Studies on Screening and Inheritance of Yellow Stem Borer (*Scirpophaga incertulas* Walker) Resistance in F2 and F3 Segregating Generations of Rice (*Oryza sativa* L.)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Original Research Article

ABSTRACT

This study investigated the screening and inheritance of resistance to yellow stem borer (*Scirpophaga incertulas*) in F2 and F3 segregating generations of rice (Oryza sativa L.) derived from six crosses viz., ADT 43 X TKM 6, ADT 43 X ASD 12, ADT 45 X TKM 6, ADT 45 X ASD 12, ASD 16 X TKM 6 and ASD 16 X ASD 12. Field screening was conducted during the 2021 and 2022 kharif seasons, evaluating white ear damage as an indicator of resistance. The F2 and F3 populations showed a range of resistance levels from highly resistant to highly susceptible across the six crosses of rice. The distribution of resistance varied, with the cross ASD 16 x TKM 6 having the highest number of highly resistant plants in F2, while ADT 45 x ASD 12 had the lowest in both

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generations. Chi-square analysis revealed that the inheritance of resistance followed a complementary gene action epistasis with a 9-resistant to 7 susceptible ratios observed in all six crosses across both generations. The screening helped identify promising yellow stem borer resistant segregants, which can be further investigated at the genomic level for molecular characterization and mapping of resistance QTLs to facilitate their introgression into rice breeding programs.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world as half of the world population feed on rice every day. Rice supplies 20 per cent of the world's dietary energy needs, while wheat and maize supply 19 and 5 per cent respectively and in some Asian countries, rice provides over 70% of calorie supply [1]. The production of rice was affected by numerous factors such as biotic stresses and abiotic stresses and the most important biotic stress is yellow stem borer of rice. Host plant resistance and screening are identified as the most effective way of yellow stem borer management in various regions. Over 100 species of insect attack and damage rice (Pathak, [2], Grist and Lever, 1969). Stem borers in the order Lepidoptera are widely prevalent and serious insect pests of rice. In India, 18 stem borer species in the family Pyralidae and three species in the family Noctuidae have been recorded (Banerjee, [3], Kapur, [4]. The yellow stem borer (YSB), *Scirpophaga incertulas* is the most dominant species in India [5]. Stem borer adults are moths and three or more generations occur in a single season. Most borer species are capable of flying only a short distance; however, they can travel 8–16 km if carried by wind [2]. A single female can lay 100–200 eggs. The larvae live and feed inside the stem or rice culm. Both traditional cultivars and the modern semi-dwarf indica varieties produce numerous tillers (15–20), and thus provide conditions conducive to stem borer infestation. The newly hatched larvae may feed externally for some time, bore into the stems, usually throughout the upper nodes and eat their way down to the base of the plants [2].They are common and serious pests in Asian countries responsible for the annual damages of 5-10 per cent of rice crops [6].The Researchers at the Central Rice Research Institute in India estimated that for every 1% increase in white ear heads due to YSB, yields were reduced by 2.2% [7]. Heavy infestation may cause yield loss up to 80 per cent [8]. The larvae of these borers cause "dead hearts" during the vegetative stage resulting in loss of productive tillers and "white ear" damage at the crop reproductive stage resulting in chaffy grain that reflects heavy economic loss in rice. The extent of damage caused by the YSB in rice ranged from 3 to 95 per cent [9]. So, it is important to identify the source for YSB resistance in rice.

2. MATERIALS AND METHODS

The field experiments were conducted during the *Kharif* season of 2021 and 2022 with F_2 and F_3 segregating generation to identify the resistant sources of rice yellow stem borer (YSB) from the six crosses using two susceptible checks TN 1 and IR 8 under field conditions. The mean of the two susceptible checks is used for the dead heart index calculation. The white ear head damage was given higher importance in screening than dead heart symptoms, this was because the white ear head damage caused by YSB occurs during the reproductive stage of the crop and drastically reduces the yield by 38 – 80 per cent while dead heart causes the yield loss of 1 – 19 per cent [10].

2.1 Assessment of White Ear Damage Rating and Scale

Observations on the incidence of yellow stem borer (YSB) in terms of white ear at reproductive stage were recorded at 70 – 75 days after transplanting (DAT). All the plants were examined for recording the incidence of yellow stem borer infection and the per cent white ear damage of YSB was calculated using the per cent white ear calculation formula, based on the damage rating scale, the status was determined by following IRRI's Standard Evaluation System (SES) for yellow stem borer [11]. The Plate 1 provides the detailed life stages and damage symptoms of the YSB.

% of White ear = Number of damaged tillers (White ear) / Total Number of tillers * 100

The percentage of white ears was converted D value,

D value (%) = Per cent White ear in Individual plant of segregating generations / Per cent White ear in susceptible checks * 100

2.2 Inheritance Studies

All the seeds harvested from the F_1 generation of six crosses were advanced to F_2 generation. All the plants in each cross of F_2 generation were screened. Similarly, F_3 seeds are harvested from F_2 generation and advanced to F_3 generation. The seeds were harvested from the F_1 plants which shows highly resistance to yellow stem borer. In F_2 and F_3 segregating populations, plants were screened against yellow stem borer and genetic ratio was worked out on using chi-square test analysis for the study of resistance character inheritance pattern in six crosses of rice. The chi-square test for goodness of fit was given by Prof. Karl Pearson for testing the significance of the discrepancy between Observed (O = Experimental) values and Expected (E = Hypothetical) values. The calculated chi-square ($χ^2$) was computed by using the following formula of Snedecor and Cochran, (1980). The degrees of freedom for Chi-square test of goodness of fit is $χ^2(n-1)$ degrees of freedom.

