Assessing different rice genotypestolerance against the major pests and identifying the biochemical basis oftolerance

Abstract:
A total of 196 rice genotypes were screened for tolerance against paddy stem borer and leaf folder pests under open field conditions. Damage assessment followed the standard evaluation procedure for rice developed by IRRI (1988). Stem borer incidence was recorded during both the vegetative and reproductive stages while leaf folder incidence was recorded at five different growth stages (30, 40, 50, 60, and 70 days after transplanting). Interestingly, tolerance to paddy stem borer varied among the genotypes. Genotypes resistant at the Vegetative Stage did not show tolerance at the Reproductive Stage. Consequently, leaf folder infestation was recorded at five different growth stages, demonstrating that although the mean infestation was considered for identifying tolerance, the rate of infestation against an accession is not consistent across all growth stages. Additionally, biochemical analysis of the resistant entries, along with a susceptible check (TN1), revealed that higher total phenol concentrations, moderate chlorophyll content, and lower sugar levels were key factors contributing to pest tolerance. The correlation between infestation percentage and biochemical parameters showed a high positive correlation between total sugars and infestation percentage and a strong negative correlation between total phenols and infestation percentage, indicating that phenols play a role in plant defense.

***Keywords*:**Screening, stem borer,leaf folder,Total phenols, Sugars

1. Introduction:

The paddy stem borer, *Scirpophaga incertulas*, inflicts significant damage to rice from seedling to maturity and accounts for a significant portion of crop losses. According to Zsögön et al. (2022), the world's population is expected to grow by 2 billion in the next 30 years due to climate change, from 7.7 billion today to 10 billion in 2050. The growing global population need more food production. Van Dijk et al. (2021) predict that global food demand will rise from 35% in 2010 to 56% in 2050.About 1,000 rice cultivars across the country lack inherent tolerance to various biotic stresses (Chatterjee et al.,2020). Another pest that causes significant damage is the rice leaf folder, *Cnaphalocrocis medinalis* (Guenee), which was once thought to be minor in many Asian countries. Extensive feeding reduces photosynthetic ability and vigor and predisposes leaves to bacterial and fungal infections (Sabir et al.,2012).Host-plant resistance plays a crucial role in creating an integrated pest management system in low-input farming environments, particularly in India (Pal et al., 2021).

Widespread use of chemical insecticides can significantly harm native natural enemy populations, potentially resulting in a resurgence of pest populations. Additionally, it leads to environmental pollution by leaving pesticide residues in the soil, air, and water, posing risks to human and animal health (Sandhu et. al., 2020). Insect-resistant plant varieties or genotypes not only decrease insect pest populations but also complement other eco-friendly pest management strategies (Rani et al., 2020). Plant traits that facilitate direct defenses have been demonstrated to reduce insect growth rates by diminishing the digestibility and nutritional quality of plant tissues (Belete, 2018; Golla et al., 2020).The ecology of rice fields has changed as a result of widespread, intensive rice farming that aims to maximize output while implementing innovative agricultural techniques, turning some little pests into significant ones.

Insecticide-based attempts to manage these pests have resulted in phytotoxicity, toxicity to beneficial organisms, resurgence, tolerance, and food residues that are over tolerance limits and pose health risks. It is imperative to reduce the usage of chemical pesticides in pest management given these drawbacks. 196 genotypes were field-tested in Karaikal to identify resistant rice genotypes against paddy stem borer and leaf folder. Chlorophyll concentration, total sugars, reducing sugars, total phenols, total soluble proteins, and proline content were all examined and linked with tolerance after resistant entries were chosen using IRRI standard evaluation procedures.

2. Materials and Methods:

The Indian Institute of Rice Research (IIRR) in Hyderabad provided rice genotypes, including two susceptible checks, Suraksha and TN 1. The IRRI-developed Standard Evaluation System was utilized to evaluate the rice varieties vulnerability to leaf folder and paddy stem borer. Throughout the growth season, the fields were regularly irrigated as needed, with water added to reach a depth of 2 to 5 cm. At a rate of 120:60:60 kg/ha, fertilizers (N: P_2O_5:K_2O), especially Urea, Diammonium phosphate (DAP), and Muriate of potash (MOP) were applied. Before transplanting, the full doses of P_2O_5 and K_2O were applied, along with half of the N. The remaining half of the N was applied in two equal amounts throughout the phases of panicle initiation and tillering. To keep weeds and crops from competing, weeds were manually pulled. After 25 days of sowing, seedlings with two replications were moved onto the main field.

