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Assessment of biophysical and biochemical attributes conferring resistance in rice accessions/varieties to yellow stem borer, *Scirpophaga incertulas* Walker (Lepidoptera: Crambidae)

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Abstract

A field trial was conducted at Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirappalli District, Tamil Nadu, India for evaluating 48 rice accessions along with four varieties as check entries (TRY 1, TN 1, IWP, TRY 3) in their level of resistance to rice yellow stem borer, during *Kharif* 2018. Five accessions (AD 16124, AD 15101, AD 16189, AD 12182 and AD 12272) recorded nil dead heart and white ear head damage and were found to be highly resistant. Three accessions (AD 16157, AD 12132, AD 16157) were found to be highly susceptible. The number of trichomes ranged from 84.80/cm² leaf to 90.60/cm² leaf on upper surface and 16.40/cm² leaf to 20.00/cm² leaf on lower surface in the highly resistant accessions/varieties. The highly susceptible entries were found to have minimum number of trichomes ranging from 11.00/cm² leaf to 15.60/cm² leaf on upper surface and 1.60/cm² leaf to 2.80/cm² leaf on lower surface. Flag leaf angle of highly resistant accessions/varieties ranged from 1.33⁰ to 4.33⁰ (decreased leaf angle) and the flag leaf attitude is erect. The penultimate leaf angle of highly resistant accessions/varieties ranged from 6.33⁰ to 8.33⁰ (erect leaf attitude). Resistant and susceptible entries had semi-erect and horizontal leaf attitudes. Highly susceptible entries had greater leaf angle ranging from 76.00⁰ to 84.67⁰ (horizontal leaf attitude). The phenol content was maximum in the resistant entries ranging from 6.26 to 7.72 mg/g and minimum in the highly susceptible entries ranging from 2.53 to 3.21 mg/g. The susceptible entries had 4.40 to 5.00 egg masses/plant as against 1.00 to 1.60 egg masses /plant.

Keywords: Rice accessions, yellow stem borer, screening, biophysical resistance, biochemical resistance

Introduction

Rice (*Oryza sativa* L.) is an ancient food grain and important crop in the world feeding more than 50 per cent of the human population (Agrawal *et al.*, 2005) [1]. The rice crop is subjected to a considerable damage by nearly 300 species of insect pests, among them only 23 species are serious pests of rice (Pasalu *et al.*, 2006). Yield loss due to insect pests of rice has been estimated about 25 per cent (Dhaliwal *et al.*, 2010) [4]. The yellow stem borer, *Scirpophaga incertulas* (Walker) is considered as the most important pest of rice ecosystem, which attack the crop from seedling stage till harvest (Deka *et al.*, 2010) [3]. The dead hearts are produced when the insect attacks at vegetative stage, while white heads are produced at the reproductive stage. The attack of yellow stem borer can cause 25-30 per cent damage to the crop (Sachan *et al.*, 2006) [17]. Globally 50 per cent of the insecticides are used for the management of insect-pests in the rice field (Huesing and English, 2004) [10]. Over reliance on synthetic pesticides causes ecological adversity and health related problems (Wakil, 2001). Heavy insecticide use had led to an exponential increase in the number of insect species developing resistance to insecticides (Georghiou, 1986) [6]. The use of resistant varieties will not only reduce insecticide application but also minimize the environmental hazards. Hence a study was taken up to screen the rice germplasms against yellow stem borer and to evaluate the resistant traits that can be utilized in the breeding programme.

Materials and Methods

A field trial was conducted at Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirappalli District, Tamil Nadu, India for evaluation of 48 rice accessions along with four varieties as check entries for their level of resistance to rice yellow stem borer, during *Kharif* 2018. The standard agronomic practices recommended by Tamil Nadu

Agricultural University were adopted except the plant protection practices. The test entries were kept unsprayed throughout the season. Incidence of dead heart (%) and white ear (%) were recorded during the vegetative and reproductive stages respectively during the peak time of infestation. The observations were recorded from ten randomly chosen hills/accession or variety.