$$χ^2 = \sum \frac{(O-E)^2}{E}$$

3. RESULTS AND DISCUSSION

3.1 Screening for Yellow Stem Borer

The screening of YSB resistance in F_2 and F_3 segregating populations of rice were studied in the following six crosses *viz.*, ADT 43 X TKM 6, ADT 43 X ASD 12, ADT 45 X TKM 6, ADT 45 X ASD 12, ASD 16 X TKM 6 and ASD 16 X ASD 12. Distribution of YSB resistance among the F_2 and F_3 segregating population in cross were represented in Fig. 1 to Fig. 12. All the plants of the parents ADT 43 and ASD 16 were susceptible to YSB, ADT 45 is highly susceptible to YSB in percentage of white ear head and D value observed, while all the plants of TKM 6 and ASD 12 were highly resistant to YSB. Screening for yellow stem borer (YSB) resistance in all the six crosses of rice revealed that, in cross ADT 43 X TKM 6, out of 350 plants screened in F_2 generation, 9 were highly resistant, 82 resistant, 107 moderately resistant, 91 moderately susceptible, 56 susceptible, and 5 highly susceptible. Out of 150 plants screened in F_3 generation, 5 were highly resistant, 34 resistant,

49 moderately resistant, 32 moderately susceptible, 24 susceptible, and 6 highly susceptible to YSB. For cross ADT 43 X ASD 12, the F_2 generation had 11 highly resistant, 75 resistant, 102 moderately resistant, 85 moderately susceptible, 69 susceptible, and 8 highly susceptible plants, while the F_3 generation had 8 highly resistant, 32 resistant, 37 moderately resistant, 39 moderately susceptible, 29 susceptible, and 5 highly susceptible plants to YSB. In cross ADT 45 X TKM 6, the F_2 generation had 8 highly resistant, 71 resistant, 111 moderately resistant, 84 moderately susceptible, 65 susceptible, and 11 highly susceptible plants, and the F_3 generation had 4 highly resistant, 37 resistant, 49 moderately resistant, 30 moderately susceptible, 22 susceptible, and 8 highly susceptible plants to YSB. For cross ADT 45 X ASD 12, the F_2 generation had 9 highly resistant, 79 resistant, 96 moderately resistant, 91 moderately susceptible, 62 susceptible, and 13 highly susceptible plants, while the F_3 generation had 3 highly resistant, 43 resistant, 47 moderately resistant, 30 moderately susceptible, 18 susceptible, and 9 highly susceptible plants to YSB. In cross ASD 16 X TKM 6, the F_2 generation had 15 highly resistant, 76 resistant, 110 moderately resistant, 82 moderately susceptible, 62 susceptible, and 5 highly susceptible plants, and the F_3 generation had 8 highly resistant, 34 resistant, 47 moderately resistant, 36 moderately susceptible, 22 susceptible, and 3 highly susceptible plants to YSB. Lastly, for cross ASD 16 X ASD 12, the F_2 generation had 12 highly resistant, 65 resistant, 109 moderately resistant, 96 moderately susceptible, 61 susceptible, and 7 highly susceptible plants, while the F_3 generation had 7 highly resistant, 34 resistant, 35 moderately resistant, 38 moderately susceptible, 32 susceptible, and 4 highly susceptible plants to YSB (Table 2). The overall white ear damage (D value) due to YSB incidence for all six crosses of rice had ranged from 0 to 70.6 per cent in F_2 generation and 0 to 63.7 per cent at F_3 generation respectively. The susceptible checks TN 1 had a white ear damage per cent of 81.12% and IR 8 had a white ear damage per cent of 69.43%. The average of these two susceptible checks were calculated (75.27%) and used to estimate D value for every individual plant of segregating populations. Similar screening for yellow stem borer (YSB) in rice varieties were reported by Justin and Preetha [12], Prasad et al. [13], Joshi et al. [14], Sudha Rani et al. (2020), Rakesh et al. [15], Nalla et al. [16], Reuolin et al. [17] and Sampathkumar et al. [18].

Table 1. Standard evaluation system for scoring yellow stem borer (YSB) resistance in rice

Scale	Percent damage of White Ear Head (WEH)	D value	Resistant status
0	No Damage	No Damage	Highly Resistant (HR)
1	1 - 5%	1 - 10%	Resistant (R)
3	6 - 10%	11 - 25%	Moderately Resistant (MR)
5	11 - 15%	26 - 40%	Moderately Susceptible (MS)
7	16 - 25%	41-60%	Susceptible (S)
9	26% and above	61-100%	Highly Susceptible (HS)

Table 2. Distribution of yellow stem borer resistance among the F_2 and F_3 segregating population in six crosses of rice

Phenotypic scoring			F_2 and F_3 segregating generation					
Scale	Status	G	ADT 43 X TKM 6	ADT 43 X ASD 12	ADT 45 X TKM 6	ADT 45 X ASD 12	ASD 16 X TKM 6	ASD 16 X ASD 12
0	Highly Resistant (HR)	F_2	9	11	8	9	15	12
		F_3	5	8	4	3	8	7
1	Resistant (R)	F_2	82	75	71	79	76	65
		F_3	34	32	37	43	34	34
3	Moderately Resistant (MR)	F_2	107	102	111	96	110	109
		F_3	49	37	49	47	47	35
5	Moderately Susceptible (MS)	F_2	91	85	84	91	82	96
		F_3	32	39	30	30	36	38
7	Susceptible (S)	F_2	56	69	65	62	62	61
		F_3	24	29	22	18	22	32
9	Highly Susceptible (HS)	F_2	5	8	11	13	5	7
		F_3	6	5	8	9	3	4

Table 3. Inheritance pattern of F_2 and F_3 segregating population for six crosses in rice for YSB resistance

Crosses		Segregation pattern of the F_2 and F_3 plants							
	G	Status of Plants Observed			Chi square value	Observed ratio	Table value at 0.05	Table value at 0.01
		R	S	Total				
ADT 43 X TKM 6	F_2	198	152	350	0.046 ns	9 : 7	3.841*	6.635**
	F_3	88	62	150	0.432 ns			
ADT 43 X ASD 12	F_2	188	162	350	0.742 ns	9 : 7	3.841*	6.635**
	F_3	77	73	150	1.325 ns			
ADT 45 X TKM 6	F_2	190	160	350	0.417 ns	9 : 7	3.841*	6.635**
	F_3	90	60	150	0.974 ns			
ADT 45 X ASD 12	F_2	184	166	350	1.669 ns	9 : 7	3.841*	6.635**
	F_3	93	57	150	2.191 ns			
ASD 16 X TKM 6	F_2	201	149	350	0.289 ns	9 : 7	3.841*	6.635**
	F_3	89	61	150	0.676 ns			
ASD 16 X ASD 12	F_2	186	164	350	1.159 ns	9 : 7	3.841*	6.635**
	F_3	76	74	150	1.731 ns			

