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Hypersensitive response and induced resistance in rice gene differentials against biotype 1 of Asian rice gall midge, *Orseolia oryzae* at Mandya, Karnataka

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Abstract

Hypersensitive reaction (HR) and induced resistance were noticed in resistant rice genotypes infested by gall midge. Detailed observations on Phalguna (*Gm2* gene), Abhaya (*Gm4* gene), ARC 5984 (*Gm5* gene) infested with gall midge biotype 1 revealed that the infestation triggered HR in the plant, leading to extensive tissue necrosis at the apical meristem and browning of central leaf. This was followed by maggot mortality and premature tillering. In susceptible genotypes this phenomenon was not evident. HR leading to necrosis is fatal to host plant but premature tillering was observed. Further, the secondary tillers were infested subsequently with the gall midge biotype 1 eggs at 7, 14, 21 and 28 days after primary infestation, and maggots failed to establish and cause silver shoot. However, HR was observed 6 days after secondary tiller infestation, when the primary tillers were infested 28 days after. But cent per cent maggot mortality was observed, regardless of the time interval between infesting primary and secondary tillers in all the HR + plants. Thus, the HR is not confined to the tillers of primary infestation but it also triggers systemic acquired resistance in other tillers in Phalguna, Abhaya and ARC 5984, whereas, in W1263 (*Gm1* gene), HR+ was not evident but antibiotic effects were observed along with maggot mortality.

Key words: *Orseolia oryzae*, rice genotypes, hypersensitivity, induced resistance.

The Asian rice gall midge, *Orseolia oryzae* (Wood-Mason) (Diptera: Cecidomyiidae) is a major insect pest of rice in several Asian countries (Bentur *et al.,* 2003). In India, gall midge has been reported from almost all the rice growing states except the Western Uttar Pradesh, Uttaranchal, Punjab, Haryana and Hill states of Himachal Pradesh and Jammu and Kashmir (Bentur *et al.,* 1992). The insect being endoparasitic, use of resistant varieties is the most economical and feasible tool for its control (Heinrichs and Pathak, 1981; Mathur *et al*., 1999; Khush, 1997). But the emergence of new virulent biotypes of gall midge in popular rice varieties is capable of overcoming resistance and this is a cause for concern. So far 7 biotypes of gall midge were identified and characterized in India (Vijayalakshmi, 2006). Widespread cultivation of high yielding varieties made a radical change in the pest status of rice gall midge in coastal Karnataka.

Early studies tried to correlate morphological difference in attributes such as color and hairiness of the leaf or compactness of the leaf sheath with resistance (Rao *et al.,* 1971). Similarly, the density and length of trichomes were negatively correlated with gall midge incidence (Joshi, 1982; Devaiah, 1984) while tillering pattern, leaf sheath compactness and inter space had

no influence. Soon it was noted that resistant varieties offered no mechanical barrier since maggots reached the apical meristem in all the varieties (Shastry *et al.,* 1972; Sain 1988). No distinct oviposition preferences were noted among resistant and susceptible varieties (Hidaka, 1974; Kalode, 1980; Kalode *et al.,* 1983; Sain and Kalode, 1994). Hidaka and Vungsilabutr (1971) observed failure of moulting of first instar maggot in W1263. A predominant antibiosis component leading to mortality of first instar larvae has been also observed by many workers (Pathak and Heinrichs, 1982; Mathur and Rajamani, 1984). Since the beginning of 19th century, hypersensitivity has been recognized as an important defense mechanism (Fernandes, 1990) and is usually controlled by an individual gene or, rarely by a few genes. Detailed observations on rice variety Phalguna, a derivative of Siam 29 with *Gm2* gene for resistance, and gall midge biotype 1 (avirulent) and biotype 4 (virulent) revealed that infestation with the former biotype leads to premature tillering and triggers a hyper sensitive reaction (HR) within 5 days (Bentur and Kalode, 1996). So, a study on the hypersensitivity and induced resistance in 6 rice genotypes against local gall midge biotype 1 (Vijaykumar *et al.,* 2008) was initiated in 2020.

