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A progressive change in the virulence spectrum of Asian rice gall midge (*Orseolia oryzae*) biotype 2 after a decade in Coastal Karnataka, India

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Abstract

Virulence composition of traditionally designated biotype 2 field population of Asian rice gall midge, *Orseolia oryzae* (Wood-Mason) (Cecidomyiidae: Diptera) was conducted a decade after in 2019 and 2020 at coastal Karnataka, India using three standard differentials *viz*., W1263 (*Gm1* gene for resistance), Phalguna (*Gm2* gene for resistance) and TN1 (susceptible without any gene). The local population of gall midge was virulent against all 16 standard rice gene differentials representing four groups identified to characterize the prevailing rice gall midge biotypes in India. The local gall midge populations in the test locations expressed their virulence against all three rice gene differentials with varied female to male sex ratio of their F_1 progenies. This confirms the prevalence of genetically heterogeneous population in coastal regions of Karnataka. Clearly, a progressive change in the virulence spectrum of local gall midge biotype 2 was noticed a decade after observations. In south coast, 73.33 to 87.27% population showed virulent attributes of traditional biotype 2 designated in 1989. Whereas in north coast, 79.69 to 86.36% population exhibited virulence attributes towards new biotype 3 for the first time in the state of Karnataka, India. These results suggested a progressive change in the traditionally designated population of biotype 2 capable of damaging resistant varieties in the region for over three decades. Further, the single female test for their F_1 progenies in all endemic locations indicated an evolution of new biotype of rice gall midge in the region.

Introduction

The Asian rice gall midge, *Orseolia oryzae* (Wood-Mason) (Cecidomyiidae: Diptera) is a serious threat for rice production in entire Asia (Singh *et al.,* 2004). In India, the infestation of rice gall midge has been reported since from the beginning of 19th century with geographically distinct population across the regions (Israel *et al.,* 1959; Mathur & Rajamani, 1984). Majority of the rice growing region in India have suffered substantial grain loss due to the repeated outbreaks of rice gall midge especially in wet seasons. However, the infestation level was not above the economic threshold level in north Indian states (Bentur *et al.,* 1992). The estimated yield loss by the rice gall midge in southern, central and

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the coastal states of India was in the range of 10-100 per cent (Siddiq, 1991). In India alone annual loss caused by rice gall midge was valued to the tuned of US dollars 80 million (Ramaswamy & Jetileksono, 1996; Bentur *et al.*, 2003). Before 1960s the pest intensity and damage were restricted to only coastal rice fields of India, but the introduction of high yielding varieties and their extensive cultivation has led the rice gall midge to become more virulent at different cultivation ecologies (Vijaykumar, 2007).

The adult rice gall midge is small delicate resembles like mosquito with long legs and pinkish red abdomen. The endoparasitic maggot is a pinkish red in color with well developed breastbone for crawling in the thoracic region. The visible sign of damage produce by the maggot of rice gall midge is changing the newly emerging leaf sheath of rice plant into an onion leaf like tubular silvery white elongated galls. This symptom is caused by maggot because of scraping and feeding on apical primordial tissue as an endoparasite and further by mixing the salivary secretions which is rich in indole acetic acid (Hatchett *et al.,* 1990). Such infected tillers convert into a sterile elongated silvery white gall without any productive rice panicle (Vijaykumar, 2007). Since the gall midge maggot attack and feed on apical primordial tissue of newly emerging rice tillers, its management through conventional agronomical practices or cultural practices and even through application of insecticides is very difficult (Vijaykumar *et al.,* 2012). Hence, most of the researchers thought of resistance breeding as one of the important, viable, eco-friendly and ecologically feasible method for its management (Heinrichs & Pathak, 1981; Nair & Devi, 1994; Khush, 1997; Mathur *et al.,* 1999). Hence, all India coordinated rice improvement program, Indian council of agricultural research, Government of India has initiated a special germplasm collection program between 1950 and 1970 for resistance against Indian population of rice gall midge and screened for resistance across the country. As a result, 56 biotype specific gall midge resistant rice varieties with specific gene for resistance have been commercially released for cultivation in gall midge endemic locations (Bentur *et al.,* 2003). These resistance varieties have occupied wide cultivable area between 1987 to 2008 in majority of the gall midge endemic locations over two decades. However, after widespread cultivation of these resistant varieties over the years, a genetically heterogeneous infectious virulent population called biotypes started evolving in the entire gall midge endemic states of India (Bentur *et al.,* 1987; Nair & Devi, 1994; Reddy *et al.,* 1997; Vijaykumar *et al.,* 2008a). But the evolution of new virulent population of rice gall midge in the form of infectious biotype in popular varieties capable of break downing resistance became the main concern and threat for rice production. After addressing the status and virulence attributes of heterogeneous population of gall midge, six new biotypes of rice gall midge have been identified and characterized through the reaction of rice standard differentials with biotype specific gene for resistance (Kalode & Bentur, 1989; Bentur *et al.,* 2003)

Karnataka is one of the important rice-producing state in India. The occurrence of gall midge on rice was first reported in 1927, but more importance was on this pest was given during 1980s by all India coordinated rice improvement project. As a result, the biotype 2 was identified in coastal parts of Karnataka (Kalode & Bentur, 1989). Since, from the beginning of the 20th century, the gall midge became major threat to rice production in entire coastal Karnataka. The maximum numbers of gall midge outbreaks in India were also reported from coastal region of Karnataka (Pasalu & Rajamani, 1996) where, yield loss due to gall midge infestation varied from 25-48 per cent (Parameshwar *et al.,* 1995; Vijaykumar *et al.,* 2008b, 2012). To know the virulence pattern of prevailing gall midge biotype population in coastal Karnataka, an extensive study was initiated between 2004 to 2009, as a result the local population of biotypes were characterized as genetically heterogeneous mixture (Vijaykumar, 2007).