G-Generations, R-Resistant, S-Susceptible (** 1% level of significance, * 5% level of significance and ns is non-significance)
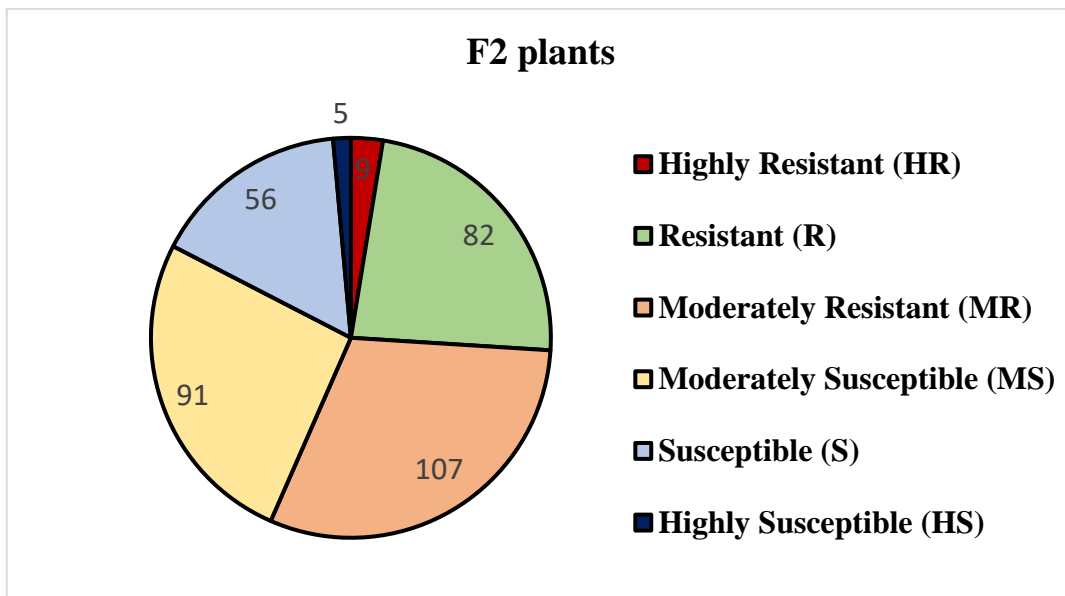
F2 plants

Fig. 1. Distribution of YSB resistance among the F2 segregating population in cross ADT 43 X TKM 6
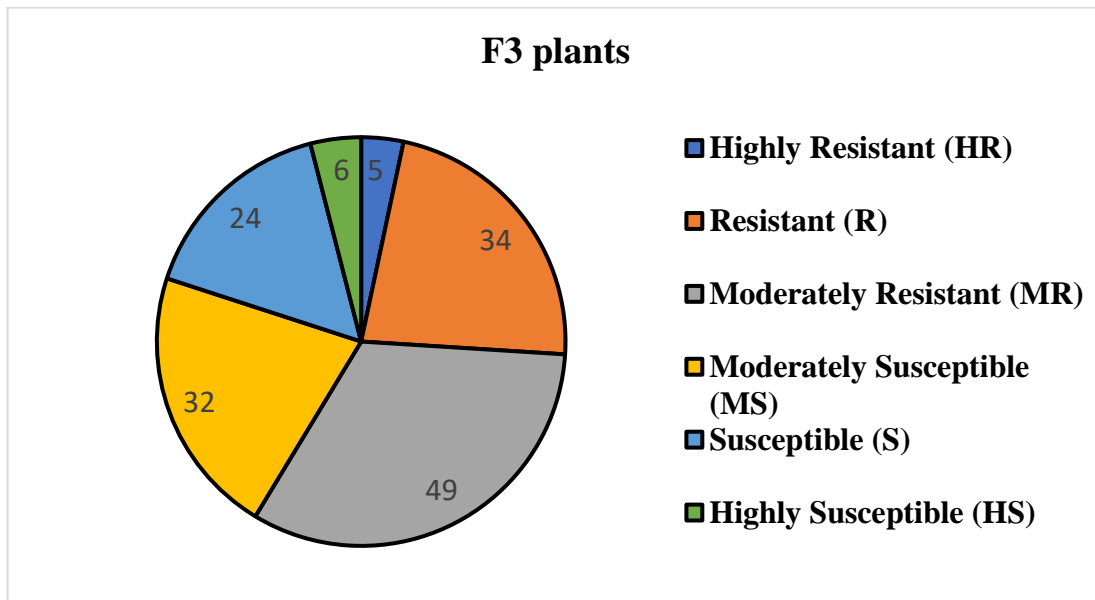
F3 plants

Fig. 2. Distribution of YSB resistance among the F3 segregating population in cross ADT 43 X TKM 6

3.2 Inheritance Studies on Yellow Stem Borer

The inheritance pattern of Yellow Stem Borer (YSB) resistance was investigated in six crosses of F_2 and F_3 segregating populations of rice. Chi-square analysis was employed to compare the observed and expected frequencies of resistant and susceptible plants. The expected ratio of 9