The damage percentage was calculated by adopting the formula developed by Heinrichs *et al.*, (1985) [7].

$$\text{Dead heart (\%)} = \frac{\text{Number of dead hearts}}{\text{Total number of tillers}} \times 100$$

$$\text{White ear (\%)} = \frac{\text{Number of white ears}}{\text{Total number of productive tillers}} \times 100$$

D values were calculated using the formula,

$$D = \frac{\text{Per cent dead or white ear in test genotype}}{\text{Percent dead heart or white ear in susceptible check}} \times 100$$

The damage rating scale 0-9 was fixed based on the D values suggested by IRRI Standard Evaluation System for screening resistance to stem borer in rice as given below, Standard evaluation system for screening resistance to stem borer in rice by IRRI

Dead heart (DH)			White ear (WE)		
Damage (%)	Scale	Resistance rating	Damage (%)	Scale	Resistance rating
0	0	Highly Resistant (HR)	0	0	Highly Resistant (HR)
1 - 20	1	Resistant (R)	1 - 10	1	Resistant (R)
21 - 40	3	Moderately Resistant (MR)	11 - 25	3	Moderately Resistant (MR)
41-60	5	Moderately Susceptible(MS)	26-30	5	Moderately Susceptible(MS)
61-80	7	Susceptible(S)	41-60	7	Susceptible(S)
81-100	9	Highly Susceptible(HS)	61-100	9	Highly Susceptible(HS)

Egg mass

Observations were made to assess the ovipositional preference of each entry by counting the number of egg mass/plant. Three replications were maintained for each rice accession/variety.

Biophysical basis of resistance

Leaf blade pubescence

Rice leaves have two kinds of trichomes, micro and macro hairs. Micro hairs are located along the stomata cells or besides motor cells, while macro hairs are located on silica cells over a thin vascular bundle (Hu *et al.*, 2013) [19]. The trichome density of rice leaf in different entries was estimated as per the procedure described by Maite *et al.*, (1980) [14]. Leaf samples collected at random in the rice plant were cut into 5 cm leaf bits in the middle of the leaf and boiled in 20 ml of water in small glass vials for 15 minutes in hot water bath at 85°C. The water was then poured out retaining the leaves and boiled after adding 20 ml of 96 per cent ethanol for 20 minutes at 80°C. Then alcohol was poured off and boiling process was repeated with alcohol to remove the chlorophyll completely from the leaves. Alcohol was again removed and 90 per cent lactic acid was added and heated at 85°C until the leaf segments cleared (approx. 30 – 45 minutes). The vials were then cooled and leaves were taken and mounted on clean slides using a drop of lactic acid to observe the trichome density. The trichomes / pubescence density per cm² on abaxial and adaxial surfaces of each leaf were counted under compound microscope (45 x magnification) for each sample. Five replications were maintained for each accession/variety. The micro hairs, macro hairs and glandular hairs on the leaf blade were counted.

All descriptors for leaves and their components were recorded on the penultimate leaf *i.e.*, the highest leaf below the flag leaf. Hence trichome density was estimated in the penultimate leaf from the randomly chosen plants. Leaf pubescence is categorised as glabrous (scale 1), intermediate (scale 2) and pubescent (scale 3), (Bioversity international, IRRI and WARDA. 2007) [2]. Leaf blade pubescence was observed at the booting stage of the plant.

Leaf angle

The angle of openness of the blade tip was measured against the culm of the leaf. The leaf angle was measured using protractor with its 90° set vertically to the culm for the flag leaf and penultimate leaf after a growth period of 39 days (Yoshida, 1981). The flag leaf attitude was scored as erect (score 1), semi erect / intermediate (score 3), horizontal (score 5) and deflexed/ descending/ drooping (scale 7). The position of the tip of the blade relative to its base, scored on the leaf below the flag leaf (penultimate leaf) is called leaf blade attitude, which was measured at the late vegetative stage (prior to heading). It is categorised as erect, horizontal and drooping (Bioversity international, IRRI and WARDA. 2007) [2].

Biochemical basis of resistance

Total phenol content

The total phenol content was estimated by the procedure described by Sadasivam and Manikkam, (1996) [18]. A quantity of 100 mg of plant sample was extracted with 80 per cent ethanol and centrifuged at 10,000 rpm for 20 minutes. The supernatant was evaporated to dryness and the residue was dissolved in 5 ml of distilled water. The aliquots of 0.2 to 2.0 ml was pipetted out and the volume was made upto 3.0 ml with distilled water. A quantity of 0.5 ml of folin ciocalteau reagent and 2.0 ml of 20 per cent sodium carbonate solution was added. It was then kept in a boiling water bath for one minute, cooled and the colour developed was measured at 650 nm wave length using spectrophotometer. The phenol content was calculated by drawing a standard graph with catechol as standard and expressed as catechol equivalents. The biophysical and biochemical parameters were assessed only for those cultures categorized as highly resistant (HR), resistant (R), susceptible (S) and highly susceptible (HS).