Leaf folder damage was recorded at five distinct times after transplantation: 30, 40, 50, 60, and 70 days. The assessment of stem borer damage was carried out at two stages: the vegetative stage (30 days after transplanting) and the reproductive stage (70 days after transplanting). Every replication's entry was evaluated for five hills that were chosen at random. Leaf folder damage was calculated by recording the total number of leaves and damaged leaves on each hill, including the susceptible checks (TN 1 and Suraksha) (Equation No.3 & 4). Stem borer damage was estimated by counting the number of tillers and damaged tillers in each hill (Equation No.1 & 2). (Heinrichs et al., 1985) assessed the percentage of damage caused by stem borer and leaf folder as follows:

Stem Borer:

$$\text{\% dead hearts/ white ears} = \frac{\text{No.of damaged tillers (Dead hearts/White ears)}}{\text{Total No.of tillers}} \times 100 \qquad \textbf{(Equation No.1)}$$

Percentage of dead heart/white ears was converted to D

$$D = \frac{\text{\% dead hearts/ white ears in test entry}}{\text{\% dead hearts/white ears in the susceptible check}} \times 100 \textbf{(Equation No.2)}$$

(Mean of two Susceptible checks)

D is converted to a 0-9 scale

(Percentage of white ears only, while percentage of dead hearts will follow the same scale as of leaf folder)

Scale	**D**	**Status**
0	No Damage	Highly Resistant
1	1-10%	Resistant
3	11-25%	Moderately Resistant
5	26-40%	Moderately susceptible
7	41-60%	Susceptible
9	61-100%	Highly Susceptible

The mean of the five hills were taken for tabulation.

(Heinrichs et al., 1985)

Leaf Folder:

$$\text{\% damaged leaves} = \frac{\text{No.of damaged leaves}}{\text{Total No.of leaves}} \times 100 \textbf{(Equation No.3)}$$

Percentage of damaged leaves is converted to D

$$D = \frac{\text{\% damaged leaves in test entry}}{\text{\% damaged leaves in the susceptible check}} \times 100 \textbf{ (Equation No.4)}$$

(Mean of two Susceptible checks)

D is converted to a 0-9 scale

Scale	**D**	**Status**
0	No Damage	Highly Resistant
1	1-20%	Resistant
3	21-40%	Moderately Resistant
5	41-60%	Moderately susceptible
7	61-80%	Susceptible
9	81-100%	Highly Susceptible

The mean of the five hills were taken for tabulation.

Following the screening process, the genotypes showing the lowest average damage from stem borers and leaf folders were singled out as resistant entries. These top-performing entries along with a susceptible one (TN 1), were selected for biochemical analysis. The objective was to identify the biochemical factors contributing to tolerance in the promising genotypes by assessing total chlorophyll, total sugars, reducing sugars, total phenols, protein and proline levels.

2.1 Estimation of Biochemical Factors:

Leaf samples were used to evaluate biochemical factors. Total chlorophyll levels were calculated using the Hiscox and Israelstam method (Hiscox et al.,1979). Total and reducing sugars were measured using the Nelson-Somogyi method, as described by Eric Fournier (Eric Fournier et al.,2001). Total phenol content was calculated using the Sadasivam and Manikkam (Sadasivam et al.,1996). Protein levels were estimated using Lowry's method (Lowry et al.,1951) and proline levels were calculated using the method described by (Bates et al.,1973).

2.2 Statistical Analysis: The data concerning biochemical parameters were subjected to analysis using AGRES software to assess their significance.

3. Results & Discussion:

3.1. Field Evaluation of Rice genotypesfor Stem borer and Leaf folder

The findings show that between 4.42 and 41.9 percent of dead hearts were caused by stem borers 30 days after transplanting (DAT) (Table 1). Table 1 shows that throughout the vegetative stage, the susceptible tests Suraksha and TN 1 showed 39.4 percent and 41.9 percent dead hearts, respectively along with similar percentages of white ears at 8.04 percent and 8.32 percent. At 30 DAT, while converting the data to D value with 0-9 scale, five entries were found resistant; 51 entries were moderately resistant, 94 were moderately susceptible,39 were susceptible and 5 were highly susceptible. With 4.42 percent dead hearts and a correspondingly low rate of white ears at 1.23 percent, accession OR2324-8 exhibited the lowest incidence. However, certain entrants with low dead heart incidence didn't always display tolerance, as they showed limited infection accompanying minimal white ears. This finding is in line with earlier studies (Mathur et al.,1978), (Srivastava, 1979), (Pathak, 1964); (Chandra Mohan et al., 1983); (Singh et al., 1997), which suggested that rice varieties resistant to paddy stem borer attack during the vegetative stage may not necessarily maintain tolerance during the reproductive stage.