resistant: 7 susceptible plants was chosen to represent complementary gene action. In all six crosses, both in F_2 and F_3 generations, there was no significant difference between the observed and expected ratios, indicating a good fit for the complementary gene action epistasis. In the cross ADT 43 X TKM 6, out of 350 F_2 plants screened, 198 showed resistance and 152 showed susceptibility. In the F_3 generation of the
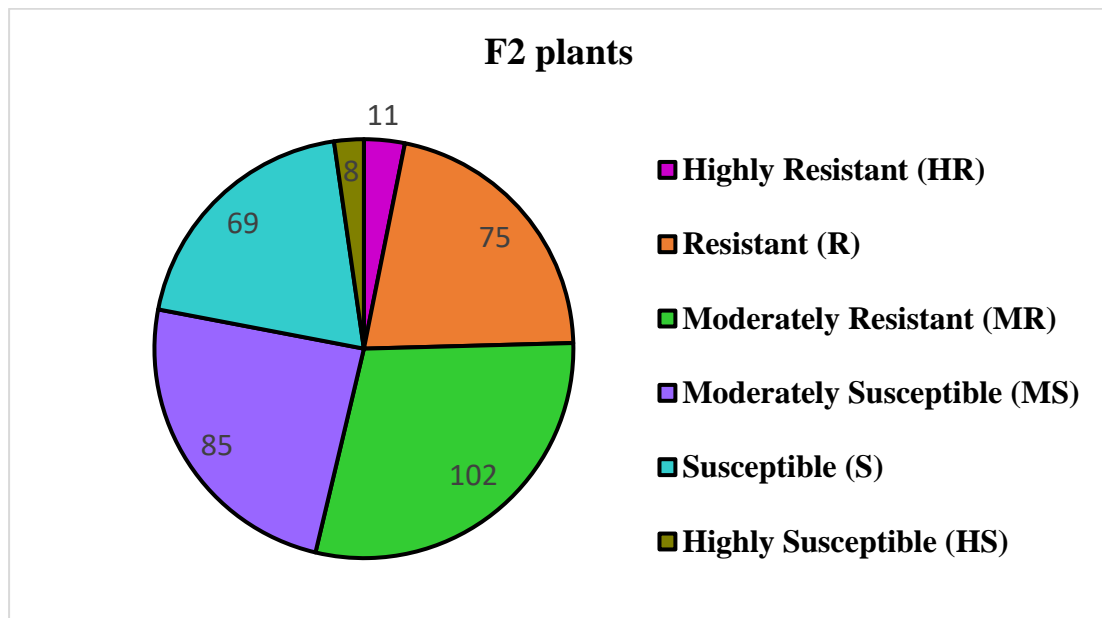
Fig. 3. Distribution of YSB resistance among the F2 segregating population in cross ADT 43 X ASD 12
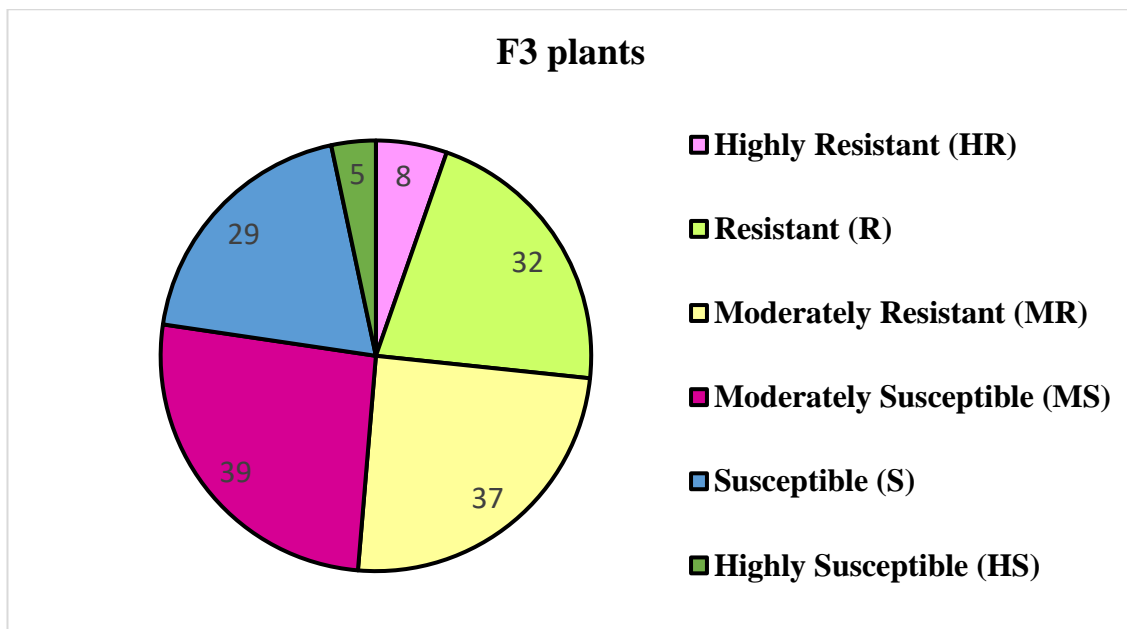

Fig. 4. Distribution of YSB resistance among the F_3 segregating population in cross ADT 43 X ASD 12