Results and Discussion

Among the 48 rice accessions/varieties screened against rice yellow stem borer, five accessions (AD 16124, AD 15101, AD 16189, AD 12182 and AD 12272) recorded nil dead heart and white ear head damage and were found to be highly

resistant. Three accessions (AD 16157, AD 12132, AD 16157) were found to be highly susceptible along with the check entry TN1. The damage rating scale for all the entries ranged from 0 to 9. (Table 2). The present findings are in accordance with the previous works. Khan *et al.* (2003) [13] reported the variety KS-282 as resistant among the eight rice varieties (JP-5, Swat-1, Swat-2, Dilrosh-97, Basmati-385, KS-282, Gomal-6 and Gomal-7) screened for their resistance against rice stem borer. Singh and Shukla (2007) [20] screened 1224 rice accessions and found 43 accessions as resistant to stem borers and 34 as susceptible. The promising genotypes ACK 14003, ACK 14004, BRNS-WP-6 recorded the scale of 1 among eight rice cultures and six varieties, (Elanchezhyan *et al.*, 2017) [5]. Observations on the number of egg mass/plant revealed that highly resistant entries were not preferred for oviposition, resistant entries recorded egg mass numbers ranging from 1.00 to 1.60 (no./plant). Highly susceptible entries had maximum number of egg masses ranging from 4.40 to 5.00 (no./plant). Results showed a significant difference among the highly resistant, resistant, susceptible and highly susceptible entries, (Table 3). All the biophysical parameters influenced the number of egg mass/plant. The oviposition was found to have a significant positive correlation with dead hearts and white heads, (Rustamani *et al.*, 2002) [16]. The leaf trichome density was negatively correlated with egg masses number ($r = -0.67$) and egg number ($r = -0.55$) in *Chilo suppressalis* (Walker) (Tabari *et al.*, 2016) [22]. The rice genotypes with the lowest number of egg masses suffer less damage from the rice stem borer (Hosseini *et al.*, 2011) [8]. The number of trichomes/cm² was maximum on the upper surface of leaf blade than the lower surface (Table 3). It ranged from 84.80/cm² to 90.60/cm² / leaf on upper surface and 16.40/cm² leaf to 20.00/cm² leaf on lower surface in the highly resistant accessions/varieties and were categorised as pubescent (scale 3). The highly susceptible entries were found to have minimum number of trichomes which ranged from 11.00/cm² leaf to 15.60/cm² leaf on upper surface and 1.60/cm² to 2.80/cm² /leaf on lower surface and was categorised as glabrous (scale 1). This is in accordance with the previous finding, that the density of trichomes is an important component of antixenosis resistance in rice genotypes to the rice striped stem borer, *Chilo suppressalis* (Walker) (Zhu *et al.*, 2008) [24]. The flag leaf angle of highly resistant accessions/varieties ranged from 1.33⁰ to 4.33⁰ (decreased leaf angle) and the flag leaf attitude as erect (scale 1). The penultimate leaf angle of highly resistant accessions/varieties ranged from 6.33⁰ to 8.33⁰ (scale 1- erect leaf attitude). Resistant and susceptible entries had semi-erect and horizontal leaf attitudes. The highly susceptible entries

had greater leaf angle ranging from 76.00⁰ to 84.67⁰, (Table 3). The leaf angle of highly resistant cultures ranged from 7.20⁰ to 8.20⁰ which was categorised as vertical/erect leaf attitude. Resistant (R) entries showed semi erect leaf attitude, susceptible (S) and highly susceptible (HS) accessions/varieties showed horizontal leaf attitude. Keulen (1986) [12] mentioned that the leaf insertion angle was one of the most important feature of the plant related to production capacity. Hence, plants with erect leaf attitude have higher silica content and have greater interception and absorption of Photosynthetically Active Radiation (PAR) and were least preferred for egg laying. The larger leaf angle in the susceptible and highly susceptible accessions/ varieties lead to semi erect/ horizontal leaf attitude of flag leaf and penultimate leaf. Plants with this horizontal leaf attitude have greater Leaf Area Index (LAI), reduced photosynthetic rate since leaves shadow one another and absorption of PAR was low and have low silica content, hence were preferred for egg laying. Kasturi Thilagam *et al.*, (2014) [11] reported that silica in rice caused vertical positioning of the leaves thus greater surface of the leaf area was exposed to sun. Hence the leaf was unpreferred for egg laying. El-Adl *et al.*, (2011) evaluated the morphological traits conferring resistance to stem borer (*Chilo agamemnon* Bles.) in some rice genotypes and found that, the flag leaf angle and stem diameter play important role in increasing the resistance to rice stem borer and used as indicator to select for stem borer resistance. The addition of silica (Si) to the nutrient solution decreased the leaf angle, modifying its architecture and favouring the predominance of more erect leaves on plants supplied with silica. (Zanao Junior *et al.*, 2010) [23].

