Original Research Article

Studies on Screening and Inheritance of ~~y~~Yellow ~~s~~Stem ~~b~~Borer (*Scirpophaga incertulas* Walker) ~~r~~Resistance in F_2 and F_3 ~~s~~Segregating ~~g~~Generations of ~~r~~Rice Plants (*Oryza sativa* L.)

Abstract

This study investigated the screening and inheritance of resistance to yellow stem borer (*Scirpophaga incertulas*) in F_2 and F_3 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 F_2 and F_3 populations showed a range of resistance levels from highly resistant to highly susceptible across the six crosses of rice. The distribution varied, with the cross ASD 16 x TKM 6 having the highest number of highly resistant plants in F_2, while ADT 45 x ASD 12 had the lowest in both generations. Chi-square analysis revealed that the inheritance of resistance followed a complementary gene action epistasis with a 9 resistant : 7 susceptible ratio in all six crosses for both the generations. The screening aided in identifying promising yellow stem borer resistant segregants that can be further studied at the genomic level for molecular characterization and mapping of resistance QTLs to facilitate their introgression into rice breeding programs.

Keywords: Yellow stem borer, Segregating generations, Rice, Inheritance study and Screening

Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops of 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 (GRiSP, 2013). 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 is identified as the most effective way of yellow stem borer management in various regions. Over 100 species of insect attack and damage rice (Pathak, 1968, 1977; 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, 1964; Kapur, 1967). The yellow stem borer (YSB), *Scirpophaga incertulas* is the most dominant species in India (Kulshreshta *et al*., 1970). 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 (Pathak, 1968). 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 for 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 (Pathak, 1968). They are common and serious pests in Asian countries responsible for annual damages of 5-10 per cent of rice crops (Pathak MD and Khan ZR, 1994). 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% (Israel and Abraham, 1967). Heavy infestation may cause yield loss up to 80 per cent (Rubia-Sanchez EG *et al*,1997). The larvae of these borers cause "dead hearts" during vegetative stage resulting in loss of productive tillers and also results in "white ear" damage at crop reproductive stage resulting chaffy grain that reflects heavy economic loss in rice. The extent of damage caused by ~~the yellow stem borer~~ YSB in rice ranged from 3 to 95 per cent (Ghose *et al*., 1960). So, it is important to identify the source for ~~stemborer~~ YSB resistance ~~of~~ in rice.

Materials and Methods

The field experiments were conducted during *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 damage 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 ~~yellow stem borer~~ occurs during 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 (Dhaliwal *et al*. 2010).

Assessment of white ear damage rating and scale

Observations on the incidence of ~~yellow stem borer~~ YSB in terms 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 YSB 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 (IRRI, 2013).

$$\% \, \boldsymbol{of\ White\ ear} = \frac{\boldsymbol{Number\ of\ damaged\ tillers\ (White\ ear)}}{\boldsymbol{Total\ Number\ of\ tillers}} \, \boldsymbol{x\ 100}$$

Percentage of white ears was converted to D value,

$$\boldsymbol{D\ value}\ (\%) = \frac{\boldsymbol{Per\ cent\ White\ ear\ in\ Individual\ plant\ of\ segregating\ generations}}{\boldsymbol{Per\ cent\ White\ ear\ in\ susceptible\ checks}} \, \boldsymbol{x\ 100}$$

Inheritance studies

All the seeds harvested from the F_1 generation of six crosses were advanced to F_2 generation. The aAll 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 borerYSB. In F_2 and F_3 segregating population, plants were screened against YSByellow stem borer and genetic ratio was worked out on using a 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 (1967). The degrees of freedom for Chi-square test of goodness of fit is (n-1) = 1?

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

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)

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1) What is your conclusion when your computed χ^2 value is significant or not significant, ie. at 5% significant level (= α = 0,05) or at 1% significant level (= α = 0,01)?

2) What is your conclusion when your computed χ^2 value is significant, ie. what is your conclusion about inheritance of YSB resistance in your F_2 and F_3 data?