Gall midge culture: Fertile soil was collected from the field and fertilizers were mixed thoroughly. Twice in a week, seeds of susceptible variety TN 1 were soaked for germination and were sown in the plastic pots of 8 diameter and 10-inch height, 2 days later at the rate of 50-75 seeds/ pot. The potted plants were kept in the greenhouse with adequate light for 10-15 days after sowing. Eight potted plants were kept inside the oviposition cage covered by polythene cover. During evening 7.00 to 10.00 p.m. the adults of rice gall midge were collected near light source using aspirator developed by ICAR-IIRR (formerly Directorate of Rice Research (DRR)), Hyderabad and were released inside the oviposition cage for infestation. Twenty-five females and 25 males were released inside the oviposition cage containing 8-10 potted plants of 15-20 days old seedlings. Two cages were daily infested for routing rearing during the study period. Adults were provided with fresh 15-20 days old potted plants daily for oviposition. Two days after adult release, the potted plants were sprayed with water periodically at 2-3 h intervals to moisten the plants for egg hatching and for better movement of newly hatched maggot to reach the apical meristem region for better establishment and development. The potted plants were transferred to shallow water tray and water level of 2-3 cm above the basal part of the plant was maintained to create optimum humidity and to prevent predation of maggot. After gall formation, the potted plants were shifted from water tray to the adult emergence cage. The adults were collected every morning between 6.00 to 9.00 AM carefully with an aspirator. Then the collected adults were used for varietal screening, to study the mechanism of resistance and also for routine culture maintenance.

Hypersensitive reaction and Induced resistance: The studies on HR and induced resistance in gall midge resistant genotypes *viz.,* W 1263 (*Gm1* gene for resistance), Phalguna (*Gm2*), Abhaya (*Gm4*), ARC 5984 (*Gm5*), Jaya and TN 1 were undertaken at V.C. Farm, Mandya following Bentur and Kalode (1996). The local Asian rice gall midge biotype 1 population cultures were collected from the field under light source in the evening and maintained in the greenhouse.

Seedlings of W 1263 (*Gm1* gene for resistance), Phalguna (*Gm2*), Abhaya (*Gm4*), ARC 5984 (*Gm5*), Jaya and TN 1 were raised separately in plastic pots (10x8 inch) containing 5 hills groups of 2 seedlings. Such 10 seedlings in a pot represent replication and such 5 replications were maintained. When the seedlings attained 10-12 days, they were artificially infested with two fertile eggs of biotype 1 and an uninfested TN1 was maintained as control. Such 3 sets were maintained. The infested plants were observed for secondary tillers at 1, 7, 14 and 21 days after infestation and total numbers of tillers in each plant were recorded in first set. In second set, the infested plants were dissected to note HR and maggot mortality. In third set, the plants were infested with 2 fertile eggs of gall midge and the observations on total numbers of tillers in each genotypes at 1, 7, 14 and 21 days after infestation was taken.

Artificial infestation: Mated females were collected from the stock culture individually and held overnight for oviposition in air-tight plastic cups lined with moist filter paper at 25-28^{0}C. Fertile eggs could be observed after 3 days. Before maggot hatching, the gall midge eggs were collected using ordinary syringe along with tiny tissue paper under binocular microscope (Nikon SMZ 800N). the collected egg was placed between the leaf sheaths of central shoot. Such infested pots were carefully maintained till the establishment of maggots and gall. For 2 to 3 days the water was sprayed at 2 h intervals using hand atomizers to create high relative humidity for egg hutching and larval establishment. Egg hatching was verified by retrieving the filter paper bit one day after infestation and observing the empty eggshell. Plants were successfully infested using this technique with a fertile egg (Bentur and Kalode 1996) (**Figure 1).**

Figure 1: Artificial infestation of rice gall midge eggs for studying hypersensitive reaction