The total phenol content in the rice accessions ranged from 7.72 to 2.53 mg/g. Higher amount of phenol content was observed in the resistant entries ranging from 6.26 to 7.72 mg/g and the highly susceptible entries had lower amount of phenol from 2.53 to 3.21 mg/g (Table 4). It was clearly evident that the resistant accessions /varieties showed maximum trichome density on the upper and lower surface of the leaf blade, lower leaf angle and high phenol content with reduced egg mass, dead heart and white ear symptoms (Fig.1). Padhi (2004) [15] recorded lower yellow stem borer incidence due to higher amount of total phenol in the rice varieties TKM 6 and PTB 18. The presence of higher amount of total phenols provided resistance by acting as a barrier to the borer larvae to utilize the plant nutrients (Suchita *et al.*, 2011) [21]. The higher phenol content was observed in resistant cultures *viz.*, ACK 14004 (4.08 mg/g fresh weight) followed by BRNS WP (3.83 mg/g fresh weight) which showed antifeedant and repellent action (Elanchezhyan *et al.*, 2017) [5].

Table 1: Screening of rice accessions/ varieties for their reaction to rice yellow stem borer, *S. incertulas*

S. No.	Rice accessions/ varieties	DH (%)	D Factor	WE (%)	D Factor	Score	Status
1	AD 16156	16.27	82.58	13.33	63.32	9	HS
2	AD 13330	13.24	67.18	8.57	40.71	7	S
3	AD 15127	8.81	44.71	6.18	29.35	5	MS
4	AD 10202	4.85	24.64	2.35	11.16	3	MR
5	AD 11168	9.90	50.23	6.25	29.69	5	MS
6	AD 16151	4.98	25.25	4.21	20.00	3	MR
7	AD 16145	14.08	71.46	11.23	53.34	7	S
8	AD 12105	2.45	12.44	1.88	8.93	1	R
9	AD 16124	0.00	0.00	0.00	0.00	0	HR
10	AD 12079	10.89	55.28	6.02	28.59	5	MS
11	AD 16168	7.07	35.89	3.33	15.81	3	MR
12	AD 16179	7.11	36.07	3.88	18.43	3	MR
13	AD 15101	0.00	0.00	0.00	0.00	0	HR

14	AD 13255	4.52	22.96	4.20	19.95	3	MR
15	AD 16302	6.09	30.92	2.83	13.44	3	MR
16	AD 12120	14.78	75.02	9.57	45.46	7	S
17	AD 16189	0.00	0.00	0.00	0.00	0	HR
18	AD 16308	9.52	48.34	6.66	31.63	5	MS
19	AD 12182	0.00	0.00	0.00	0.00	0	HR
20	AD 12228	12.14	61.60	10.47	49.73	7	S
21	AD 07302	10.70	54.29	5.73	27.22	5	MS
22	AD 13263	7.25	36.82	3.73	17.71	3	MR
23	AD 09493	3.74	18.98	1.12	5.32	1	R
24	AD 12180	12.86	65.26	11.76	55.86	7	S
25	AD 12205	12.50	63.45	10.15	48.21	7	S
26	AD 16268	5.37	27.24	3.44	16.34	3	MR
27	AD 13310	15.15	76.91	9.73	46.22	7	S
28	AD 16258	0.98	4.98	2.17	10.30	1	R
29	AD 12178	1.44	7.32	0.84	3.99	1	R
30	AD 13299	4.76	24.17	3.06	14.53	3	MR
31	AD 13298	6.83	34.67	2.91	13.82	3	MR
32	AD 15093	14.29	75.52	8.33	39.57	7	S
33	AD 16121	4.78	24.29	3.00	14.25	3	MR
34	AD 13211	3.98	20.20	2.23	10.59	3	MR
35	AD 13258	8.80	44.65	6.79	32.25	5	MS
36	AD 12272	0.00	0.00	0.00	0.00	0	HR
37	AD 12132	18.42	93.51	16.30	77.43	9	HS
38	AD 13280	3.50	17.77	1.85	8.78	1	R
39	AD 16157	19.59	99.43	13.86	65.84	9	HS
40	AD 15088	13.40	68.03	9.64	45.79	7	S
41	ACK 12001	12.24	62.16	10.78	51.21	7	S
42	ACK 13005	7.69	39.05	3.20	15.20	3	MR
43	ACK 12001	7.14	36.26	4.93	23.42	3	MR
44	ACK 14001	6.50	32.99	4.34	20.61	3	MR
45	TRY 3	8.96	45.49	6.00	28.50	5	MS
46	TRY 1	0.00	0.00	0.00	0.00	0	HR
47	I. White ponni	12.69	64.42	10.83	51.44	7	S
48	TN 1	19.70	100.00	21.05	100.00	9	HS