3) What is your conclusion when your computed χ^2 value is non-significant (in fact, that is what your data show - Table 3 below), what is your conclusion about inheritance of YSB resistance in your F_2 and F_3 data?

7	16 - 25%	41-60%	Susceptible (S)
9	26% and above	61-100%	Highly Susceptible (HS)

Results and Discussion

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 F~~f~~ig. 1 to F~~f~~ig. ~~1~~2. 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, out of 350 plants screened i~~I~~n F_2 generation of cross ADT 43 X TKM 6, ~~out of 350 plants screened,~~ 9 plants were highly resistant, 82 resistant, 107 moderately resistant, 91 moderately susceptible, 56 susceptible, and 5 highly susceptible to YSB. Out of 150 plants i~~I~~n F_3 generation, ~~out of 150 plants,~~ 5 plants 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. 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.

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 (Table 2). The similar screening for ~~yellow stem borer~~ YSB in rice varieties were reported by Justin and Preetha (2014), Prasad *et al*. (2015), Joshi et al. (2019), Sudha Rani *et al*. (2020), Rakesh *et al*. (2021), Nalla *et al*. (2020), Reuolin *et al*. (2019) and Sampathkumar *et al*. (2022).

Inheritance studies on yellow stem borer

The inheritance pattern of Yellow Stem Borer (YSB) resistance was investigated in six crosses of F2 and F3 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 F2 and F3 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 F2 plants screened, 198 showed resistance and 152 showed susceptibility. In the F3 generation of the 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, 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). The similar kind of inheritance studies were reported by Ram *et al*. (2010), Ali *et al*. (2012) and Meshram *et al*. (2020).

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 -YSB~~yellow stem borer~~ 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 programmes of rice plant cultures.

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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 = χ^2	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, $\chi^2_{0.05}$ = 3.841, $\chi^2_{0.01}$ = 6.635 (** = significant at 1% level; of significance * = significant at 5 % level, ^{ns} = non-significant at 5 % level of significance).

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It is quite uncommon to put levels of significance mark(s) at the chi-square tables like that. Putting ** or * at the chi-square table value can only cause unecessary confusions.

So, I suggest that:
• The significant mark(s), ie "ns" be put at the calculated chi-square (= χ^2 values).
(It is shown that all of your computed χ^2 values are NOT significant at α = 0.05).
• The last two-columns of your chi-square table values (= the critical chi-square values) be deleted – and, instead, their values be put once as the bottom note.

• put chi-square value of 0.05 and chi-square value of 0.01 at the table note as the added notes.

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Fig. 1. Distribution of YSB resistance among the F_2 segregating population in cross ADT 43 X TKM 6

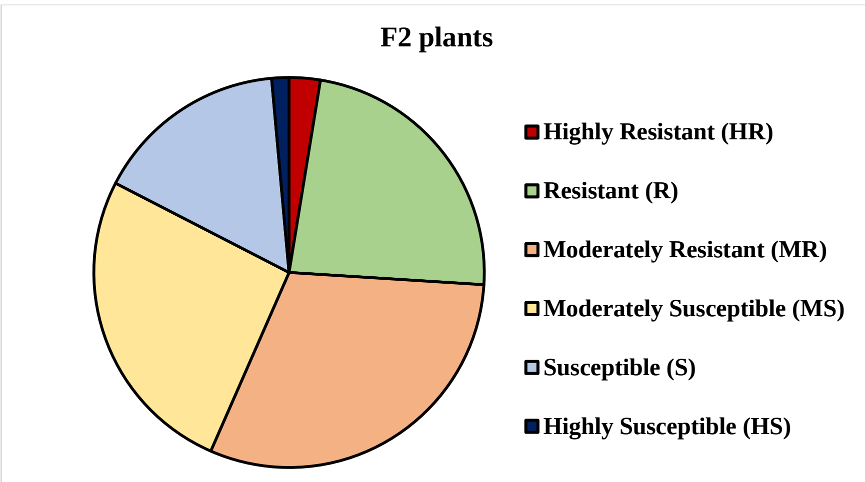

Fig. 2. Distribution of YSB resistance among the F_3 segregating population in cross ADT 43 X TKM 6

