Biochemical basis of resistance in rice to leaf folder complex

Abstract: To identify the resistant entries against rice leaf folder complex (*Cnaphalocrocis medinalis, Marasmia patnalis, Marasmia ruralis* and *Marasmia exigua)*, 196 rice accessions were field evaluated at Pandit Jawaharlal Nehru College of Agriculture and Research Institute, Karaikal. The biochemical factors such as total chlorophyll, total sugars, reducing sugars, total phenols, total soluble protein and proline were assessed for the entries that showed resistance against the leaf folder complex along with a susceptible check TN1. A high level of total phenols, moderate chlorophyll content, and low sugar content were identified as factors that conferred resistance to this pest.

Keywords: Rice leaf folder complex, field evaluated, biochemical factors and resistance.

Introduction:

The rice leaf folder complex previously considered as a minor pest, has gained major pest status and caused considerable yield loss (Khan *et al.*, 1999; Singh *et al.*, 2003). In certain cases it has been recorded to cause 63 to 80 per cent loss in rice yield (Rajendran *et al.*, 1986).The use of more nitrogenous fertilizers and indiscriminate use of insecticides have been attributed as the causes of this minor pest gaining major pest status (Dhaliwal *et al.*, 1979). 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. 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.

Materials and Methods:

Rice accessions have been received from the Directorate of Rice Research (DRR), Hyderabad with two controls (TN 1 and Suraksha). 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. 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.

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.

So 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 s leaf folder.

Reference

Ashrith, K.N., Kalleshwaraswamy, C.M., Dushyantha kumar, B.M. and Dhananjaya, B. 2020. Morphological and biochemical basis of resistance in rice genotypes against two major lepidopteran pests. *Indian Journal of Entomology*. 82(4). DoI No.: 10.5958/0974-8172.2020.00158.3.

Bates, L.S., R.P. Waldran and I.D. Teare. 1973. Rapid determination of free proline for water stress studies. *Plant Soil*. 39:205-208.

Chandramani, P., Rajendran, R., Sivasubramanian. P. and Muthiah, C. 2009. Management of hoppers in rice through host nutrition- A novel approach. *Journal of Biopesticides*. 2(1):99-106.

Dhaliwal, G.S., Shaki, H. N., Gill, P.S and Maskina, M.S. 1979. Field reaction of rice varieties to leaf folder at various nitrogen levels. *International Rice Research Newsletter*. 4(3):7.

Eric Fournier. 2001. Current Protocols in Food Analytical Chemistry E1.1.1-E1.1.8.

Fabro, G. 2004. Proline accumulation and AtP5CS2 gene activation are induced by plant-pathogen incompatible interactions in *Arabidopsis*. *Molecular Plant-Microbe Interactions*. 17:343-350.

Garcia-Olmedo, F., G. Salcedo, R. Sanchez-Monge, L. Gomez, J. Royo, and P. Carbonero, 1987. *Oxford Surveys of Plant Molecular and Cell Biology*. 4:275-334.

Haudecoeur, E. (2009) Proline antagonizes GABA-induced quenching of quorum sensing in *Agrobacterium tumefaciens*. *Proceedings of the National Academy of Sciences of the United States of America.* 106: 14587-14592.

Hiscox, J.D. and G.F. Israelstam. 1979. A method for the extraction of chlorophyll from leaf tissues without maceration. *Canadian Journal of Botany***.** 57:1332-1334.

Khan, Z. H., S. L. Gupta, V. V. Ramamurthy and Debjani Dey. 1999. Biodiversity inventory of lepidopterous insects associated with rice agroecosystem. *Shashpa*. Special Issue No.2:107.

Laszlo Szabados and Arnould Savoure. 2004. Proline: a multifunctional amino acid. *Trends in Plant Science*. 15(2): 89-97.

Loka Reddy, K., Pasalu, I. C., Sreenivasa Raju, A. and Reddy, D. D. R. 2004. Biochemical basis of resistance in rice cultivars to brown plant hopper *Nilaparvata lugens* (Stal.). *Journal of the Entomological Research.* 28(1):79-85.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. 1951.J.Biol.Chem., (193) : 265 (The original method). *N. lugens. Indian Journal of Entomology*. 62:239-241.

Nanda, U. K., Dash, D. and Rath, L. K. 2000. Biochemical basis of resistance in rice to BPH.

Padhi, G. 2004. Biochemical basis of resistance in rice to yellow stem borer, *Scirpophaga incertulas* Wlk. *Madras Agricultural Journal*. 91(4-6):253-256.

Rajendran, R., B. Rajendran and P.C. Sundrababu, 1986.Varietal resistance of rice to leaffolder. *International Rice Research Newsletter*. 11(4):17-18.

Rath, L. K., Mishra, D. S. and Panda, SK. 1999. Effect of certain micronutrient fertilizers on the biochemical changes in rice and their correlation with *Sogatella furcifera* Howrath. *Annals of plant Protection Sciences*. 7: 8-12.

Rathika, M. 2008. Studies on the tritrophic interaction of rice leaf folder. M.Sc. Thesis.Tamil Nadu Agricultural University, Coimbatore.

Sadasivam, S and Manikkam, A. 1996.Biochemical methods. 2nd Edition, New Age International (P) Limited Publishers .20-21 & 193-194.

Singh, H. M., R. K. Srivastava, S. M. A. Rizvi, F. A. Elazegui, N. P. Castilla and S. Savary.2003. Yield reduction due to brown spot and leaf folder injuries at varying levels of fertilizers and water supply to rice crop. *Annals of plant Protection Sciences*. 11: 16-19.

Suchita, R. G., Barkhade, U. P., Moharil, M. P. and Ugale, T.B. 2011. Biochemical basis of resistance to cotton cultivars against mealy bugs (*Phenococcus solenopsis* Tinsley). *Crop Research*. 42 (1, 2 & 3): 320-323.

Sujatha, G., Reddy, G. P. V. and Murthy, M. M. K. 1987.Effects of certain biochemical factors on the expression of resistance of rice varieties to brown planthopper (*Nilaparvata lugens* Stal.). *Journal of Research APAU*. 15: 124-128.

Xu, J., Qi-Xiang, W and Jin-Cai, W. 2010. Resistance of cultivated rice varieties to *Cnaphalocrocis medinalis* (Lepidoptera: Pyralidae). *Journal of Economic Entomology*. 103(4): 1166-1171.