DH- Dead Heart; WE- White Ear

Table 2: Evaluation of biophysical attributes conferring resistance in rice accessions/varieties to yellow stem borer, *S.incertulas*

S. No.	Rice accessions/ varieties	Resistance status	Flag leaf angle (°)*	Penultimate leaf angle (°)*	Leaf blade pubescence (no./cm ² / leaf)#		Egg mass (no./plant) #
					Upper surface	Lower surface	
1	AD 16124	HR	1.33	7.00	86.80	17.60	0.00 (0.71)a
2	AD 15101	HR	1.33	8.00	84.80	20.00	0.00 (0.71)a
3	AD 16189	HR	2.33	8.33	86.20	18.20	0.00 (0.71)a
4	AD 12182	HR	2.67	7.67	90.60	18.80	0.00 (0.71)a
5	TRY 1	HR	4.33	8.33	88.00	16.40	0.00 (0.71)a
6	AD 12272	HR	3.33	6.33	85.40	19.80	0.00 (0.71)a
7	AD 12105	R	6.67	10.33	63.80	10.20	1.20 (1.30)bc
8	AD 09493	R	10.67	14.33	60.20	9.80	1.40 (1.38)bc
9	AD 16258	R	10.00	12.33	52.60	9.60	1.00 (1.22)b
10	AD 12178	R	7.67	15.67	50.20	10.00	1.40 (1.38)bc
11	AD 13280	R	8.67	14.67	57.00	10.60	1.60 (1.45)c
12	AD 15088	S	41.00	24.33	29.60	6.80	3.40 (1.97)def
13	ACK 12001	S	45.33	17.67	31.00	5.60	3.00 (1.87)d
14	AD 15093	S	50.33	19.33	33.20	5.40	3.80 (2.07)fg
15	I.White ponni	S	58.33	18.67	32.20	6.40	3.60 (2.02)ef
16	AD 13310	S	42.33	25.33	26.80	5.40	3.20 (1.92)def
17	AD 12180	S	55.33	29.33	31.60	6.60	3.80 (2.07)fg
18	AD 12205	S	56.67	26.67	37.60	6.80	3.40 (1.97)def
19	AD 12228	S	40.67	24.33	38.00	5.00	3.00 (1.87)d
20	AD 12120	S	43.00	21.33	35.60	6.80	3.40 (1.97)def
21	AD 16145	S	51.33	20.33	27.40	6.20	3.20 (1.92)de
22	AD 13330	S	55.00	20.00	29.80	7.40	3.20 (1.92)def
23	AD 16156	HS	76.00	34.33	11.00	2.40	4.80 (2.30)h
24	AD 12132	HS	82.33	35.67	13.60	2.00	4.40 (2.21)gh
25	AD 16157	HS	79.67	36.00	15.60	2.80	4.80 (2.30)h
26	TN 1	HS	84.67	37.00	15.00	1.60	5.00 (0.81)h
SEd	-	-	-	-	-	-	0.08
CD (p=0.05)	-	-	-	-	-	-	0.16

*Mean of three replications; #Mean of five replications

Figures in parenthesis () are square root transformed values.