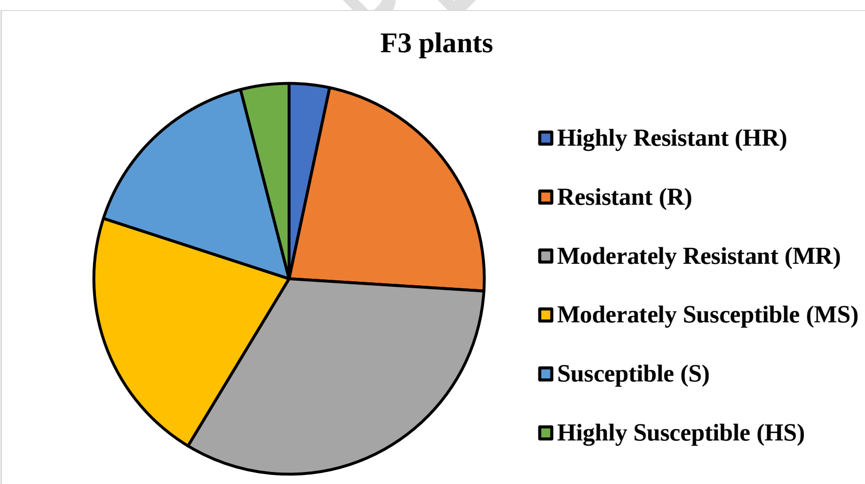

Fig. 3. Distribution of YSB resistance among the F_2 segregating population in cross ADT 43 X ASD 12

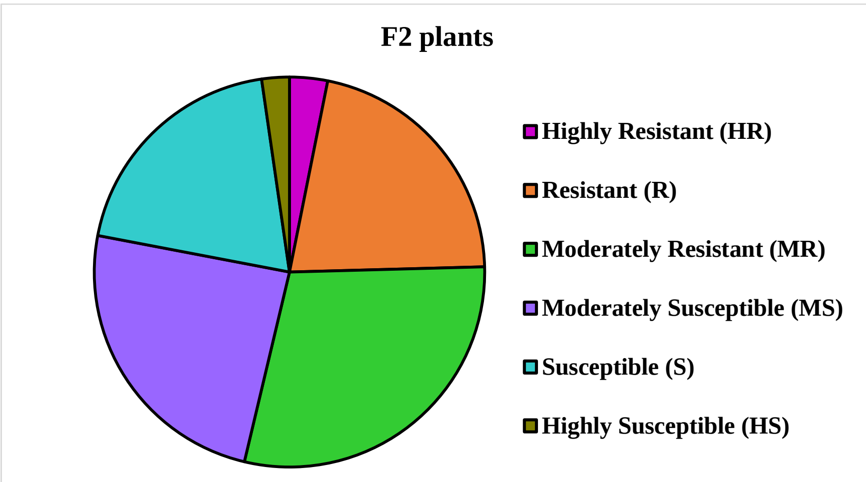

Fig. 4. Distribution of YSB resistance among the F_3 segregating population in cross ADT 43 X ASD 12