In view of the complexity and dynamic rapid changes in the pest status and virulence attributes of prevailing rice gall midge biotype population in endemic and new locations of Karnataka, not only rural poor rice farmers but also entomologists, breeders and policy makers were at bay in tackling the problem posed by the rice gall midge in these regions. The reports from different major rice growing parts of the country suggested that gall midge resistant rice genotypes breaking down the resistance due to extensive cultivation of more than one resistant rice varieties having different genes for resistance leading to the evolution of new virulent biotypes in India (Bentur *et al.,* 2003). This situation has exacerbated the rural poverty-stricken rice farmers of coastal region of Karnataka affecting their livelihood and socio-economic conditions. Since 2009, nearly 35 to 90 per cent infestation of rice gall midge was reported in several new locations of coastal Karnataka in both nursery and main field (Vijaykumar *et al.,* 2012). Keeping this in view, and also to know the present status of virulent gall midge population after a decade, the present investigation was undertaken at rice gall midge endemic and new locations in coastal regions of Karnataka.

Materials and Methods

Evaluation of standard rice gene differentials

The present study on identification of virulent rice gall midge biotypes prevailing in coastal Karnataka (Figure 1a) was initiated in the wet seasons of 2019 and 2020, after a decade in 2009. Sixteen standard set of rice gene differentials having known gene for resistance under 4 groups to detect and characterize the prevailing gall midge biotypes in India developed by Indian institute of rice research, Hyderabad was obtained and sown in the test locations. The seedlings of rice gene differentials with 20-25 d old were planted in 4 rows representing 20 hills following the standard spacing of 20×15 cm between plants and rows, respectively. At 50 d after transplanting, the observations on plant damage on hill basis and number of healthy and infested tillers with white elongated galls (silver shoots) were recorded on 20 hills of each differential (Figure 1b-e).

All 16 standard rice differentials representing 4 groups were scored as resistance (R) with plant damage of less than 10 per cent and as susceptible (S) with more than 10% plant damage (Kalode & Bentur, 1989). On the basis of susceptibility or resistance pattern, the prevailing biotype in respective location were identified as biotype 1(R-R-R-S), biotype 2 (S-R-R-S), biotype 3 (R-S-R-S), biotype 4 (S-S-R-S), biotype 5 (R-R-S-S) and biotype 6 (R-S-S-S) (Kalode & Bentur, 1989; DRR, 1998). Likewise, at 50 d after transplanting, the per cent damage in the form of silver shoot was recorded and processed using standard evaluation system for rice developed by International Rice Research Institute (IRRI), Los Banos, Philippines (IRRI, 2016). Due to continuous cultivation of gall midge resistance varieties such as Phalguna, Triguna, MO-4 in entire coastal region of Karnataka, most of the resistance rice varieties released for commercial cultivation became susceptible. Hence, the prevailing local virulent gall midge population were monitored during 2009, 2019 and in 2020 with 3 standard rice gene differentials with respective resistance gene *viz*., W1263 (*Gm1*) (Reddy *et al.* 1997), Phalguna (*Gm2*) (Mohan *et al.* 1994) and TN1 (no gene) were sown in a plastic screening box (42×30×8 cm).

Quantification of virulence pattern in local biotypes

To quantify the composition of gall midge population in terms of virulence spectrum in new locations of southern and northern coastal Karnataka (Figure 2), the populations were monitored in the wet season of 2020 using three standard rice differentials *viz*., W1263 with *Gm1* gene for resistance (Reddy *et al.,* 1997), Phalguna with *Gm2* gene for resistance (Mohan *et al.,* 1994) and susceptible check TN1 (no gene for resistance) received from Indian institute of rice research, Hyderabad. The seeds of differentials were sown separately in plastic tray (42×30×8 cm), two weeks prior to anticipated peak population of gall midge at each test locations.

One week-old young seedling was transplanted in small plastic pots of about 25 cm in height and 10 cm in diameter, containing 750 g of soil. One hill of 3 standard gene differentials of rice, namely W1263, Phalguna and TN1 were planted in triangular fashion in each pot (Figure 3a,b). One hill containing 5 seedlings was represented by each rice gene differential. Precautions were taken before infestation to protect plants from natural infestation by holding the potted plants in a net cage (2.0×1.5×1.5 m). The pots with 3 differentials were covered with locally prepared cylindrical plastic cages on the day of infestation. Each cage was placed on the potted gene differential, and the upper rim of the cage in each pot was covered with muslin cloth and tightened for good ventilation with rubber bands. At least 20 to 30 cm was the height of the cage to leave enough room above the plant. Each pot with three differentials was infested with one gravid female gall midge (presumed to be mated) which was collected between 19.30 to 23.30 IST near light source using aspirator in the rice farm. When the plants reached 3 leaf stage or two weeks old, the collected midge was released inside the pot through a small slit, and the pots with adult midge were sealed in a cage for 3 d for egg laying.