Table 1. Field reaction of resistant rice genotypes against paddy stem borer

Resistant Entries at 30 DAT		Resistant Entries at 70 DAT	
Accession	**% Dead Heart**	**Accession**	**%White Ear**
OR 2324-8	4.42	**OR 2324-8**	1.23
RTN 62-6-7-1	5.62	**RTN 62-6-7-1**	1.67
CR 2698	7.12	**R 1138-688-3-533-1**	1.82
HUR-913	7.94	**CR 2698**	1.94
CN 1561-70-19-35-9-MLD 1	8.1	**HUR-913**	2.14
***Suraksha**	39.4	**Suraksha**	8.04
***TN1**	41.9	**TN1**	8.32

DAT: Days after transplantation ***Control Checks – Suraksha & TN1**

Table 1 shows that by 70 days after transplanting (DAT), white ear damage varied from 1.23 percent to 8.32 percent. The study found that OR 2324-8 had the lowest incidence of white ears which has also lowest incidence of dead hearts. At 70 DAT, while converting the data to D value with 0-9 scale,no entry was found to be resistant; four entries were moderately resistant, 64 were moderately susceptible, 94 were susceptible, and 32 were highly susceptible (Table 2). (Panigrahi et al., 2010) screened 118 deep-water rice accessions and identified 64 as resistant during the vegetative stage. Of the 196 entries, none were found to be resistant at this point. Sujay Pandey et al., 2011) screened 60 germplasms and found 35 germplasms to be resistant. By screening 41 rice germplasms, (Singh et al., 1997) found ten to be resistant during the vegetative stage and one during the reproductive stage. Previous research (Tiwary et al., 1988; Mishra et al., 1990; Gubbaiah et al., 1993; Balasubramanian et al., 2000; Sarao et al., 2009; Padhi, 2009; Rath et al., 2010 have documented varying responses in rice lines to stem borer infestation, classifying them as resistant, moderately resistant, susceptible, moderately susceptible, and highly susceptible, based on damage scales. For biochemical analysis, the top five genotypes were selected based on their ranking.

Table 2: Rice genotypes classified by percentage of white ear against paddy stem borer