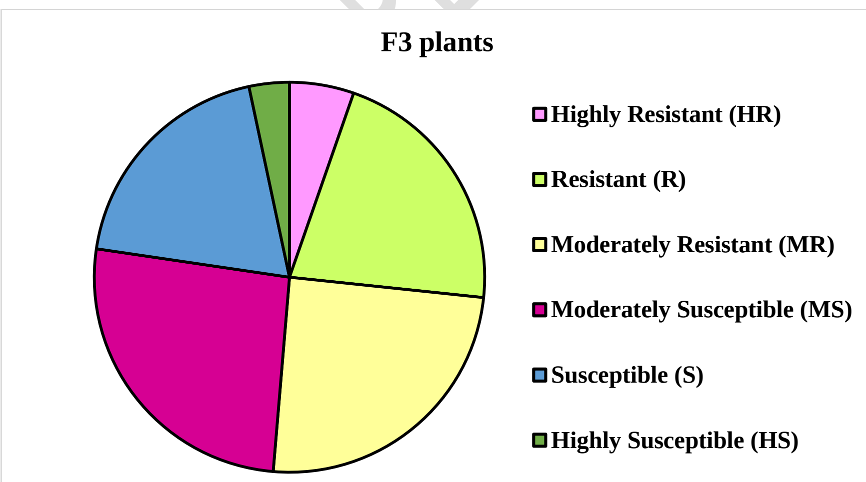

Fig. 5. Distribution of YSB resistance among the F_2 segregating population in cross ADT 45 X TKM 6

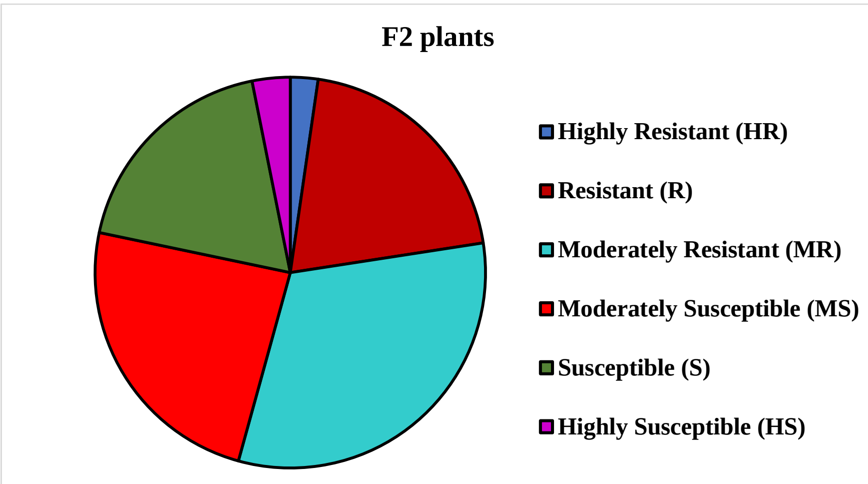

Fig. 6. Distribution of YSB resistance among the F_3 segregating population in cross ADT 45 X TKM 6

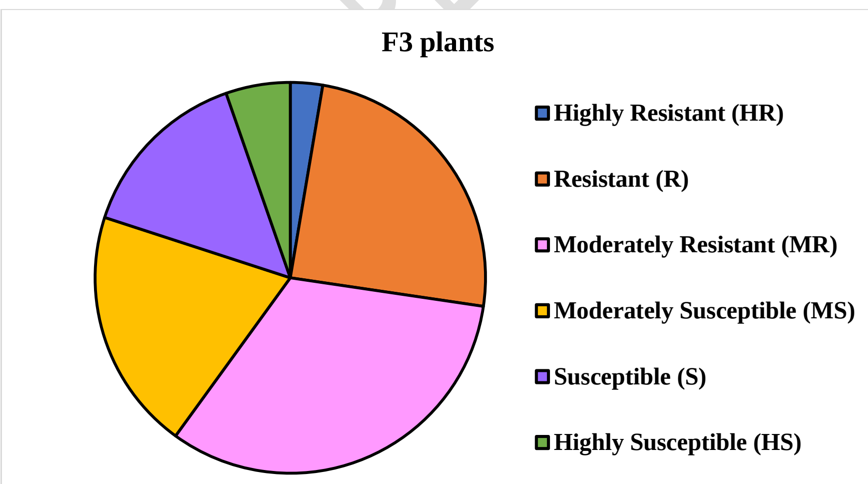

Fig. 7. Distribution of YSB resistance among the F_2 segregating population in cross ADT 45 X ASD 12