The cages were removed on the fourth day, and plants were regularly sprayed with clean water using a hand atomizer for 2 to 3 d at 2 h intervals to achieve high relative humidity (>95 per cent) for egg hatching and maggot establishment. Alternatively, for 2 d after watering, the pots were sealed with a plastic cage. The observations on the number of gall midge damaged plants for each of the differentials and the number of galls in W1263, Phalguna and TN 1 have been recorded when differentials in all the pots show galls. During 2009, 660 females were tested in replicated trial representing 220 in each. Likewise, in 2020, 330 females were tested in replicated trial representing 110 in each. The biotypes in each infested pot are differentiated based on the reaction pattern of resistance (R) susceptibility (S) per single infested female. Biotype 1 (R-R-S), biotype 2 (S-R-S), biotype 3 (R-S-S) and biotype 4 (S-S-S) were verified and the number of females expressing biotype1, 2, 3 and 4 reaction attributes were counted in each pot checked and the percentage expression of each biotype pattern was determined (Vijaykumar *et al.,* 2008a).

Similarly, the sex of the emerging gall midge was recorded in each pot containing 3 differentials infested by single females. This can be done by covering the infested pots with plastic cages again prior to the emergence of adults. The sex was identified by analyzing the pupae by dissecting after 20 to 27 d of infestation under a binocular microscope. The size and color of the abdomen will easily distinguish the male and female pupae (Perera & Fernando, 1970; Panda & Mohanthy, 1970). The male pupas are tiny and

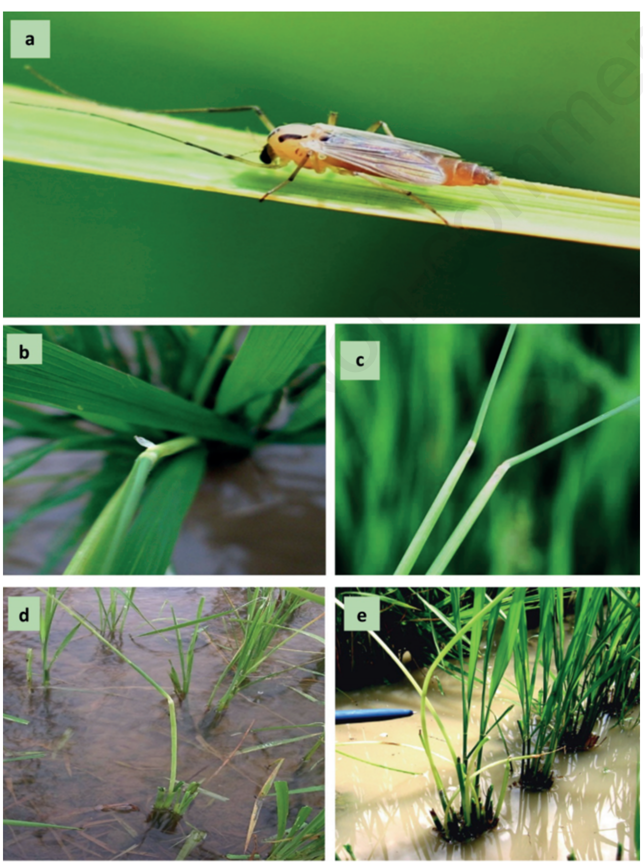

Figure 1. A) Adult of rice gall midge (*Orseolia oryzae*), B-E) Tillers without rice panicles due to internal feeding by maggots.

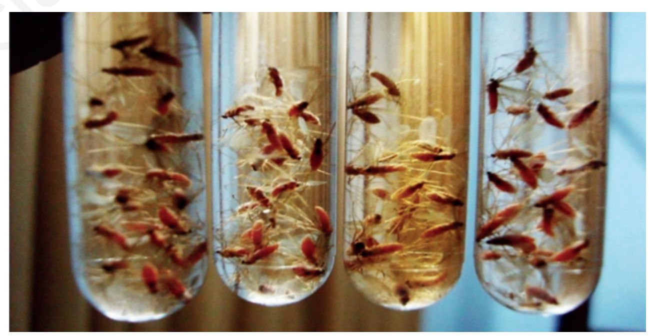

Figure 2. Virulent population of rice gall midge (*Orseolia oryzae*) prevailed across coastal regions of Karnataka, India.

Figure 3. A-B) Single female test technique by using standard rice gene differentials to quantify the virulence spectrum in local population of gall midge (*Orseolia oryzae*) across different endemic locations in coastal Karnataka, India.

brown in color, while the females are larger and pinkish red in color. Generally, all the evolving population (F_1) would be of one sex if a single female infests each pot, unless the populations are genetically non-homogeneous (Sahu *et al.,* 2004). Therefore, the reaction of a single female's offspring would assist in determining its role as a biotype. In addition, the response of all females tested at the test locations will help to measure the composition of the gall midge population.

Statistical analysis

The data set on the number of females infested on each gene differentials were transformed before analysis to log (n+0.50). The data on the number of females tested on each gene differential were analyzed using one-way analysis of variance (ANOVA) with randomized complete block design. The means were separated by Tukey's HSD (P=0.05) (Tukey, 1965) using SPSS 24.0 Armonk, NY (IBM Corp. Released, 2016). Further, the analysis was performed using SAS 9.4 (SAS Institute Inc., 2015). Likewise, the data set on the per cent virulence pattern by tested females were transformed before analysis to arcsine. The data on the percentage of females expressing virulence spectrum of different biotypes were analyzed using one-way analysis of variance (ANOVA) with randomized complete block design. The means were separated by Tukey's HSD (P=0.05) using SPSS 24.0 Armonk, NY (IBM Corp. Released, 2016). Further, the analysis was performed using SAS 9.4 (SAS Institute Inc., 2015).