Damage % (range)	**Damage rating**	**Reaction**	**Genotypes**
1-10	1	Resistant	Nil
11-25	3	Moderately resistant	OR 2324-8, RTN 62-6-7-1, R 1138-688-3-533-1, CR 2698
26-40	5	Moderately susceptible	HUR-913, CN 1561-70-19-35-9-MLD 1, ARRH-3626, R 1528-1058-1-110-1, UPR 3506-7-1-1, HUBR 10-9, PAU 3761-26-3-1, UPR 3413-8-2-1, CR 2715-13-IR-84887-B-154, JGL 17183, Swarna ,UPR 3425-11-1-1, HRI-169, HRI-171, R 1529-1183-1-1041-1, NDR 9543, R 1535-1382-1-1667-1, NWGR 3132, NP- 3112, NDR 6244, CR 2304-5-3-7-1, NK6303, Swarna Dhan, Rewa 1103, ARRH-3585, TJP 48, RP 5125-5-9-1 (IR 84898-B-171-19), IR 36, OR 1946-2, RP 5125-2-4 (IR 84898-B-165-10), OR 2328-5, NK6320, CR 2701-1-47-2-IR 84882-B-120, NPG-209, NPG-210, PA 6201, CR 2641-26-1-2-2, CR 2644-2-6-4-3-2, CR 2707, Kalanamak, OR 2324-2, Dinesh, KPH-371, Purnendu, CR 2702, PAU 3879-87-1-1, TaraoriBasmati, UPR 3411-1-1-1, PA 6129, KAU-PTB- Vaisakh, RH09011, UPR 3425-14-3-1, MAS 946, Pusa 1121, NP- 124-8, RGL 7001, CR 2717-10-IR 84899-B-185, VNR-203, OR 2163-14, Ajaya, CN 1446-5-8-17-1-MLD 4, CB 05022, NDR 9542, Benibhog
41-60	7	Susceptible	R 1570-418-1-149-1, RNR 2354, Pusa 1612-07-6-5, ORS-327, IR 50, WGL-480, Pusa Basmati, HR 12, JGL 17196, IR78091-6-2-3-1-1, VRH-639, 25 P 25, C1446-5-18-17-2-MLD 2, CB 08-504, HRI-172 (H), Jalmagna, Badshabhog, NDR 359, TN 1, Nidhi, CN 1646-6-11-9, OR 1895-2, WGL 407, CR 2613-1-5-2-7-2, PAU 3105-45-3-2, SYE-4-5-73-28-6-13, CR 2616-3-3-3-1, R1570-2649-1-1546-1, CR 2543-83, TRC 2008-1, 27P31, OR 2336-1, MEPH-106, PAU 3371-26-1-3, CR 2304-12-9-7-4, PAU 3386-31-1-2-5, NDR 6311, NK-6355, CR 2695-10-1-2-3, NP-5151, CR 2729-4-1-IR 84899-B-182-CRA, KPH-272, OR 2405-KK-9, CR 2696- IR 83920-B-B-CRA-103-14-1-1-1, UPR 3330-9-1-2,KPH-216, NDR 8002, RP 5130-12-3-5-21-3, HKR 06-47, CR 2716-10-IR 84898-B-165, CR 2683-28-45-1-5, RTN 8-4-2-1-2, PSB RC 18 (IR 31672-62-1-2-2-2-3), NDR 370133, CR 2649-7, CR 2718-10-IR 83927-B-B-279, CB 05-031, SYE - 2-3-16-65-31-82, R 2085-RF-69, OM 5240, Sabita, PNPH-24, WGL 365, KMP-148, Pusa Sugand 5, RP 5130-136-5-5-33-5, CR 2496, CB 06-124, CR 2611-3-2-2-1, RP 3644-1-19-5-5, HRI-173, IR 64, BPT 2511, RP 5127-9-3 (IR 93376-B-B- 130), OR 2331-14, Pusa 1592-06-5-2, CR 2656-11-3-4-2, R 1124-69-1-45-1, CR 2652-14, Pusa 1509-03-3-9-5, Vikramarya, CR 2547-62-316, RAU 467-79-60, GK5016, Triguna, HUR –ASG-KN-23 S, NDR 4058-7, UPR 3426-3-1-1, Improved Samba Mahsuri, NDR 370135, WGL-451, R1566-2577-2-1530-1, TM 05091, US314 (H)
61-100	9	Highly Susceptible	TRC 2008-5, KRH 2, CN 1448-5-2-5-5-MLD 6, KJT 1-11-15-23-26-22, R 1532-1101-1-119-1, Pusa 1509-03-1-7-2, NVSR-178, NP- 218, SKL-32-70-15-10, MGD -107, AD 04022, APH-111, OR 2172-7, RP Bio 4918-2485, CN 1223-5-4-3-2, RP 5124-11-6-2 (IR 83876-BF3 Bulk), CR 2482-10-4-3-2, CH 45, CR 2241-7-2-3-1, CB 05-754, RH-1531, Shabagi Dhan, NDR 1107, XR-99982, CR 2687-4-13-2-1, 27P52, R 1570-2644-2-1547, NVSR-176, CR 2682-4-2-2-2-1, CR 2699, CR 2721-81-3-IR 83380-B-B-124-1, 27P88

*Checks-Suraksha and TN 1

The findings showed that at 30 days after transplantation (DAT), leaf folder damage ranged from 0.49 to 12.10 percent, with an increasing trend up to 70 DAT (Table 3). The mean damage percentages for Suraksha and TN 1 control checks were 43.96 percent and 45.82percent respectively (Table 3). On the other hand, ARRH-3626 had a low damage withmean value 6.82 percent (Table 3). Because of little pest pressure, at 30 DAT its incidence was just 0.78 percent, at 40 DAT, it rose to 4.69 percent, at 50 DAT it was 7.53 percent,at 60 DAT it was 11.43 percent and at 70 DAT it was 9.65 percent (Table 3). Interestingly, NDR 370135 which is having highest mean value of 8.83 percent showed the lowest occurrence among the 10 resistant entries at 30 DAT as 0.49 percent (Table 3). The number of pests increased trend over time. Pest populations are dynamic, so variations are normal and depend on entry characteristics. At 40 DAT, CR 2698 had the lowest damage percentage in the group as 3.72 percent (Table 3). On the other hand, ARRH-3626 consistently showed minimal incidence percentages of 7.53 percent, 11.43 percent, and 9.65 percent at 50, 60, and 70 DAT, respectively (Table 3).

