Understanding the Biochemical basis of resistance in rice to leaffolder (*Cnaphalocrocis medinalis)*

Abstract: Rice leaf folder has been recorded to cause major loss in rice yield**.** To know the relevance of biochemical factors in conferring tolerance to rice leaffolder *Cnaphalocrocis medinalis*, a study was conducted utilizing 196 rice accessions at Pandit Jawaharlal Nehru College of Agriculture and Research Institute, Karaikal. Standard evaluation system developed by IRRI for leaf folder complex was followed for screening the rice accessions and entries were ranked accordingly based on the leaf damage. Among these entries, top five entries along with the susceptible check (TN1) were selected for analysis of biochemical factors such as total chlorophyll, total sugars, reducing sugars, total phenols, total soluble protein and proline. Results revealed that high level of total phenols, moderate chlorophyll content, and low sugar content in leaves conferred resistance to this pest in rice.

Keywords: Total sugars, reducing sugars, phenol content, total soluble protein, proline

Introduction:

The rice leaffolder *Cnaphalocrocis medinalis* (Guenee) is one of the destructive insect pests of rice. It was considered as minor or sporadic pest in the past in many Asian countries. Due to this pest, loss in seed yield have been reported from 20 per cent at Tillering stage to as high as 50 per cent at Flowering stage (Manjunatha et al.2002, Padmavathi et al.2013). Extensive feeding results in reduction in photosynthetic ability, vigour and predisposes the leaves to bacterial and fungal infection (Sabir et al.2012). In certain cases it has been recorded to cause 63 to 80 per cent loss in rice yield (Rajendran *et al.*, 1986). Intensive and extensive cultivation of rice for maximization of yield and use of new strategies in agriculture, have led to complete changeover in the ecology of rice field and because of the changed agro ecosystem, some minor pests have attained major status.

The mechanism of resistance may be due to physical, biochemical or both combined factors. Accumulation of defense enzymes, chemicals and resistant proteins by insect feeding has been reported in many insect-plant interactions (Radja Commare et al, 2002). The plant strategy to deter feeding herbivore has become an important aspect of insect-plant interaction studies and is gaining tremendous importance. Among the plant chemicals, presence of increased or decreased amount of both the nutritional compounds and non nutritional secondary substances influences the resistance or susceptibility of plants to insects (Bharathi, 1996). According to Kogan and Paxton (1983), many changes that occur following herbivory result in accumulation of phenolic compounds. Also, the direct defense is known to reduce insect growth rates by interfering with the digestibility and nutritional quality of plant tissues (Johnson et al, 1989). To identify the resistance genotypes in rice to leaf folder complex, 196 entries were taken for conducting the field trial at Karaikal. Resistant entries were selected based on the screening procedure (IRRI standard evaluation procedure). To know the relevance of biochemical factors responsible for the resistance mechanism in these genotypes, biochemical analysis such as total chlorophyll, total sugars, reducing sugars, total phenols, total soluble protein and proline were carried out. Biochemical studies of rice varieties will be helpful in confirming the physiological antibiosis of the new rice germplasm. Feeding activities of herbivorous insects often result in physiological, morphological and chemical changes in the form of accumulation of the compounds having defensive properties. The biochemical factors are chemicals that affect insect behaviour, physiology and growth. Some biochemical factors are associated with repellence, feeding deterrence toxicity or adverse effects on insects (Saxena, 1986).

Materials and Methods:

Rice accessions have been received from the Indian Institute of Rice research (IIRR) Hyderabad with two susceptible check (TN 1). Standard evaluation system was followed for screening the rice accessions developed by IRRI for leaf folder complex such as *Cnaphalocrocis medinalis, Marasmia patnalis, M. ruralis* and *M. exigua.* Standing water was maintained continuous to a height of 2 to 5 cm throughout the crop season by irrigating the field on need basis. Fertilizers *viz.,* N: P_2O_5:K_2O were applied @ 120:60:60 kg/ha in the form of Urea, Diammonium phosphate (DAP) and Muriate of potash (MOP) respectively. Full doses of P_2O_5 and K_2O along with half dose of N were applied before transplanting as a basal application, while remaining half dose of N was applied in two equal splits at tillering and panicle initiation stages of the crop. Weeds were manually removed from experimental field to avoid crop-weed competition during crop period.

After screening the accessions, the resistant entries with least mean leaf folder damage were identified. Top five entries based on the ranking and a susceptible entry TN 1 was taken for biochemical analysis. To determine the biochemical factors responsible for imparting resistance in the promising genotypes, estimation of total chlorophyll, total sugars, reducing sugars, total phenols, protein and proline were carried out.