same cross, out of 150 plants, 88 were resistant and 62 were susceptible. In the cross ADT 43 X ASD 12, out of 350 F_2 plants, 188 were resistant and 162 were susceptible. In the F_3 generation of the same cross, out of 150 plants, 77 were resistant and 73 were susceptible. In the cross ADT 45 X TKM 6, out of 350 F_2 plants, 190 were resistant and 160 were susceptible. In the F_3 generation of the same cross, out of 150 plants, 90 were resistant and 60 were susceptible. In the cross ADT 45 X ASD 12, out of 350 F_2 plants, 184 were resistant and 166 were susceptible. In the F_3 generation of the same cross, out of 150 plants, 93 were resistant and 57 were susceptible. In the cross ASD 16 X TKM 6, out of 350 F_2 plants, 201 were resistant and 149 were susceptible. In the F_3 generation of the same cross,
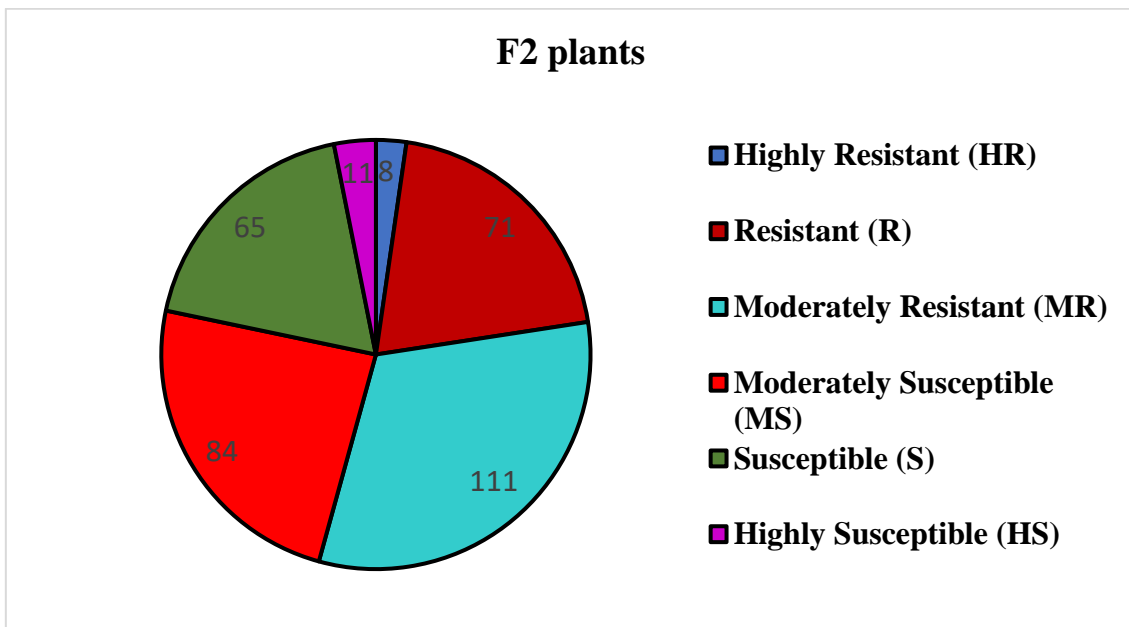
Fig. 5. Distribution of YSB resistance among the F2 segregating population in cross ADT 45 X TKM 6
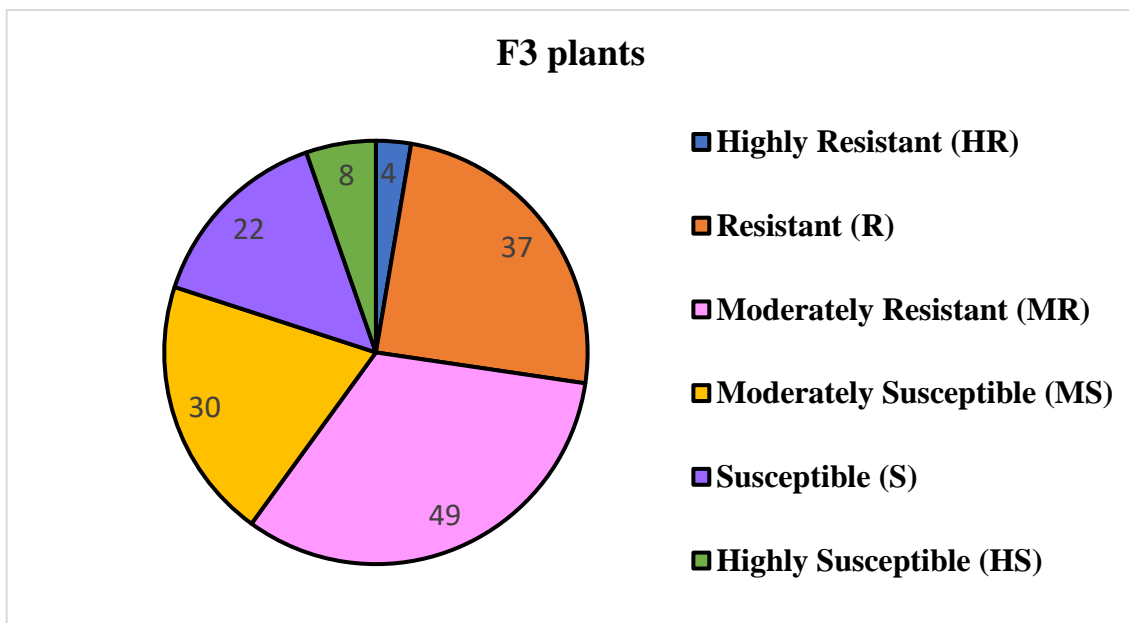

Fig. 6. Distribution of YSB resistance among the F3 segregating population in cross ADT 45 X TKM 6

