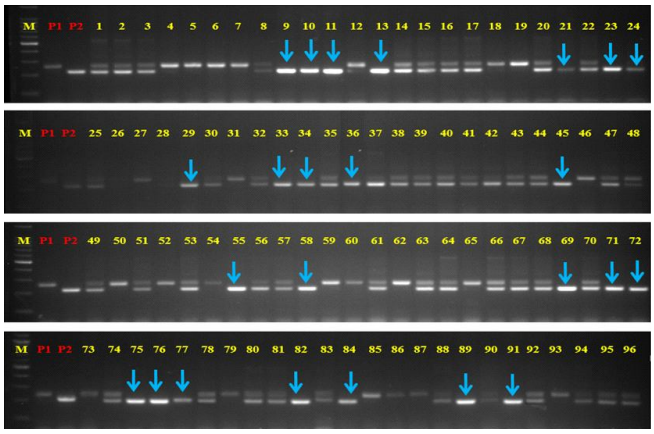

Fig. 3 : Foreground selection in BC_2F_2 plants from TS///TS// TS/M229 using *qbph-1-1* specific RM11069 marker, P1-Telangana Sona, P2-M229.

The results clearly demonstrate that *qbph-1-1* can be successfully introgressed, fixed, and phenotypically expressed within a limited number of backcross generations. The availability of homozygous BC_2F_2 lines combining BPH resistance with the desirable characteristics of Telangana Sona represents a significant advancement towards sustainable pest management. These lines constitute valuable pre-breeding material for varietal release or for pyramiding with other resistant genes to enhance durability against evolving BPH populations, as suggested by Bentur *et al*. (2016) and Du *et al*. (2020).

Conclusion

The study demonstrates the effectiveness of marker-assisted backcross breeding as a practical strategy for improving brown planthopper resistance in elite rice cultivars. Introgression of the resistance QTL *qbph-1-1* into the Telangana Sona genetic background resulted in stable expression of resistance without compromising the variety's identity. The approach highlights the value of integrating molecular tools with targeted phenotypic validation for managing complex insect pests. The resistant lines developed provide a strong foundation for sustainable pest management in rice-based production systems. These lines also serve as valuable genetic resources for future resistance gene pyramiding. The work reinforces the role of precise molecular selection in accelerating crop improvement. Overall, the study contributes to the development of durable and environmentally sound solutions for brown planthopper management in rice.

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