Table 3. Field reaction of resistant rice genotypesagainst leaf folder during different growth stages

Accession	% Damaged leaves					
	30DAT	40 DAT	50 DAT	60 DAT	70 DAT	Mean
ARRH-3626	0.78	4.69	7.53	11.43	9.65	6.82
OR 2324-8	0.65	4.36	7.80	12.01	9.90	6.94
CR 2698	0.78	3.72	9.70	13.21	11.20	7.72
UPR 3506-7-1-1	0.82	4.72	8.34	13.91	11.30	7.82
HUBR 10-9	0.91	3.78	9.80	12.40	12.20	7.82
R 1528-1058-1-110-1	2.29	4.70	8.10	13.86	10.82	7.95
CN 1561-70-19-35-9-MLD 1	0.85	3.81	8.82	13.77	12.51	7.95
CR 2652-14	0.58	4.47	9.95	15.41	13.45	8.77
PAU 3371-26-1-3	0.55	5.46	8.74	15.76	13.56	8.81
NDR 370135	0.49	5.26	10.18	14.54	13.67	8.83
*Suraksha	11.82	29.20	56.78	64.20	57.80	43.96
*TN-1	12.10	34.90	59.40	63.60	59.10	45.82

DAT: Days after transplantation ***Control Checks – Suraksha & TN1**

Significant differences in average leaf damage were found among the 196 entries evaluated in comparison to the control checks Suraksha and TN1. Ten entries were classified as resistant, 145 as moderately resistant, 31 as moderately susceptible, and seven as susceptible (Table 4). In contrast, (Patnaik et al. 1987) examined 22 medium-duration and 24 medium-late-duration cultivars and found that none were completely free from infestation. Damage extent varied significantly among the resistant entries at different levels.

The results showed a variety of trends in rice entries with respect to the lowest and maximum incidence at particular intervals. In a similar vein, (Shah et al., 2008) reported that IRRI-6 demonstrated moderate susceptibility to leaf folder with a score of 5, while Basmati-385 and KSK-282 were susceptible to it with a score of 7. Changes in environmental conditions could explain the discrepancies in results. It was reported that IRRI-6, KSK-282, and DR-83 also demonstrated tolerance to rice leaf folders, whereas Basmati-385 was identified as the most susceptible variety (Muhammad et al., 2013). These findings align with previous research.