Results

Reaction of standard rice gene differentials

In wet season of 2019, out of 16 standard rice differentials representing four groups identified to characterize the prevailing biotype population in the country, all the differential groups exhibited susceptible reaction. The plant damage and silver shoot at 50 d after planting was varied between 0.00 to 55.00 and 0.00 to 27.18 per cent, respectively. Likewise, in 2020 wet season also similar trend in infestation was observed where, plant damage and silver shoot at 50 d after planting was varied between 0.00 to 50.00 and 0.00 to 27.33 per cent, respectively. The reaction pattern of these standard rice gene differentials under 4 groups indicated the virulence pattern of R-S-R-S exhibiting prevalence of new biotype 3 in the coastal region of Karnataka, South India. Interestingly, in both the season in 2019 and 2020, few differentials in group I and III recorded lower level of incidence, and this revealed consistent presence of both biotype 2 and biotype 3 mixtures in the region. But the traditional biotype population characterized three decades back was biotype 2, which was avirulent/non-infective against group II and III rice gene differentials with the reaction pattern of S-R-R-S (Kalode & Bentur, 1989). These results indicated an evolution of new virulent biotype population capable of damaging all differentials in group II which got specific gene/s for resistance (Table 1).

Virulence composition of biotypes

The results on the virulence spectrum of local gall midge populations in coastal regions of Karnataka during 2009 and 2020 revealed the presence of heterogenous mixed population of biotypes deviating from traditional biotype 2. The rapid surveys conducted in wet seasons of 2009 and 2020 in major rice growing areas of southern coastal region *viz*., Puttur, Bantwala, Mangalore, Belthangadi indicated higher infestation (18.56-29.20%) on commercially released gall midge rice varieties *viz*., Uma, MO-4, MO-21, Mahaveera and Phalguna. Likewise, at Karkala, Udupi, Bramhavara and Kundapura of north coastal region, the infestation level was 12.26 to 34.99%. This indicated more virulence among local biotype population against resistant rice varieties released for commercial cultivation in the region (Vijaykumar *et al*., 2009a; Vijaykumar, 2020). Further, the results of the single female test to quantify the virulence spectrum of local population in south coast revealed existence of two biotype population. The percentage of females expressing virulence spectrum of gall midge biotype 3 was varied between 68.03 to 75.60 (F=9.32; df=7, 14; P<0.0001). The F_1 progeny of the virulent populations were found heterogeneous

Table 1. Reaction of standard rice differentials against local population of gall midge (*Orseolia oryzae*) at coastal regions of Karnataka in 2019 and 2020.

Entry No.	Group	Differential	Gene	2019[a] DP (%)	2019[a] SS (%)	2020[a] DP (%)	2020[a] SS (%)	GMB reaction[b]	No. NPT under greenhouse condition	No. NPT under field conditions
1	I	KAVYA	*Gm1*	10.00	1.12	5.00	0.55	R?	4	3
2		W 1263	*Gm1*	5.00	0.54	0.00	0.00	R	2	2
3		ARC 6605	*(?)*	5.00	0.00	0.00	0.00	R	2	2
4	II	PHALGUNA	*Gm2*	45.00	23.30	40.00	25.00	S	2	2
5		ARC5984	*Gm 5*	40.00	22.50	35.00	27.33	S	2	2
6		DUKONG 1	*Gm 6*	10.00	20.13	45.00	21.62	S	2	2
7		RP 2333-156-8	*Gm 7*	25.00	27.27	45.00	26.95	S	2	2
8		MADHURI L 9	*Gm9*	5.00	26.87	40.00	23.80	S	2	2
9		BG 380-2	*Gm 10*	40.00	25.00	40.00	21.85	S	2	2
10	III	RP 2068-18-3-5	*gm3*	0.00	0.00	0.00	0.00	R	2	2
11		ABHAYA	*Gm 4*	0.00	0.00	0.00	0.00	R	2	2
12		INRC 3021	*Gm 8*	10.00	1.05	5.00	1.07	R?	4	3
13		AGANNI	*Gm 8*	0.00	0.00	0.00	0.00	R	2	2
14		INRC 15888	*Gm 8*	0.00	0.00	0.00	0.00	R	2	2
15		B 95-1	*None*	15.00	0.00	0.00	0.00	R	2	2
16	IV	TN1	*None*	55.00	27.18	50.00	16.36	S	2	2

[a]Observations at 60 d after transplanting in Wet seasons. DP, damaged plant (hill basis); SS, silver shoot (tiller basis); GMB, gall midge biotype; NPT, No. of promising tests conducted for confirmation of biotype population; R, resistance; S, susceptible. [b]Reaction pattern of 4 groups of standard rice gene differentials against biotype 1=R-R-R-S, biotype 2 =S-R-R-S, biotype 3=R-S-R-S, biotype 4=S-S-R-S, biotype 5=R-R-S-S, biotype 6=R-S-S-S.

with more male progeny on Phalguna (*Gm 1*) compared to W 1263 (*Gm 2*) and TN 1 (no gene). However, 26.36 to 30.00 per cent population expressed virulence spectrum of biotype 2 at Puttur, Bantwala, Mangalore and Belthangadi of south coastal region (F=24.21; df=7, 14; P<0.0001). Likewise, in north coastal region, significantly the local population of rice gall midge expressed more virulence attributes of biotype 3 (69.09 to 75.60%) (F=9.32; df = 7, 14; P<0.0001). In this region, the per cent female found expressing virulence attributes of new biotype 3 was varied between 69.09 to 75.60 percent and, 21.36 to 29.54 per cent test population (F=24.21; df=7, 14; P<0.0001). exhibited virulence spectrum of traditionally designated biotype 2 with more male progenies in the F_1 population (Table 2).