The observations on HR, maggot mortality and tiller bearing capacity were recorded on Phalguna, Abhaya and ARC5984. The presence of necrotic tissue in one of the tillers of the plant was considered enough to classify it as hypersensitive reaction (HR) and plants with two dead larvae in primary tiller was considered to show antibiosis. The plants were observed for HR reaction and maggot mortality at 2, 3, 4, 5 and 6 days after infestation. In third set, the tillers were infested with 2 fertile eggs of gall midge biotype 1 and the observations on total numbers of tillers in each genotypes at 1, 7, 14 and 21 days after infestation was taken. Further, in each genotype, the secondary tillers of Phalguna, Abhaya and ARC5984 were again infested by two more fertile eggs of gall midge biotype 1, at 7, 14, 21 and 28 days after primary tiller infestation. In each week of secondary infestation, the observations on HR and living larvae was taken (7, 14, 21 and 28) at 3, 4, 5, 6 and 7 days after secondary tiller infestation on 50 plants. Further, the collected data were subjected to Analysis of Variance (ANOVA) and means were separated by Tukey's HSD test (Tukey, 1953) for interpretation.

The resistant donors, Phalguna, Abhaya and ARC 5984 showed varying degrees of central leaf browning, including at times death of the entire leaf of young seedlings. The dissection of the apical region of the seedlings revealed extensive necrosis of meristem tissue. Dead first instar larvae were observed at the vicinity of the necrotic tissue. This response resembled a typical hypersensitive reaction (HR) (Fernandes, 1990) and this was observed in Phalguna, Abhaya and ARC 5984 resistant donors. In all the three resistant genotypes (Phalguna, Abhaya and ARC 5984) at 3, 4, 5, 6 and 7 days after secondary tiller infestation in each week after primary infestation, there was no expression of hypersensitive reaction but 100 cent per cent maggot mortality was observed. In W 1263 genotypes this phenomenon (HR) was not observed but antibiotic effects were seen with maggot mortality, while in susceptible check Jaya and TN 1 the insect completed life cycle successfully.

Studies indicated both extensive tissue necrosis at the apical meristem of the young seedlings, succeeded by maggot mortality and premature tillering. There was no significant difference among the resistant and susceptible donors with respect to number of tillers after a day of infestation. But, at 7 days after infestation, pre mature tillering was observed in all the resistant donors, except W1263. Significantly higher number of tillers were observed in Phalguna (1.42±0.20) followed by Abhaya (1.38±0.10) and ARC 5984 (1.34±0.00), while, it was not observed on susceptible TN 1, Jaya and also un-infested TN 1. Similar results were observed at 14 and 21 days after infestation indicating significant level of premature tillering on these resistant donors (**Table 1**).

Table 1. Effect of gall midge infestation on tiller bearing capacity in resistant and susceptible rice genotypes

Genotypes	No. tested*	Number of tillers/plant at DAI			
		1	7	14	21
Phalguna (*Gm2*)	50	1.00±0.00[a]	1.42±0.20[a]	1.72±0.40[a]	2.41±0.12[a]
Abhaya (*Gm4*)	50	1.00±0.00[a]	1.38±0.10[b]	1.64±0.20[b]	2.09±0.10[b]
ARC 5984 (*Gm5*)	50	1.00±0.00[a]	1.34±0.00[c]	1.61±0.24[b]	1.98±0.20[b]
Jaya (S)	50	1.00±0.00[a]	1.00±0.00[d]	1.00±0.00[c]	1.10±0.22[c]
TN 1 (S)	50	1.00±0.00[a]	1.00±0.00[d]	1.00±0.00[c]	1.20±0.20[c]
TN 1 (un-infested)	50	1.00±0.00[a]	1.00±0.00[d]	1.00±0.00[c]	1.00±0.00[c]

DAI-Days after infestation; *-@ 2eggs/seedling; *Gm2*, *Gm4*, *Gm5* are genes for resistance; S-Susceptible; Means in a column followed by different letter are significantly different as per Tukey's HSD (Tukey, 1953).