Estimation of biochemical factors:

The biochemical factors were estimated from the leaf samples. Total chlorophyll was estimated following Hiscox and Israelstam, 1979. For total and reducing sugars Nelson-Somogyi method was followed (Eric Fournier, 2001), while total phenol was estimated following Sadasivam and Manikkam (1996). For estimation of protein, Lowry’s method was followed (Lowry *et al.,* 1951) and proline was estimated employing Bates *et al.,* 1973.

Statistical analysis:

Data on biochemical parameters analyzed using AGRES software for its significance.

Results and Discussion:

Since leaf folder complex feeds on the leaves, to find out whether the intensity of greenness has a role on attracting the pest, total chlorophyll content was analyzed in the selected resistant entries along with a susceptible check. Results implied that susceptible entry TN 1 has 4.83 mg/g while the resistant entries having comparatively less amounts of chlorophyll (Table 1). Similar type of result was obtained by Xu *et al.,* 2010 against the leaf folder incidence. This result suggests that greenness has an influence in attracting leaf folder pest.

Table 1. Total chlorophyll of selected rice genotypes showing differential reaction to rice leaf folder

S.No.	Accession	(%) leaf damage	Total chlorophyll (mg/g)
1.	ARRH-3626	6.82	2.97
2.	OR 2324-8	6.94	2.21
3.	CR 2698	7.72	2.72
4.	UPR 3506-7-1-1	7.81	3.28
5.	HUBR 10-9	7.81	2.29
6.	R 1528-1058-1-110-1	7.93	3.09
7.	CN 1561-70-19-35-9-MLD 1	7.93	3.37
8.	TN-1	45.82	4.83
Mean	**--**	**--**	**3.09**
C.D (P=0.05)	**--**	**--**	**0.49**
C.V%	**--**	**--**	**9.08**

As leaf folder complex is a chewing pest, to know the role of sugars on the palatability of leaves, total reducing sugars was estimated from the leaves of resistant and susceptible entries. Significant variation was observed among the tested entries for total sugars. Total sugars were higher in the susceptible entry TN 1 (129.86 mg/g) than resistant ones (Table 2). These findings were in accordance with the Nanda *et al.*, 2000; Padhi, 2004; Chandramani *et al.,* 2009. Higher amounts of total sugars were reported in brown planthopper susceptible entries, Tellahamsa and Jaya (Sujatha *et al.,* 1987). *Sogatella furcifera* populations were positively correlated with the total sugars and amino acids (Rath *et al.,* 1999).

Amount of reducing sugars also influenced the incidence of pest. Amount of reducing sugars in resistant entries ranged from 27.20 to 56.02 mg/g and the susceptible check TN 1 has 53.57 mg/g. Among the resistant entries, UPR 3506-7-1-1 showed 56.02 mg/g which was higher than the susceptible check. The cause of resistance may be due to high phenolic content (15.66 mg/g) (Table 3).Similar results were reported by Nanda *et al.,* 2000; Padhi 2004, Chandramani *et al.,* 2009 and Ashrith *et al*., 2020.

Table 2. Total sugars of selected rice genotypes showing differential reaction to rice leaf folder

S.No.	**Accession**	**(%) leaf damage**	**Total sugars (mg/g)**
1.	ARRH-3626	6.82	23.18
2.	OR 2324-8	6.94	51.90
3.	CR 2698	7.72	53.02
4.	UPR 3506-7-1-1	7.81	25.49
5.	HUBR 10-9	7.81	17.96
6.	R 1528-1058-1-110-1	7.93	38.62
7.	CN 1561-70-19-35-9-MLD 1	7.93	26.82
8.	TN-1	45.82	129.86
Mean	**--**	**--**	**45.86**
C.D (P=0.05)	**--**	**--**	**1.69**
C.V%	**--**	**--**	**2.10**

Table 3. Reducing sugars of selected rice genotypes showing differential reaction to rice leaf folder

S.No.	**Accession**	**(%) leaf damage**	**Reducing sugars (mg/g)**
1.	ARRH-3626	6.82	27.81
2.	OR 2324-8	6.94	47.31
3.	CR 2698	7.72	34.93
4.	UPR 3506-7-1-1	7.81	56.02

5.	HUBR 10-9	7.81	25.46
6.	R 1528-1058-1-110-1	7.93	27.20
7.	CN 1561-70-19-35-9-MLD 1	7.93	34.21
8.	TN-1	45.82	53.57
Mean	--	--	**38.31**
C.D (P=0.05)	--	--	**1.82**
C.V%	--	--	**2.72**

Entries with low amount of phenols were prone to attack by leaf folder larva. Amount of total phenols ranged from 5.67 to 15.66 mg/100g. Susceptible entry TN 1 (5.67 mg/100g) has lesser amount of phenolic compounds (Table 4). These results were in concurrence with the findings of Loka Reddy *et al.,* (2004) and Chandramani *et al.,* (2009) in brown plant hopper affected leaves. Similar results were reported by Rathika (2008) and Ashrith *et al*. (2020) in rice for leaf folder indicating the presence of higher phenols in resistant entries.