The detection on the prevalence of local rice gall midge biotype population in entire coastal region was studied in 2009 (Vijaykumar *et al.*, 2012). A decade after preliminary observations on the prevalence of local biotype population in wet 2020 at coastal Karnataka significantly indicated the existence of gall midge population with more virulence towards new biotype 3 in north coast (F=553.87; df=7, 14; P<0.0001), and biotype 2 in south coast (F=692.39; df=7, 14; P<0.0001). At south coast, there was a rapid change in the virulence attributes of local population towards traditional biotype 2 after 10 years. Where, the local population exhibited 73.33, 83.93, 77.27 and 87.27 per cent virulence attributes of biotype 2 at Puttur, Bantwala, Mangalore and Belthangadi, respectively (F=692.39; df=7, 14; P<0.0001) (Figure 4). In these locations, the results on the quantification of virulence through single female test confirmed the existence of biotype 2. Further, the female to male sex ratio of F_1 population varied between 2.93:1 to 3.18:1 on W1263 (*Gm 1*), indicating homogenous population.

Interestingly at north coast in 2020, there was an improvement in virulence spectrum of local population towards new biotype 3 with more virulence on differential Phalguna (*Gm 2*) over the decade. The per cent female population expressing virulence spectrum of new biotype 3 at Karkala, Udupi, Bramhavara and Kundapura was 84.54, 86.36, 81.81 and 79.69, respectively coast (F=553.87; df=7, 14; P<0.0001) (Figure 5). These results were further confirmed by the F_1 progenies of virulent female where, the female to male sex ratio at Karkala, Udupi, Bramhavara and Kundapura on Phalguna (*Gm2*) was 2.95:1, 2.86:1, 2.91:1 and 3.05:1, respectively. Likewise, the female to male sex ratio of virulent female population on W1263 (*Gm1*) was 1.61:1, 1.36:1, 1.54:1 and 1.42:1, respectively at Karkala, Udupi, Bramhavara and Kundapura. Thus, a greater number of male progenies in the F_1 population on differential W1263 (*Gm1*) indicated the presence of avirulent population against *Gm1* gene. However, in all test locations of south and north coastal region of Karnataka, the female to male sex ratio of F_1 population on TN1 (no gene for resistance) was more than 3:1 (Table 2).

Table 2. Virulence spectrum in local population of gall midge (*Orseolia oryzae*) at endemic locations of Coastal Karnataka in 2009 and 2020.

Location	No. females tested^{a}	No. Females infested on^{a} TN 1 (No gene)	W 1263 (*Gm 1*)	Phalguna (*Gm 2*)	*Gm1*+ *Gm2*	Virulence pattern (%) by test females towards^{b} GMB 1	GMB 2	GMB 3	GMB 4	Sex ratio on^{c} TN 1 F:M	W 1263 F:M	Phalguna F:M
2009												
Puttur	220(14.83)^{a}	220(14.83)^{a}	66.00(8.13)^{a}	149.67(12.25)^{ab}	4.33(2.19)^{b}	0.00	30.00 (33.21)^{a}	68.03(55.55)^{b}	1.96(8.13)^{b}	2.60:1	2.26:1	1.88:1
Bantwala	220(14.83)^{a}	220(14.83)^{a}	58.67(7.69)^{bc}	161.33(12.72)^{b}	0.00(0.70)^{b}	0.00	26.66(31.11)^{a}	73.33(58.89)^{b}	0.00(0.00)^{b}	2.47:1	3.20:1	1.83:1
Mangalore	220(14.83)^{a}	220(14.83)^{a}	61.33(7.86)^{ab}	158.67(12.61)^{b}	0.00(0.70)^{b}	0.00	27.87(31.88)^{a}	72.12(58.12)^{b}	0.00(0.00)^{b}	2.53:1	3.00:1	2.06:1
Belthangadi	220(14.83)^{a}	220(14.83)^{a}	58.00(7.61)^{bc}	162.00(12.72)^{ab}	0.00(0.70)^{b}	0.00	26.36(30.92)^{a}	73.63(59.08)^{b}	0.00(0.00)^{b}	3.68:1	2.90:1	2.12:1
Karkala	220(14.83)^{a}	220(14.83)^{a}	54.67(7.42)^{c}	160.33(12.68)^{b}	5.00(2.24)^{b}	0.00	24.84(29.87)^{a}	72.88(58.63)^{b}	2.27(8.72)^{b}	3.17:1	3.28:1	1.93:1
Udupi	220(14.83)^{a}	220(14.83)^{a}	54.67(7.42)^{c}	160.00(12.65)^{b}	5.33(2.41)^{b}	0.00	24.85(29.87)^{a}	72.72(59.50)^{ab}	2.42(8.91)^{b}	2.73:1	2.68:1	2.07:1
Bramhavar	220(14.83)^{a}	220(14.83)^{a}	65.00(8.06)^{a}	152.00(12.33)^{ab}	3.00(1.74)^{b}	0.00	29.54(32.90)^{a}	69.09(56.23)^{b}	1.36(6.55)^{c}	3.02:1	2.15:1	1.42:1
Kundapura	220(14.83)^{a}	220(14.83)^{a}	47.00(6.85)^{d}	166.33(12.89)^{a}	6.67(2.67)^{a}	0.00	21.36(27.56)^{b}	75.60(60.40)^{a}	3.03(9.98)^{a}	3.44:1	2.28:1	1.56:1
F	NS	NS	23.68	10.29	44.38		24.21	9.32	64.03			
P			<0.0001	<0.0001	<0.0001	-	<0.0001	<0.0001	<0.0001	-	-	-
df			7, 14	7, 14	7, 14		7, 14	7, 14	7, 14			
2020												
Puttur	110(10.49)^{a}	110(10.49)^{a}	80.67(9.00)^{c}	29.33(5.46)^{ab}	0.00	0.00	73.33(58.89)^{b}	26.66(31.05)^{c}	0.00	3.18:1	3.05:1	1.96:1
Bantwala	110(10.49)^{a}	110(10.49)^{a}	92.33(9.63)^{ab}	17.67(4.26)^{c}	0.00	0.00	83.93(66.34)^{a}	16.06(23.66)^{d}	0.00	2.98:1	3.14:1	1.38:1
Mangalore	110(10.49)^{a}	110(10.49)^{a}	85.00(9.22)^{bc}	25.00(5.00)^{b}	0.00	0.00	77.27(61.48)^{b}	22.72(28.45)^{c}	0.00	3.11:1	3.09:1	1.56:1
Belthangadi	110(10.49)^{a}	110(10.49)^{a}	96.00(9.80)^{a}	14.00(3.74)^{c}	0.00	0.00	87.27(69.04)^{a}	12.72(20.88)^{d}	0.00	3.08:1	2.93:1	1.84:1
Karkala	110(10.49)^{a}	110(10.49)^{a}	17.00(4.12)^{ef}	93.00(9.64)^{a}	0.00	0.00	15.45(23.11)^{cd}	84.54(66.81)^{ab}	0.00	3.22:1	1.61:1	2.95:1
Udupi	110(10.49)^{a}	110(10.49)^{a}	15.00(3.87)^{f}	95.00(9.74)^{a}	0.00	0.00	13.63(21.64)^{d}	86.36(68.28)^{a}	0.00	3.16:1	1.36:1	2.86:1
Bramhavar	110(10.49)^{a}	110(10.49)^{a}	21.00(4.58)^{de}	90.00(9.48)^{a}	0.00	0.00	19.09(25.92)^{c}	81.81(64.75)^{ab}	0.00	3.27:1	1.54:1	2.91:1
Kundapura	110(10.49)^{a}	110(10.49)^{a}	22.33(4.77)^{d}	87.67(9.38)^{a}	0.00	0.00	20.30(26.78)^{c}	79.69(63.22)^{b}	0.00	3.38:1	1.42:1	3.05:1
F	NS	NS	599.52	691.72			692.39	553.87				
P			<0.0001	<0.0001	-	-	<0.0001	<0.0001	-	-	-	-
df			7, 14	7, 14			7, 14	7, 14				