Table 4: Rice genotypes classified by percentage of damage against leaf folder

Damage % (range)	Damage rating	Reaction	Genotypes
0-20	1	Resistant	ARRH-3626, CN 1561-70-19-35-9-MLD 1, CR 2652-14, CR 2698, HUBR 10-9, NDR 370135, OR 2324-8, PAU 3371-26-1-3, R 1528-1058-1-110-1, UPR 3506-7-1-1
20-40	3	Moderately resistant	27P31, 27P52, 27P88, AD 04022, Ajaya, APH-111, ARRH-3585, Badshabhog, Benibhog, BPT 2511, CB 05022, CB 05-031, CB 05-754, CB 06-124, CH 45, CN 1448-5-2-5-5-MLD 6, CN 1646-6-11-9, CR 2241-7-2-3-1, CR 2304-12-9-7-4, CR 2304-5-3-7-1, CR 2482-10-4-3-2, CR 2496, CR 2543-83, CR 2547-62-316, CR 2611-3-2-2-1, CR 2613-1-5-2-7-2, CR 2616-3-3-3-1, CR 2644-2-6-4-3-2, CR 2649-7, CR 2656-11-3-4-2, CR 2682-4-2-2-2-1, CR 2683-28-45-1-5, CR 2687-4-13-2-1, CR 2695-10-1-2-3, CR 2696- IR 83920-B-B-CRA-120, CR 2701-1-47-2-IR 84882-B-120, CR 2716-10-IR 84898-B-165, CR 2717-10-IR 84899-B-185, CR 2718-10-IR 83927-B-B-279, CR 2721-81-3-IR 83380-B-B-124-1, CR 2729-4-1-IR 84899-B-182-CRA-12-1, GK5016, HKR 06-47, HRI-169, HRI-171, HRI-172 (H), HRI-173, HUR –ASG-KN-23 S, HUR-913, Improved Samba Mahsuri, IR 50, IR78091-6-2-3-1-1, Jalmagna, KAU-PTB- Vaisakh, KPH-216, KPH-272, KRH 2, MEPH-106, MGD -107, NDR 359, NDR 370133, NDR 4058-7, NDR 6244, NDR 6311, NDR 8002, NDR 9542, NDR 9543, Nidhi, NK6320, NK-6355, NK-6355, NP- 124-8, NP- 218, NP- 3112, NP- 3112, NP-5151, NPG-209, NPG-210, NWGR 3132, OR 1895-2, OR 1946-2, OR 2163-14, OR 2172-7, OR 2324-2, OR 2328-5, OR 2331-14, OR 2336-1, OR 2405-KK-9, PA 6129, PA 6201, PAU 3105-45-3-2, PAU 3386-31-1-2-5, PAU 3761-26-3-1, PAU 3879-87-1-1, PNPH-24, PSB RC 18 (IR 31672-62-1-2-2-2-3), Purnendu, Pusa 1509-03-1-7-2, Pusa 1509-03-3-9-5, Pusa 1592-06-5-2, Pusa 1612-07-6-5, Pusa Sugand 5, R 1124-69-1-45-1, R 1138-688-3-533-1, R 1529-1183-1-1041-1, R 1532-1101-1-119-1, R 1535-1382-1-1667-1, R 1570-418-1-149-1, R1570-2649-1-1546-1, RAU 467-79-60, Rewa 1103, RGL 7001, RH09011, RH-1531, RNR 2354, RP 3644-1-19-5-5, RP 5125-2-4 (IR 84898-B-165-10), RP 5125-5-9-1 (IR 84898-B-171-19), RP 5127-9-3 (IR 93376-B-B-130), RP 5130-12-3-5-21-3, RP Bio 4918-2485, RTN 62-6-7-1, RTN 8-4-2-1-2, Sabita, SKL-32-70-15-10, Swarna, Swarna Dhan, SYE - 2-3-16-65-31-82, SYE-4-5-73-28-6-13, TRC 2008-1, TRC 2008-5, Triguna, UPR 3330-9-1-2, UPR 3411-1-1-1, UPR 3425-11-1-1, UPR 3426-3-1-1, Vikramarya, VNR-203, VRH-639, WGL 365, WGL 407, WGL-451, WGL-480, XR-99982
40-60	5	Moderately susceptible	25 P 25, C1446-5-18-17-2-MLD 2, CB 08-504, CN 1223-5-4-3-2, CN 1446-5-8-17-1-MLD 4, CR 2641-26-1-2-2, CR 2699, CR 2702, CR 2707, CR 2715-13-IR-84887-B-154, IR 36, JGL 17183, JGL 17196, KJT 1-11-15-23-26-22, KPH-371, NDR 1107, NK6303, NVSR-176, NVSR-178, ORS-327, Pusa Basmati, R 1570-2644-2-1547, R 2085-RF-69, R1566-2577-2-1530-1, RP 5124-11-6-2 (IR 83876-BF3 Bulk), RP 5130-136-5-5-33-5, TJP 48, TM 05091, UPR 3413-8-2-1, UPR 3425-14-3-1, US314 (H)
60-80	7	Susceptible	Dinesh, HR 12, IR 64, Kalanamak, Pusa 1121, Shabagi Dhan, Taraori Basmati
***Checks-Suraksha and TN 1**			

3.2. Biochemical analysis:

Damage from rice genotype screening against paddy stem borer and leaf folder ranges from 1.23% to 8.32% (Table 5)and from 6.82% to 45.82% (Table 6). Analyses of biochemical components such as total chlorophyll, total sugars, reducing sugars, phenols, proteins, and proline were conducted to look into the mechanisms influencing tolerance against these pests.

By examining the total chlorophyll content in a few resistant entries, the attraction-luring quality of greenness (which draws insects) was investigated for its potential function in paddy stem borer infestation (Table 5). The examined entries levels of chlorophyll varied significantly from one another. In comparison to the resistant kinds, the susceptible variety TN 1 exhibited a higher chlorophyll content, suggesting that a higher chlorophyll content draws insects for eating. Likewise, the leaf folder which is a leaf-eating pest, the significance of greenness in luring this insect was also investigated through the use of a susceptibility check and chlorophyll content analysis. According to the results, the resistant entries had lower quantities of chlorophyll (Table 6), whereas the susceptible variety TN 1 had 4.83 mg/g. This result is consistent with (Xu et al. 2010), which hypothesizes that leaf folder attractiveness is influenced by greenness.

The impact of total sugars, which aid in the survival and spread of the paddy stem borer, was examined. Table 5 shows that the susceptible variety TN 1 had substantially larger levels of total and reducing sugars than the resistant entries. The resistant entries total sugar content was considerably lower than that of the susceptible check TN 1 (129.86 mg/g), ranging from 11.53 to 51.90 mg/g. According to these findings, the total sugar concentrations of the sensitive TN 1 were higher than those of the resistant entries. In a similar vein, total sugars were determined in order to determine their effect on the leaf folder infestation. The total sugar content in the susceptible entry (TN 1) was higher than that of the resistant entries (129.86 mg/g), according to the results (Table 6).These results align with research by (Nanda et al. 2000), (Padhi., 2004), (Chandramani,et al. 2009), and (Dharshini et al. 2014) which found that the resistant control Ptb-33 had the lowest total sugar content and TN1 and Jaya had the highest. Nutrients, especially sugar and certain amino acids may act as potent sucking stimulants for stem borers.