Table 4. Total phenols of selected rice genotypes showing differential reaction to rice leaf folder

S.No.	Accession	(%) leaf damage	Phenols (mg/100g)
1.	ARRH-3626	6.82	6.89
2.	OR 2324-8	6.94	12.76
3.	CR 2698	7.72	12.49
4.	UPR 3506-7-1-1	7.81	15.66
5.	HUBR 10-9	7.81	9.57
6.	R 1528-1058-1-110-1	7.93	10.49
7.	CN 1561-70-19-35-9-MLD 1	7.93	14.82
8.	TN-1	45.82	5.67
Mean	--	--	**10.77**
C.D (P=0.05)	--	--	**3.74**
C.V%	--	--	**19.83**

Amount of total protein was higher in the entries which were resistant against the leaf folder while the susceptible entry had comparatively lesser amounts. Amount of total protein ranged from 5.80 mg/g to 23.08 mg/g (Table 5). Amount of protein in the susceptible check TN1 was 11.84 mg/g implying that protein content did not influenced the resistance against leaf folder. These results were in contrast to the findings of Suchita *et al.*, 2011 in cotton against Mealybugs that protein content is higher in susceptible entries.

Table 5. Total soluble protein of selected rice genotypes showing differential reaction to rice leaf folder

S.No.	Accession	(%) leaf damage	Protein (mg/g)
1.	ARRH-3626	6.82	5.80
2.	OR 2324-8	6.94	12.85
3.	CR 2698	7.72	20.21
4.	UPR 3506-7-1-1	7.81	23.08
5.	HUBR 10-9	7.81	11.21
6.	R 1528-1058-1-110-1	7.93	11.62
7.	CN 1561-70-19-35-9-MLD 1	7.93	18.44
8.	TN-1	45.82	11.84
Mean	**--**	**--**	**14.38**
C.D (P=0.05)	**--**	**--**	**0.92**
C.V%	**--**	**--**	**3.6**

The data from previous studies suggested that proline has regulatory function, controls plant development and act as signal molecules (Laszlo Szabados and Arnould Savoure, 2004). Proline metabolism can also influence programmed cell death in plants. In *Arabidiopsis*, incompatible plant-pathogen interactions trigger a hypersensitive response (HR) via reactive oxygen species (ROS) signals, which is accompanied by local activation of *P5CS2* and proline accumulation (Fabro, G. 2004). Proline was recently proposed to modulate the plant defence response to *Agrobacterium tumefaciens.* Proline accumulates in plant tumours, and functions as a competitive antagonist of gamma-aminobutyric (GABA)-dependent plant defence, interfering with the GABA-induced degradation of quorum-sensing signal (Haudecoeur, *et al.*, 2009).

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

S.No.	Accession	(%) leaf damage	Proline (ppm)
1.	ARRH-3626	6.82	33.90
2.	OR 2324-8	6.94	24.95
3.	CR 2698	7.72	52.72
4.	UPR 3506-7-1-1	7.81	36.75
5.	HUBR 10-9	7.81	36.07
6.	R 1528-1058-1-110-1	7.93	47.72
7.	CN 1561-70-19-35-9-MLD 1	7.93	51.65
8.	TN-1	45.82	113.03
Mean	**--**	**--**	**49.59**
C.D (P=0.05)	**--**	**--**	**9.38**
C.V%	**--**	**--**	**10.80**

Very few or nil reports are found for the role of proline against pathogen or pest incidence. In order to investigate the role of proline against pest damage, the proline content was analysed in the rice entries (Table 6). Interestingly the susceptible check TN 1 found to have significantly higher level of proline when compared to resistant entries implying the fact that more damage induce the synthesis of proline which may act as a signal molecule for plant defence mechanism. By further studies this may be proved.

Conclusion

By the present study, it is suggested that rice genotypes having high phenolic compounds, moderate chlorophyll content and lower sugar content could be utilized in the breeding programme for developing resistant varieties for leaf folder. Varieties with high phenolic compounds, moderate chlorophyll content and lower sugar content may be recommended for the areas with high leaf folder infestation in rice.

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