out of 150 plants, 89 were resistant and 61 were susceptible. In the cross ASD 16 X ASD 12, out of 350 F_2 plants, 186 were resistant and 164 were susceptible. In the F_3 generation of the same cross, out of 150 plants, 76 were resistant and 74 were susceptible. In all cases, the calculated chi-square values were lower than the table Chi-square values for significance at both 5% and 1% levels, indicating no significant difference between observed and expected ratios. This suggests that the inheritance of YSB resistance in these crosses follows a complementary gene action, with no major deviations from the expected ratios (Table 3). Similar kinds of inheritance studies were reported by Ram et al. [19], Ali et al. [20] and Meshram et al. [21].
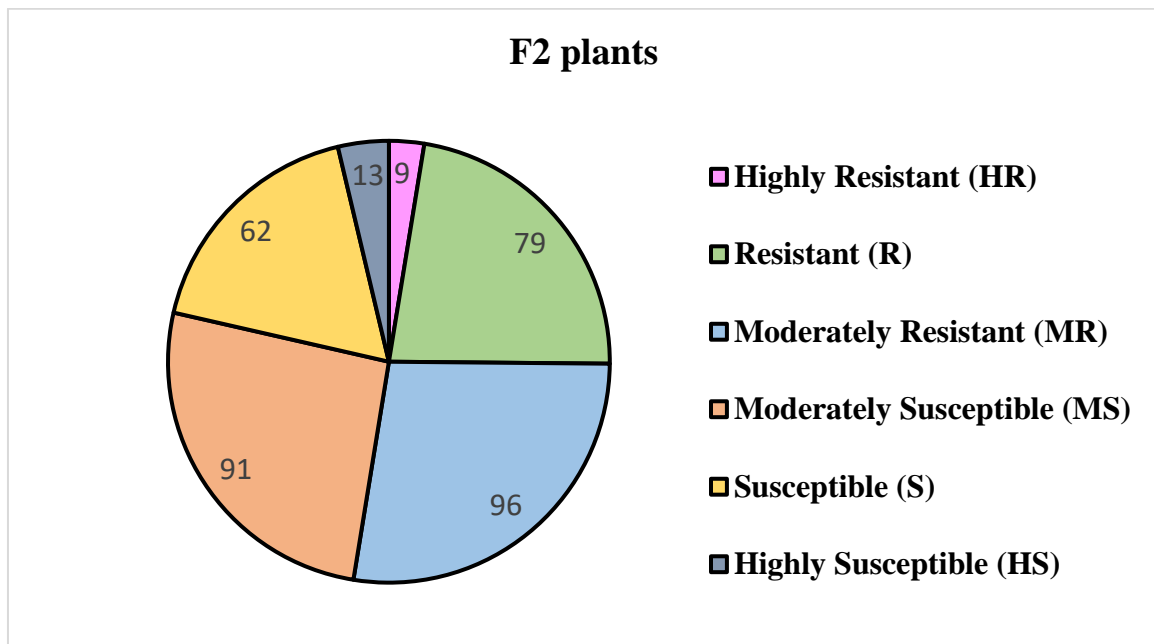
Fig. 7. Distribution of YSB resistance among the F2 segregating population in cross ADT 45 X ASD 12
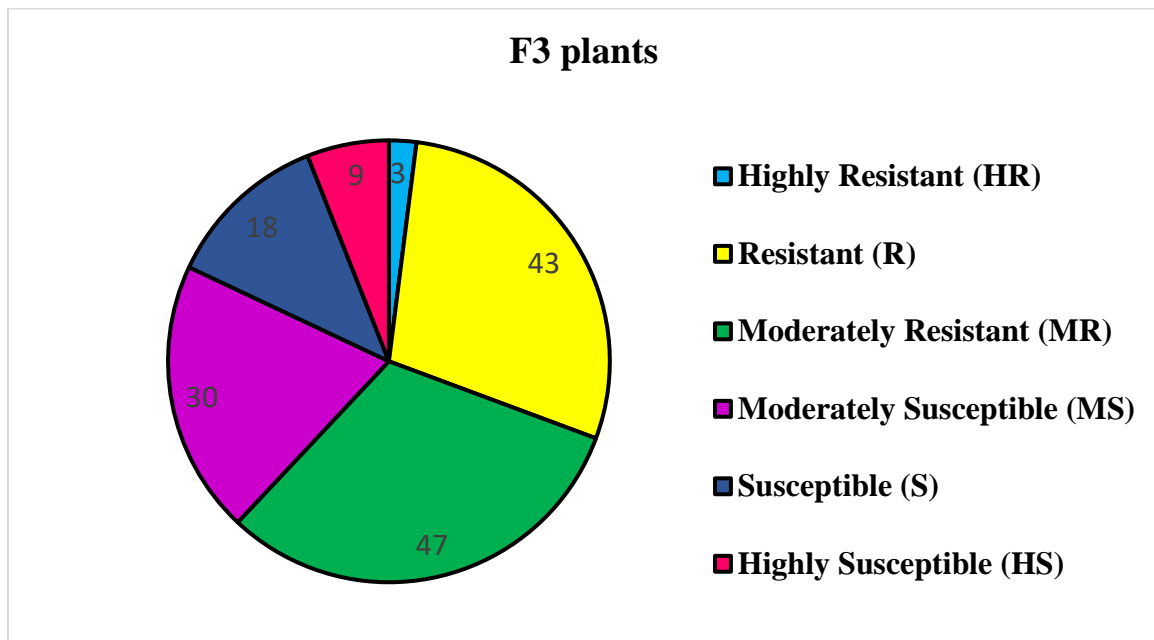

Fig. 8. Distribution of YSB resistance among the F3 segregating population in cross ADT 45 X ASD 12

The complementary epistasis is also called as duplicate recessive epistasis. In this type of gene interactions, the production of one of the two phenotypes of a trait required the presence of dominant alleles of both the genes controlling the concerned trait. The resistance was governed by dominant gene 'A' and 'B' [22,23]. when these genes were in separate individuals (AAbb or aaBB) or recessive (aabb) they produce susceptible plants. The observed segregation pattern suggests that the female parents ADT 43, ADT 45 and ASD 16, which showed susceptibility, have homozygous recessive alleles for susceptibility (aabb). In contrast, the male parent TKM 6 and ASD 12 likely have homozygous dominant allele (AABB) for
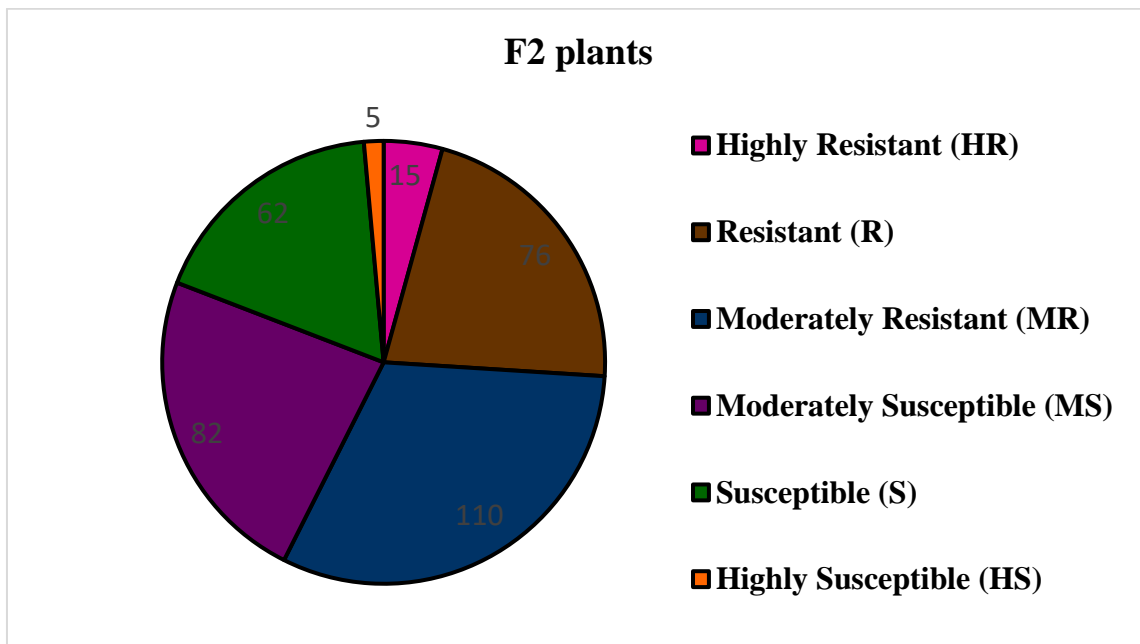
F2 plants