To determine the effect of this component against the infestation of leaf folder and paddy stem borer, reducing sugars were also analyzed. The susceptible check TN 1 has 53.55 mg/g among the resistant entries against the paddy stem borer, while the resistant entries had values ranging from 19.22 to 47.31 mg/g (Table 5). Remarkably, UPR 3506-7-1-1, one of the resistant entries, contains more reducing sugars (56.02 mg/g) than the susceptible check TN 1, according to the results against the leaf folder pest. It implies that this tolerance might be brought on by UPR 3506-7-1-1's high phenolic content (15.66 mg/g) (Table 6). Similar results were reported by (Nanda et al. 2000), (Chandramani et al. 2009), and (Ashrith et al. 2020).

Table 5. Biochemical factors of selected rice genotypes showing differential reaction to paddy stem borer

S.No.	Accession	(%) white ear	Total chlorophyl l (mg/g)	Total sugars (mg/g)	Reducing sugars (mg/g)	Phenols (mg/100g)	Protein (mg/g)	Proline (ppm)
1.	OR 2324-8	1.23	2.21	51.90	47.31	12.76	12.85	24.95
2.	RTN 62-6-7-1	1.67	4.16	12.35	32.81	15.71	19.36	31.26
3.	R 1138-688-3-533-1	1.82	3.22	17.62	19.22	14.03	15.76	32.68
4.	CR 2698	1.94	2.72	53.02	34.93	12.49	20.21	52.72
5.	HUR-913	2.14	2.29	11.53	27.72	17.67	10.66	34.30
6.	TN-1	8.32	4.83	129.86	53.55	5.67	11.84	113.03
Mean	--	--	**3.24**	**46.051**	**35.93**	**13.05**	**15.11**	**48.15**
C.D (P=0.05)	--	--	**0.42**	**4.80**	**1.08**	**2.85**	**1.96**	**9.39**
C.V%	--	--	**7.06**	**5.73**	**1.66**	**12.01**	**7.15**	**10.72**

Higher concentrations of phenolic compounds confer tolerance on the plant, as these compounds form a barrier that keeps plant nutrients from being utilized by borer larvae (Kind PRN, 1954). As a result, the overall phenol content was calculated for both paddy stem borer and leaf folder. It was discovered that total phenols in paddy stem borer resistant entrieshad considerably greater values (Table 5) and lower in the susceptible entry (TN 1), confirming the conclusions of (Panda et al., 1975);(Padhi., 2004) and (Suchita et al.,2011). More phenolic compounds have been found in rice types resistant to sucking pests, according to several investigations (Pathak et. al., 1977; Grayer et. al., 1994). Most resistant and somewhat resistant cultivars experience increased phenolic production as a result of brown plant hopper (BPH) infestation, but the total phenol content is reduced in the BPH-sensitive variety, TN 1 (Loka Reddy et al. 2004). Additionally, this phenomenon has been noted in sorghum (Kalappanavar et al. 2000) and tomatoes (Sivaprakasam et al. 1996) are examples of other crops. Both susceptible checks and resistant landraces showed an increase in phenolic content following infestation, according to Dharshini et al. (2014) who also noted that the increase was injury-specific. Similarly, the larvae of leaf folders were more likely to attack entries with low phenol concentration. The reslts also suggested the same that susceptible entry TN 1 had the lowest total phenol concentration (5.67 mg/100g) and the resistant entries with the range spanning from 5.67 to 15.66 mg/100g (Table 6). These results are in line with those of (Rathika, 2008) and (Ashrith et al. 2020) in rice for leaf folder tolerance, which shows higher phenol levels in resistant entries, as well as those of (Loka Reddy et al.,2004) and (Chandramani et al.,2009) in brown plant hopper-affected leaves.

Since proteins are crucial for defense against insect pests, the total soluble protein in rice leaves was examined (Garcia Olmedo *et al.*,1987);(Ryan,1990) and(Lawrence et.al.,2002). The susceptible check had a protein concentration that was comparable to certain resistant types, despite the substantial range seen.In contrast to TN 1 (5.67 mg/100g), the entries OR 2324-8 and HUR-913, which had protein contents comparable to the susceptible check TN 1, however had greater phenol contents (12.49 and 15.71 mg/100g) (Table 5). This implies that the defensive mechanism in resistant entry was aided by a higher phenolic content. Similarly,the entries that were resistant to the leaf folder had higher total protein content, whereas susceptible entries had relatively lower levels. Table 6 shows that the protein level varied from 5.80 mg/g to 23.08 mg/g. The susceptible check TN 1 had a protein content of 11.84 mg/g, suggesting that the protein content did not affect tolerance against the leaf folder. These outcomes are in opposition to those of (Suchita et al. 2011), who discovered that vulnerable entries against mealybugs had a higher protein content.