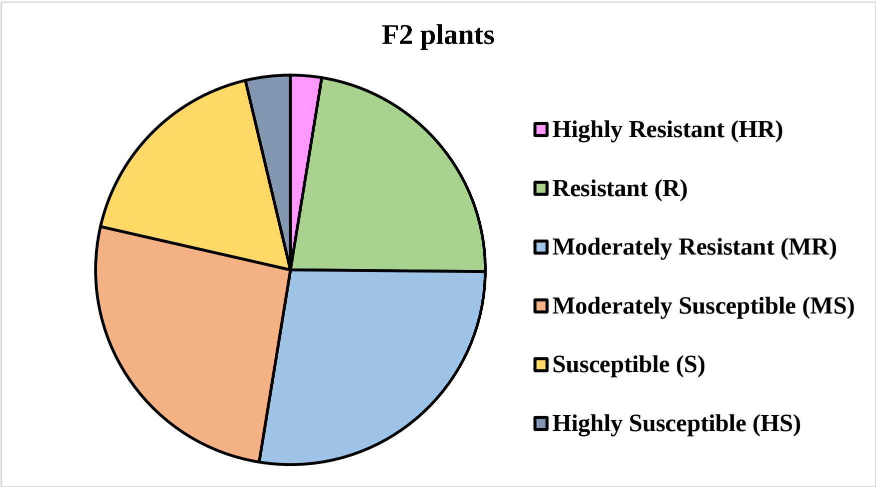

Fig. 8. Distribution of YSB resistance among the F_3 segregating population in cross ADT 45 X ASD 12

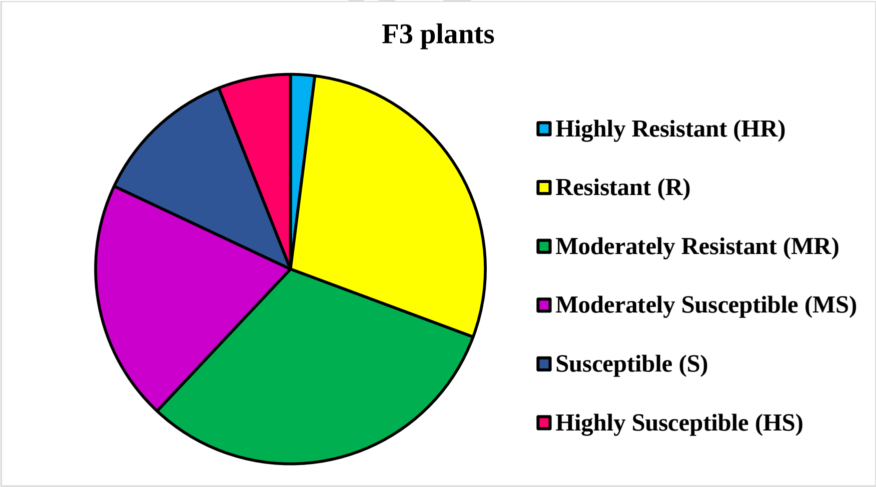

Fig. 9. Distribution of YSB resistance among the F_2 segregating population in cross ASD 16 X TKM 6