The detailed studies on HR and expression of maggot mortality in relation to gall midge infestation in Phalguna indicated that the HR expression started on third day of infestation (8.33 %) and 75 per cent of the plants infested with gall midge showed HR on the fifth day, and 100 per cent larval mortality within the same period (fifth day) (**Figure 2)**. The necrosis of the young apical meristem of the seedling when infested with gall midge is fatal to the plant (host plant) for further growth. The premature tillering was observed in resistant donor Phalguna after tissue necrosis. Further, the secondary tillers were again infested with the gall midge biotype 1 eggs at 7, 14, 21 and 28 days after primary infestation. However, HR was observed 6 days after secondary tiller infestation, when secondary tiller infestation followed by primary

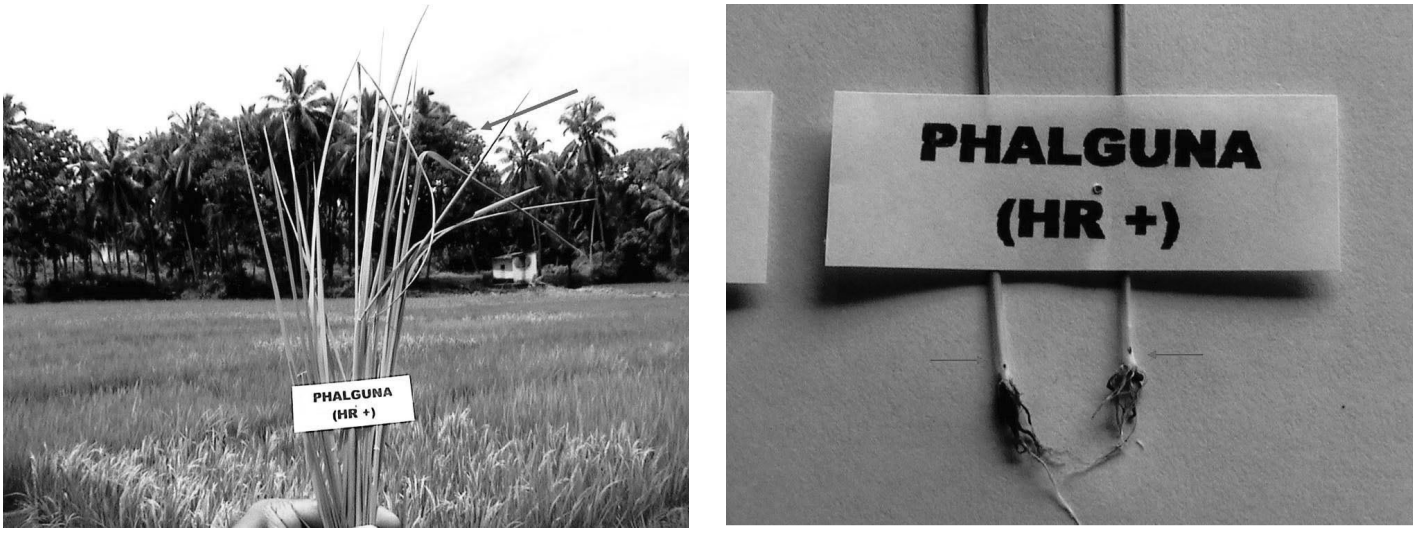

Figure 2: Expression of hypersensitive reaction (HR +) in Phalguna (*Gm2 gene*) in response to gall midge infestation

tiller infestation by 28 days. But cent per cent maggot mortality was observed, regardless of the time interval between infesting primary and secondary tillers. Mortality in secondary tillers without the expression of HR suggested that HR is not a prerequisite for maggot mortality. Further, a systemic induced resistance by primary infestation was evident which probably prevented other tillers from expressing HR. Thus, this study indicated that the HR was confined to the tillers of primary infestation and it also triggered systemic acquired resistance in other tillers (secondary tillers), and the duration and stability of the systemic acquired resistance in secondary tillers needs to be studied.