GMB, gall midge biotype; *Gm 1* (W1263) and *Gm 2* (Phalguna) genes for resistance; F, female, M, male; Standard rice gene differentials tested (W1263; Phalguna; TN 1). [a]Data were square root-transferred before subjected to one-way ANOVA; [b]Data were arc sin transformed before subjected to one-way ANOVA; Means within a column followed by the same letter are not significantly different (P<0.05; Tukey's HSD); [c]Sex ratio calculated based on test females in respective gene differential; R, resistance; S, susceptibility by single infested female test; Reaction pattern for biotype 1 =R-R-S; biotype 2 =S-R-S; biotype 3=R-S-S; biotype 4 =S-S-S.

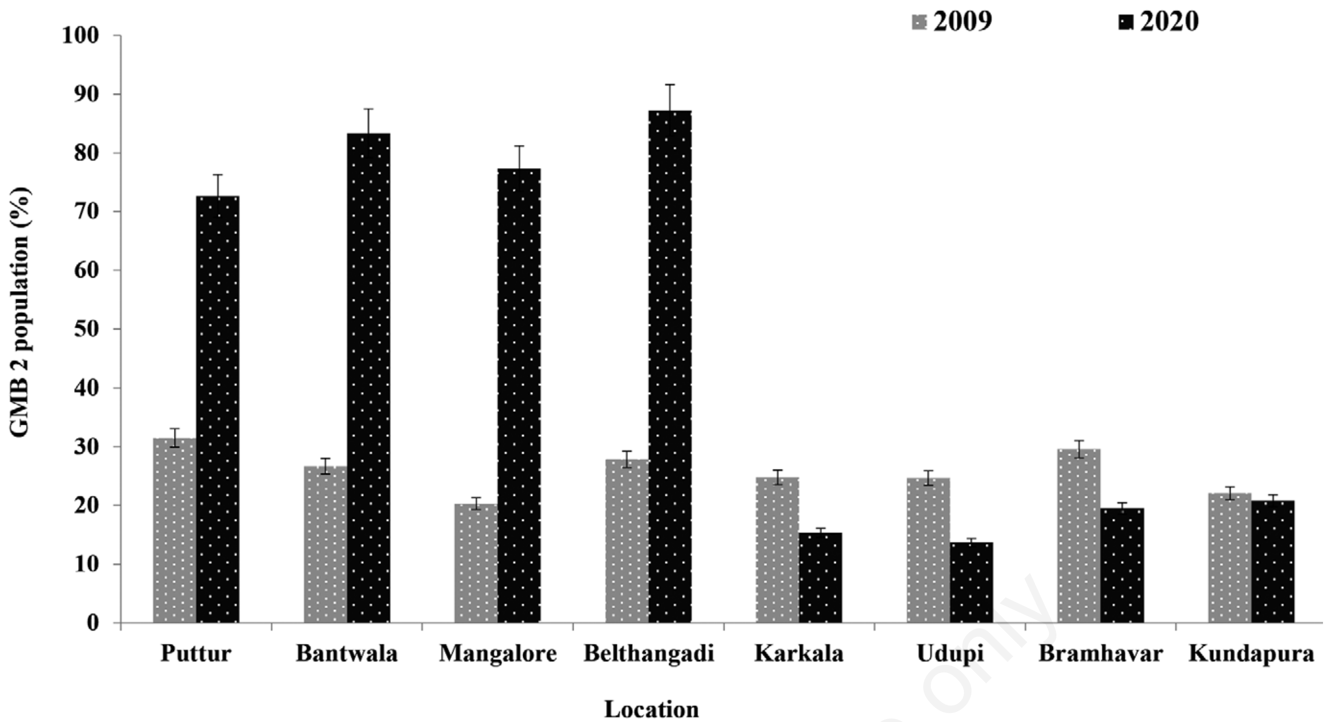

Figure 4. Virulence composition of local biotype population of rice gall midge (*Orseolia oryzae*) at coastal regions of Karnataka, 2009 and 2020 towards biotype 2; GMB=Gall midge biotype; ANOVA testing for differences for biotype 2 in 2009, $F = 24.21$, df = 7, 14, $P = < 0.0001$; for the year 2020, $F = 692.39$, df = 7, 14, $P = <0.0001$.

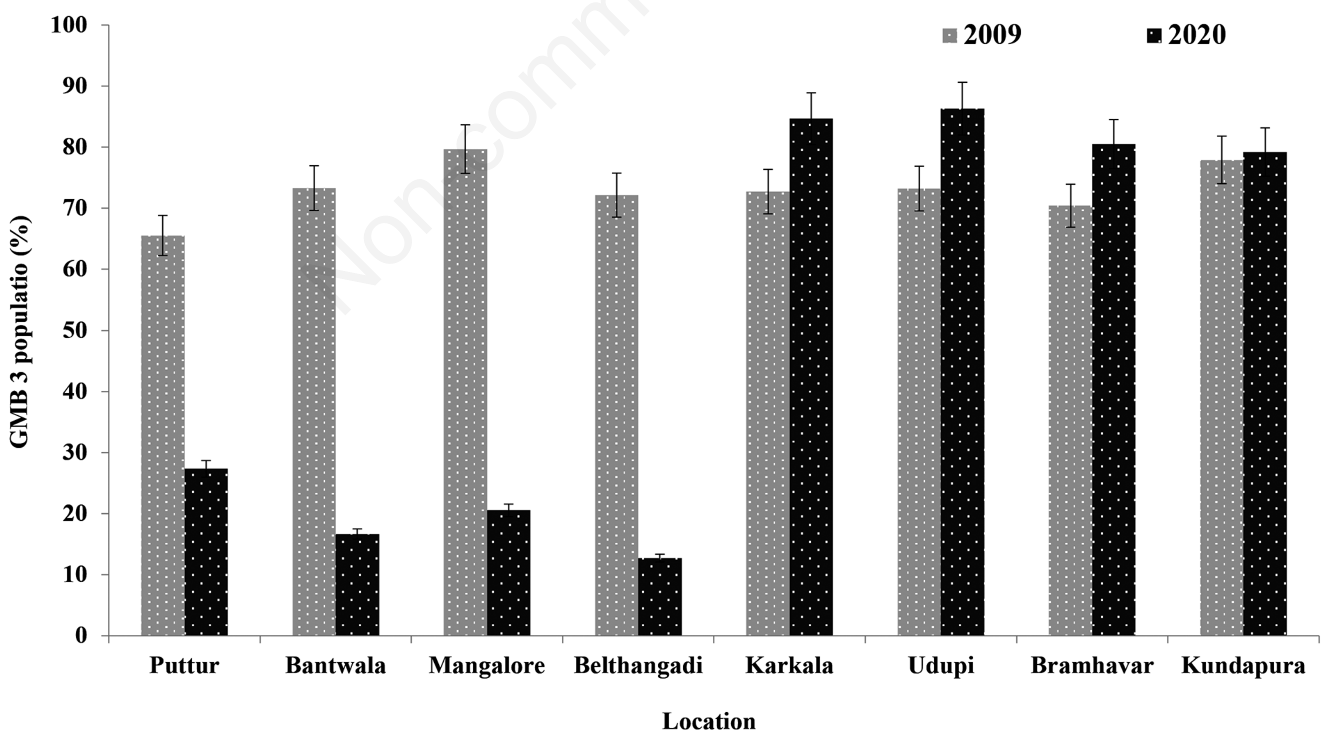

Figure 5. Virulence composition of local biotype population of rice gall midge (*Orseolia oryzae*) at coastal regions of Karnataka, 2009 and 2020 towards biotype 3; GMB=Gall midge biotype; ANOVA testing for differences for biotype 3 in 2009, $F = 9.32$, df = 7, 14, $P = < 0.0001$; for 2020, $F = 553.87$, df = 7, 14, $P = <0.0001$.

Discussion

Breeding resistant rice varieties has been a viable and ecologically acceptable approach for management of gall midge. Since 1970, more than 56 resistant rice varieties have been developed and released for commercial cultivation in India. Of these, Phalguna, Surekha in Andhra Pradesh, Jyothi in Kerala, and Phalguna, Shakti, Mahaveera, Nethravathi, Vikram and MO-4 in coastal Karnataka became very popular and were cultivated extensively year after year (Bentur *et al.,* 2003; Vijaykumar *et al.,* 2006). Due to large scale cultivation of gall midge resistant rice cultivars in coastal rice belt of Karnataka over decades, has signaled the development of virulent biotype causing 40 to 65 per cent plant damage (DRR, 2002; Vijaykumar *et al.,* 2009a).