Table 6. Biochemical factors of selected rice genotypes showing differential reaction to leaf folder.

S.No.	Accession	(%) Leaf Damage	Total chlorophyll (mg/g)	Total sugars (mg/g)	Reducing sugars (mg/g)	Phenols (mg/100g)	Protein (mg/g)	Proline (ppm)
1.	ARRH-3626	6.82	2.97	23.18	27.81	6.89	5.80	33.90
2.	OR 2324-8	6.94	2.21	51.90	47.31	12.76	12.85	24.95
3.	CR 2698	7.72	2.72	53.02	34.93	12.49	20.21	52.72
4.	UPR 3506-7-1-1	7.81	3.28	25.49	56.02	15.66	23.08	36.75
5.	HUBR 10-9	7.81	2.29	17.96	25.46	9.57	11.21	36.07
6.	R 1528-1058-1-110-1	7.93	3.09	38.62	27.20	10.49	11.62	47.72
7.	CN 1561-70-19-35-9-MLD 1	7.93	3.37	26.82	34.21	14.82	18.44	51.65
8.	TN-1	45.82	4.83	129.86	53.57	5.67	11.84	113.03
Mean	--	--	**3.09**	**45.86**	**38.31**	**10.77**	**14.38**	**49.59**
C.D (P=0.05)	--	--	**0.49**	**1.69**	**1.82**	**3.74**	**0.92**	**9.38**
C.V%	--	--	**9.08**	**2.10**	**2.72**	**19.83**	**3.6**	**10.80**

According to earlier research, proline regulates plant development, serves as a signalling molecule, and has regulatory properties (Laszlo Szabados et.al., 2004). Plants' programmed cell death may also be influenced by proline metabolism. Reactive oxygen species (ROS) signals in *Arabidopsis* cause an incompatible plant-pathogen response (HR), which is accompanied by local P5CS2 activation and proline buildup (Fabro G, 2004). By building up in plant tumors and functioning as a competitive antagonist of gamma-aminobutyric acid (GABA)-dependent plant defense by obstructing the GABA-induced degradation of quorum-sensing signals, proline has been proposed to modify plant defense responses to *Agrobacterium tumefaciens* (Haudecoeur, 2009). Reports on proline's function in disease or pest occurrence are scant or nonexistent. The proline content of rice entry was examined in order to look into the role of proline in pest damage (Table 5,6). The study revealed that the sensitive check TN 1 exhibited notably elevated quantities of proline in contrast to the resistant entry. This implies that heightened damage triggers the manufacture of proline, which might potentially function as a signal molecule within the plant's defensive mechanism. Additional research is required to validate this concept.

3.3 Correlation Analysis

A correlation analysis between percent infestation and various biochemical parameters revealed that total sugars (r = 0.88; n = 4; p > 0.01) had a positive correlation with percent infestationwhile phenol (r = -0.85; n = 4; p > 0.01) had a negative correlation. This suggests that genotypes with low total sugar content and high phenol content exhibited tolerance to these pests.

4. Conclusion

A field research was conducted for screening of 196 rice genotypes againstpaddy stem borer (*Scirpophaga incertulas*) and leaf folder (*Cnaphalocrocis medinalis*). A variety of crop stages, including the vegetative and reproductive phases (30 and 70 DAT), as well as the five growth stages (30, 40, 50, 60, and 70 DAT) for leaf folder tolerance, were evaluated for stem borer and leaf folder tolerance. The results indicated that entries that were resistant to the paddy stem borer during the vegetative stage did not show tolerance during the reproductive stage and leaf folder infestation is not consistent in all growth phases.In order to determine the causes of tolerance, further research was done on the biochemical factors. The results were significant and suggested that rice genotypes with high phenolic content, moderate levels of chlorophyll and low sugar content could be used in breeding programs to create resistant varieties against leaf folder and stem borer. These types might also be advised for areas where these pests are highly prevalent.

Disclaimer (Artificial intelligence)

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts.

Details of the AI usage are given below:

1. We agreethat we have used generative AI in a limited way, to clarify topic sentences and improve grammar, punctuation, and concision.

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