Fig. 9. Distribution of YSB resistance among the F_2 segregating population in cross ASD 16 X TKM 6
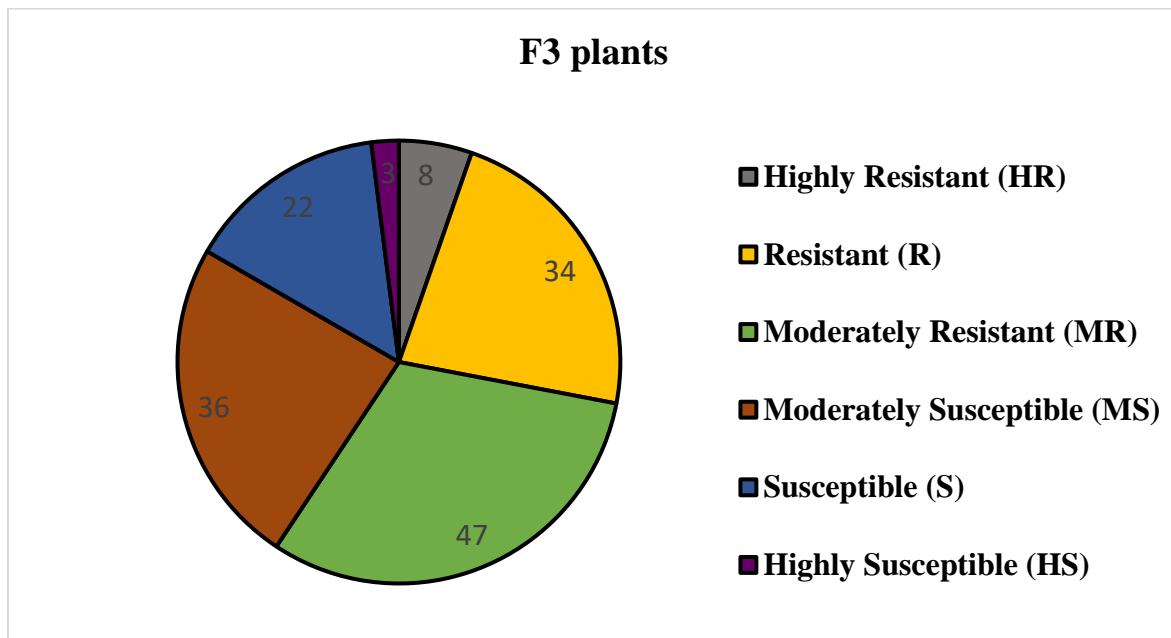
F3 plants

Fig. 10. Distribution of YSB resistance among the F3 segregating population in cross ASD 16 X TKM 6

resistance. The crosses between ADT 43 X TKM 6, ADT 43 X ASD 12, ADT 45 X TKM 6, ADT 45 X ASD 12, ASD 16 X TKM 6 and ASD 16 X ASD 12 would result in heterozygous progeny having both dominant and recessive allele (AaBb) and since dominant allele is contributing for resistance, all the plants in F_1 generation showed resistance to YSB of rice. In a genetic study, the segregation process resulted in two categories, which exhibited a 9:7 ratio, indicating gene interactions. This is because of, recessive allele 'a' is epistatic to B/b alleles and mask the expression of these alleles. Another recessive allele 'b' is epistatic to A/a alleles and mask their expression. Hence in segregating generations, the plants with two dominant
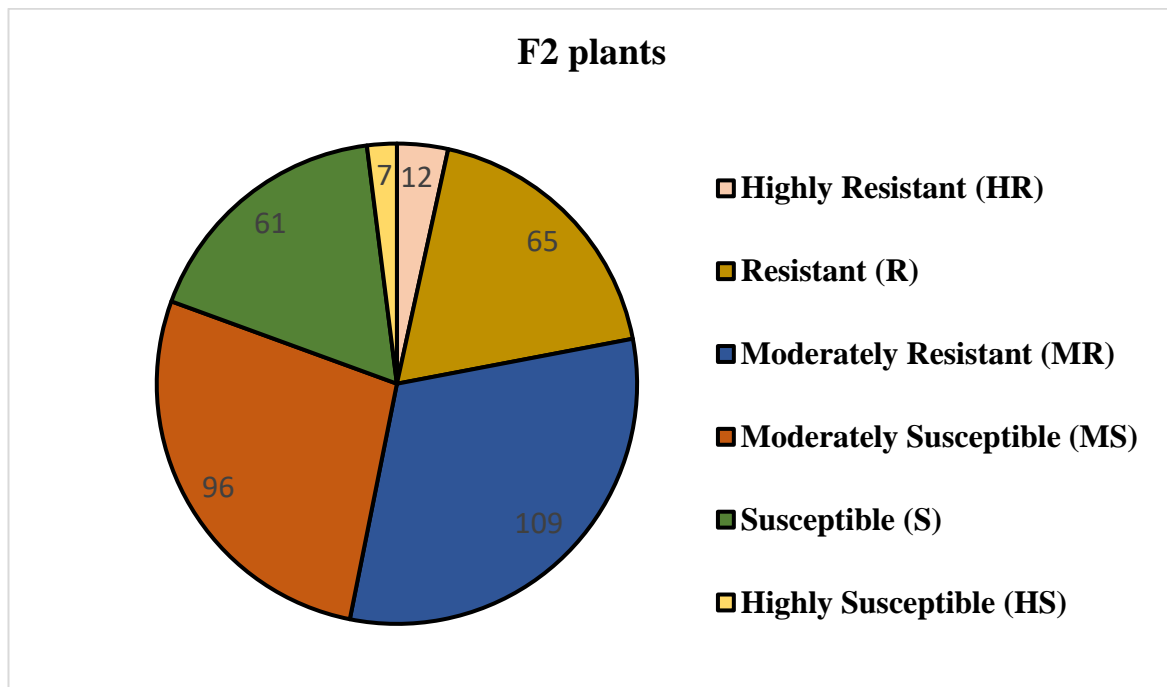
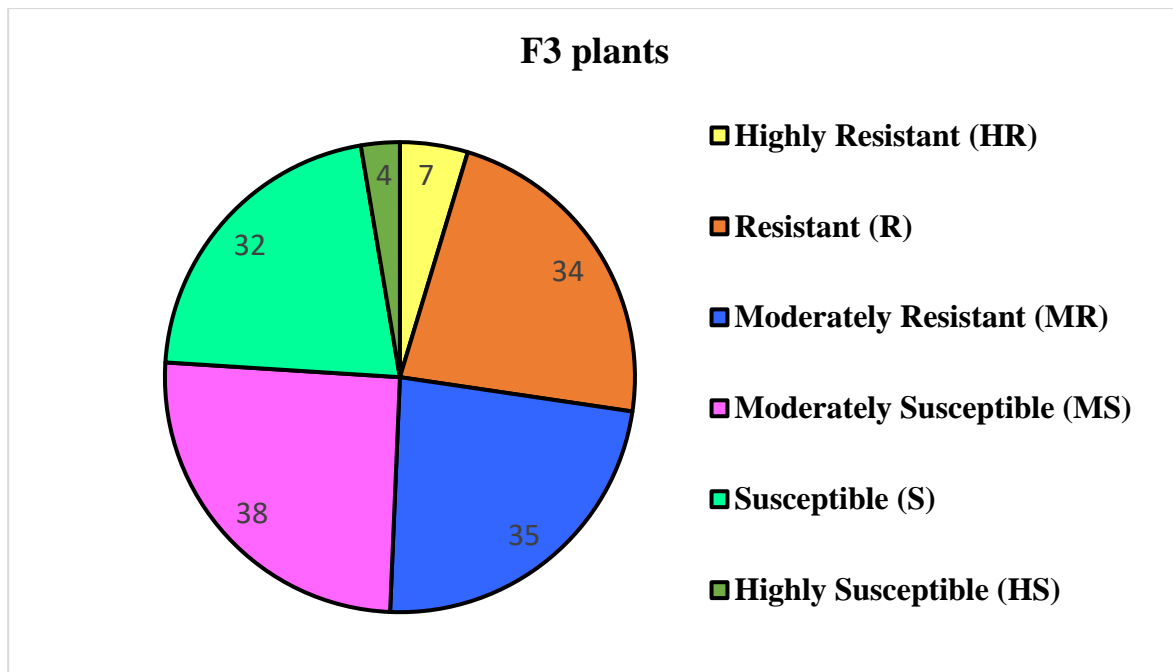**Fig. 11. Distribution of YSB resistance among the F2 segregating population in cross ASD 16 X ASD 12**