In a column, means followed by similar letter(s) are not different statistically (p=0.05)

Table 3: Evaluation of phenol content in rice accessions/varieties conferring resistance to yellow stem borer, *S. incertulas*

S. No.	Rice accessions/ varieties	Resistance status	Phenol content (mg/g fresh weight)
1	AD 16124	HR	6.26
2	AD 15101	HR	7.44
3	AD 16189	HR	7.72
4	AD 12182	HR	6.83
5	TRY 1	HR	6.45
6	AD 12272	HR	6.74
7	AD 12105	R	5.19
8	AD 09493	R	5.63
9	AD 16258	R	5.51
10	AD 12178	R	5.82
11	AD 13280	R	5.66
12	AD 15088	S	3.98
13	ACK 12001	S	4.62
14	AD 15093	S	4.61
15	I.White ponna	S	4.48
16	AD 13310	S	4.75
17	AD 12180	S	4.31
18	AD 12205	S	4.77
19	AD 12228	S	3.86
20	AD 12120	S	4.42
21	AD 16145	S	4.66
22	AD 13330	S	4.52
23	AD 16156	HS	3.11
24	AD 12132	HS	2.53
25	AD 16157	HS	2.65
26	TN 1	HS	3.21

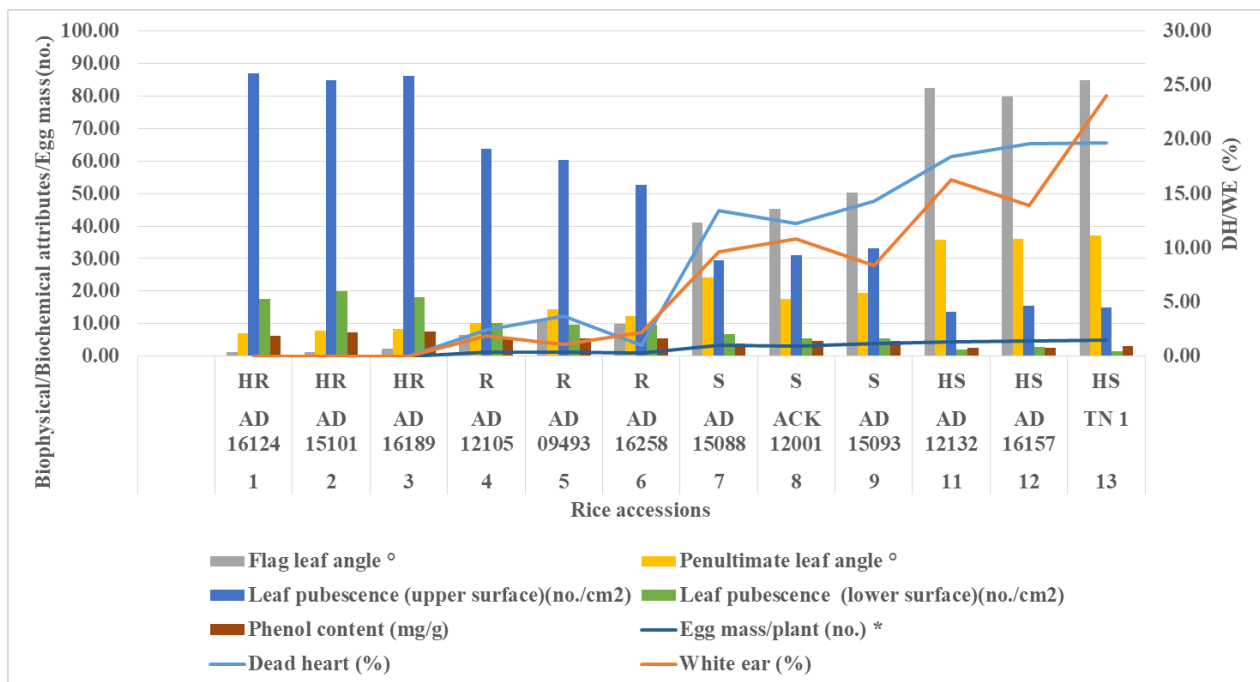


Fig 1: Influence of biophysical and biochemical attributes on the preference of yellow stem borer, *S. incertulas* on rice

Conclusion

The rice accessions with maximum trichome density on upper and lower surface of leaf blade, lower leaf angle and high phenol content conferred resistance to yellow stem borer with minimum dead heart and white ear since they are least preferred by adults for oviposition.

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