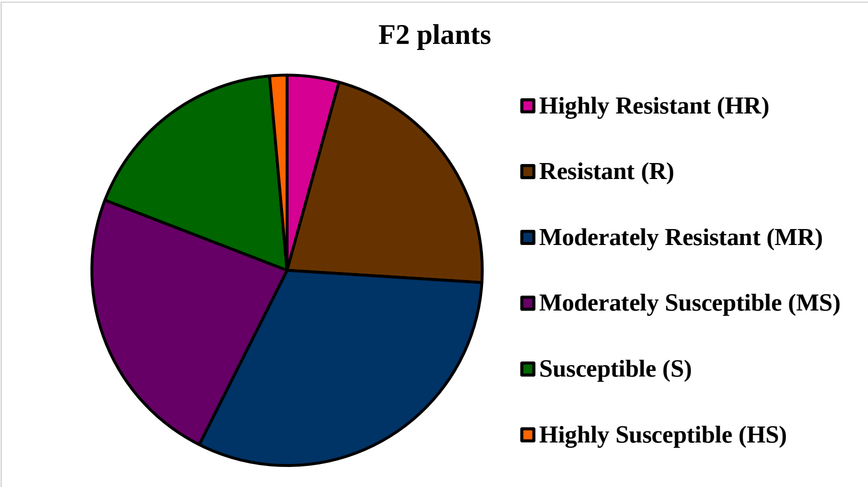

Fig. 10. Distribution of YSB resistance among the F_3 segregating population in cross ASD 16 X TKM 6

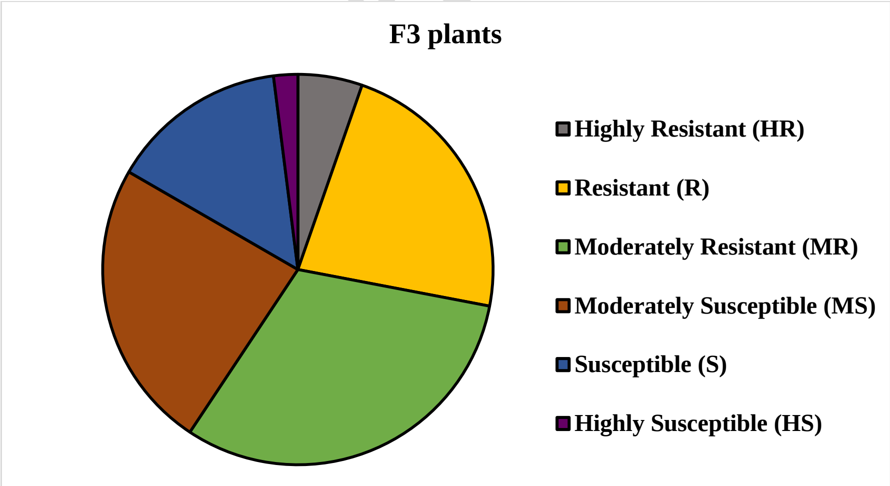

Fig. 11. Distribution of YSB resistance among the F_2 segregating population in cross ASD 16 X ASD 12

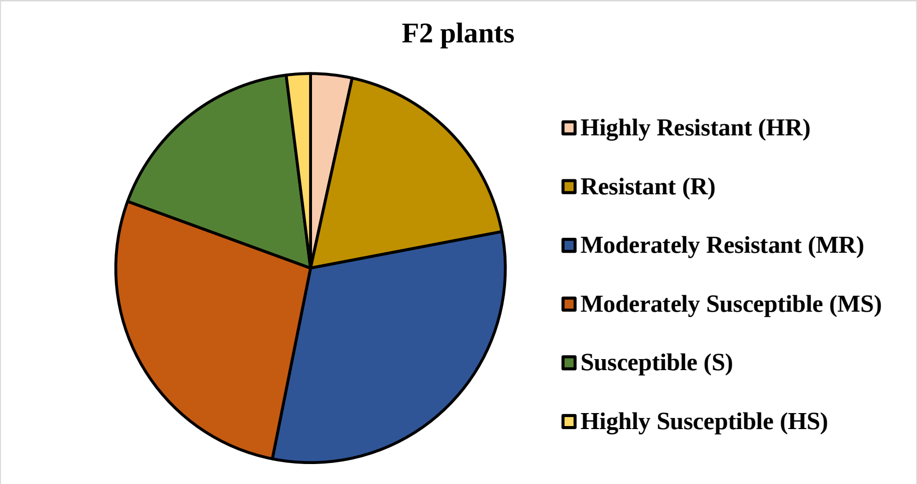

Fig. 12. Distribution of YSB resistance among the F_3 segregating population in cross ASD 16 X ASD 12