However, the reports on development of virulent biotypes of gall midge resulting in breakdown of resistance in the popular gall midge resistant varieties started appearing since 1987 (Kalode & Bentur, 1989). This added alternative dimension to breeding for resistance to the gall midge. Soon it became apparent that majority of the resistance genes deployed in the popular gall midge rice varieties have either been overcome or there is evidence of building-up of virulence against resistance genes (*Gm1* and *Gm2*) among gall midge population. Similar situation was observed in Karnataka from 1996 to till date (Pasalu & Rajamani, 1996; Vijaykumar & Shivanna, 2020). To overcome the problem in detecting virulent population of gall midge populations in the region, the frequent monitoring and continuous testing of standard rice gene differentials in endemic and new locations are essentially required.

Majority of rice farmers adapted gall midge resistant rice varieties *viz*., Phalguna, Shakti, Mahaveera, Nethravathi and MO-4 over decades in coastal region of Karnataka. Similarly, the resistant rice varieties released against other insect pests of rice has also resulted a change in pest status of rice gall midge in certain regions (Vijaykumar *et al.,* 2009a). The occurrence of gall midge biotypes in India was first suspected by Khan & Murthy (1955) even when no gall midge resistant varieties were developed. Subsequently, Roy *et al.* (1969) observed differential reaction pattern in gall midge resistant donors and cultivars at two endemic locations *viz*., Sambalpur in Orissa and Warangal in Andhra Pradesh, India. Presence of two biotypes was further confirmed by national and international germplasm testing programs (Chatterji *et al.,* 1975; Roy *et al.,* 1971). So similar situation was observed across coastal Karnataka in the present study.

The development of a new virulent biotype was first reported in 1986 from the north coastal districts of Andhra Pradesh, India in response to extensive cultivation resistant variety Phalguna (*Gm2*) (Bentur *et al.,* 1987). These results substantially supported the present investigations at coastal Karnataka. Since then, reports of development of new virulent biotypes of gall midge have been reported in the state of Maharashtra (Prakasarao & Kandalkar, 1992), Manipur (Singh, 1996) and Kerala (Nair & Devi, 1994) and in Karnataka (Vijaykumar *et al.,* 2009a). In all these regions, the popular gall midge resistant varieties have become susceptible against local virulent population. In 1993, the appearance of a new virulent population capable of overcoming resistance in rice varieties such as Phalguna and Surekha was reported from a new region, Telangana 500 km south-west of north coastal region in Andhra Pradesh (Srinivas *et al.,* 1994). This population differed from that of north coast in its virulence pattern.

Similar situation was observed across different locations of north coastal Karnataka, where the local gall midge population were more virulent as biotype 3 coast (F=553.87; df=7, 14; P<0.0001). On the other hand, reports of biotype 4 in north-coastal Andhra Pradesh (Bentur *et al.,* 1987), Maharashtra (Prakasarao and Kandalkar 1992) biotype 3 in northern Telangana state (Srinivas *et al.,* 1994) were preceded by introduction and extensive cultivation of gall midge resistant rice varieties. These reports strongly corroborate results of the present investigations. Likewise, at Ragolu, Andhra Pradesh (India), the population was more virulent against *Gm1* gene (W 1263) followed by *Gm2* gene (Phalguna), while 24.7 per cent population expressed virulence against both *Gm1* and *Gm2* gene indicating pattern of biotype 4 (DRR, 2004). At Raipur (India), during wet 2004, 7.5 per cent of the populations were found heterogeneous (Modi *et al.,* 2004). This composition has been observed to vary from location to location and from year to year at the same test location (Bentur *et al.,* 2003). The biotype 5 in Kerala (Nair & Devi, 1992) also preceded by extensive cultivation of locally bred rice varieties that were resistant to gall midge. The results of the present investigations also strongly supported by reports of the earlier workers across Indian states. Where, the evolution of new virulent population was attributed to the extensive cultivation of resistant rice varieties against gall midge biotype population. The constant and meticulous monitoring of biotype populations across gall midge endemic locations of Indian states is urgently required to track the changing scenario of gall midge biotype populations. This will help in developing rational management practices against virulent population of rice gall midge across Indian states.

Conclusions

The precise identification of rice gall midge biotypes hitherto in Karnataka, India was a stumbling block for the management of gall midge in rice since 1987. In the present investigations, a progressive change in the local gall midge biotype 2 was observed. Further, a rapid development in the virulence attributes of local gall midge 2 population towards new biotype 3 was observed in north coastal Karnataka. To date, identification and virulence patterns of the prevailing rice gall midge populations went unrecorded especially in new locations of southern and coastal regions of Karnataka. The results of the present studies not only establish virulence composition of biotypes in the region, but also going to indicate the continuous cultivation of resistant gall midge rice genotypes intensifying virulence. The new observation will have profound practical implications and new ways of managing biotype complex in the state. The documentation of changing scenario of prevailing virulent gall midge biotypes in new and endemic locations of coastal Karnataka will provide a clear picture on the extent and rate of variations among biotypes. These information needs to be documented throughout rice growing regions of Indian subcontinent. The precise identification virulent biotype population will greatly assist in developing and deploying location specific resistant rice varieties and resistant donors against this pest. Further, the identification of biotype specific resistant donors will help in developing "durable gall midge resistant varieties" through molecular breeding wherever, such populations emerge across the country. This will lead to the successful evolution of resistant rice genotypes that will thwart the rice gall midge for a considerable time to come. This will bring some socio-economic changes among farming communities and other stakeholders, not only in Karnataka but, also in entire rice growing regions of India.

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