Fig. 12. Distribution of YSB resistance among the F3 segregating population in cross ASD 16 X ASD 12

alleles [A_B_] genotypes would produce resistant plants (9/16) to rice yellow stem borer and plants with [aaB_] genotype (3/16), [A_bb] genotype (3/16) and [aabb] genotype would produce (1/16) susceptible plants. Thus, there are only two phenotypic classes *viz.*, susceptibility and resistance are produced and normal dihybrid ratio is modified to 9:7 ratio of complementary gene action [24,25].
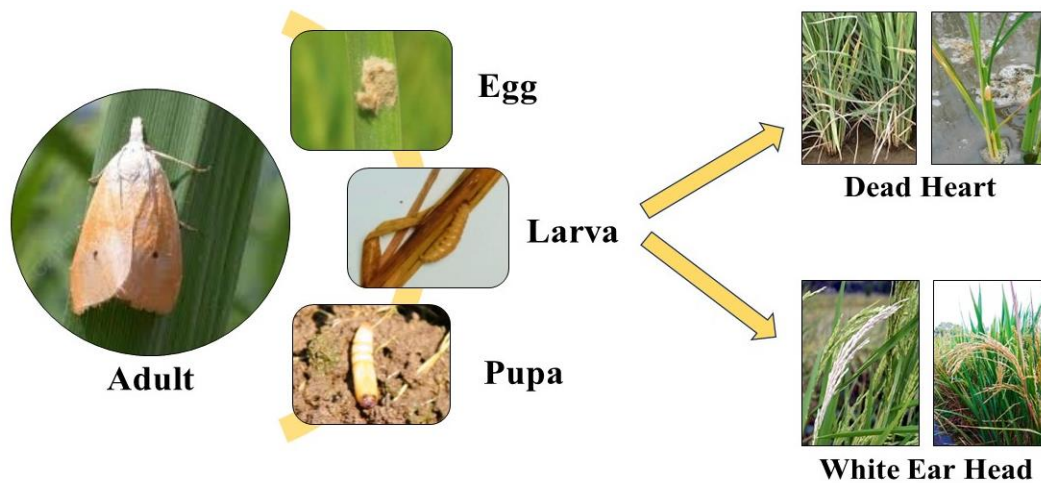
Plate 1. Life stages and damage symptoms of yellow stem borer (*Scirpophaga incertulas)*

4. CONCLUSION

All crosses had plants in each resistance category, ranging from highly resistant to highly susceptible. In most crosses, the moderately resistant category had the highest number of plants in both F_2 and F_3 generations. The cross ASD 16 X TKM 6 had the highest number of highly resistant plants in the F_2 generation, while cross ADT 45 X ASD 12 had the lowest. In case of F_3 generation, cross ASD 16 X TKM 6 and cross ADT 43 X ASD 12 had the highest number of highly resistant plants, while cross ADT 45 X ASD 12 had the lowest. The distribution of plants across resistance categories varied among the crosses, suggesting differences in the inheritance pattern of YSB resistance. The screening studies and identification of yellow stem borer (YSB) resistant plants in segregating generations aided in coupling the resistant characters with the high yielding traits. The selected promising rice segregants against yellow stem borer (YSB) from the present investigations can be further studied at genomic level. The molecular characterization and identification of QTLs for resistance against YSB through molecular markers may be utilized for introgression of resistant genes in the breeding programs of rice cultures.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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