Despite many examples of HR of plants against pathogens such as fungi (De Wilt, 1992), bacteria (Jakobek *et al.,* 1993), and viruses (Ponz and Brueing, 1986; Zaitlin and Hull, 1987), there are only few examples of HR having any importance against insect herbivores (Fernandes, 1990). The known examples of plant hypersensitivity against insect herbivores come from gall forming aphids and tephritid flies (Anderson *et al.,* 1989). The present study may be the third instance of cecidomyiid gall formers after the reports of Bentur and Kalode (1990 and 1996). Tissue necrosis has also been described in wheat cultivars following attack by avirulent biotypes of the Hessian fly, *Mayetiola destructor* Say (Shukle *et al.,* 1992). Systemic acquired resistance is very well documented in pathogen-plant interactions (Bell, 1981). Necrosis of the apical meristem in all of the infested tillers would be fatal to the host plant. Hence, systematic acquired resistance rendering all other tillers resistant without necrosis has distinct survival value for the host plant. In pathogen-plant interactions the phenomenon of hypersensitivity is noted only against race specific defense and not in pathogen and non-host plant interactions (Bell, 1981). Likewise, the weed gall midge *Oreseolia fluvialis,* which can survive on *Paspalidium geminatum* and *Echinochloa crusgalli* but not on rice (Sain, 1988), did not elicit HR either in Phalguna or TN1 varieties of rice (Bentur and Kalode, 1996). Genetic diversity with (Phalguna, Abhaya and ARC 5984) or without (W1263) the expression of HR against rice gall midge was noted in the present study. Such diversity is known against pathogen (Dixon and Lamb, 1990).

These results on the HR and induced resistance by artificial infestation of biotype 1 indicated varied degrees of central leaf browning in Phalguna, Abhaya and ARC 5984 resistant genotypes. The dissection of the apical region of these seedlings revealed extensive necrosis of meristematic tissue. This response resembled a typical HR (Fernandes, 1990; Bentur and Kalode, 1990, 1996). While in W1263, this phenomenon was not observed but antibiotic effect was seen with maggot mortality. These results corroborate with the study made by Bentur and Kalode (1996). The detailed studies on HR in Phalguna, Abhaya and ARC 5984 indicated both extensive tissue necrosis at the apical meristem of the young seedlings, succeeded by maggot mortality and premature tillering. Significantly higher number of tillers was noticed in Phalguna followed by Abhaya and ARC 5984 at 7, 14 and 21 days after infestation compared to susceptible TN1 and un-infested TN1. Further studies indicated that the HR expression started on third day after infestation (8.33%) and 75 per cent of the infested plants showed HR on the fifth day along with 100 per cent maggot mortality in Phalguna (**Figure 3)**.

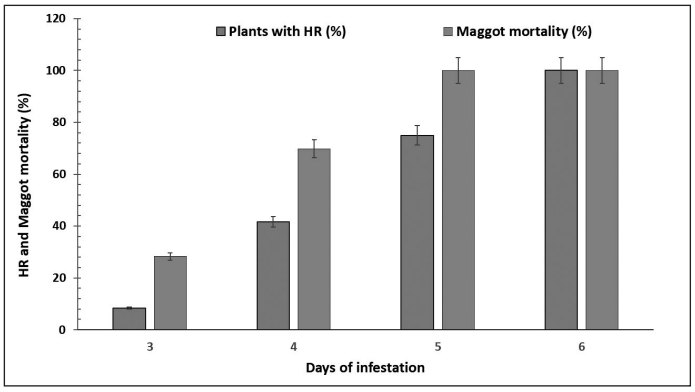

Figure 3: Hypersensitive reaction expression and maggot mortality in primary tillers of var Phalguna in response to gall midge infestation

Bentur and Kalode (1990, 1996) observed plants with cent per cent HR, results in maggot mortality on 5th day after infestation in Phalguna. But premature tillering was observed in resistant donors Phalguna, Abhaya and ARC 5984 subsequent to the tissue necrosis. Further studies, by infesting secondary tillers with gall midge eggs at 7, 14, 21 and 28 days after primary

infestation indicated 100 per cent maggot mortality, regardless of the time interval between infesting primary and secondary tillers.

Thus, the mortality in secondary tillers without the expression of HR suggested that HR is not a prerequisite for maggot mortality as reported by Bentur and Kalode (1996). Furthermore; a systemic induced resistance by primary infestation was evident which probably prevented other tillers (secondary tillers) from expressing HR. The present study revealed that the HR was confined to the tillers of primary infestation and it also triggered systemic acquired resistance in other tillers. Identification, field evaluation and utilization of such potential genotypes in rice breeding will lead to the suppression of virulent gall midge populations and stabilize the yields.

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