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# Phenotypic and Genotypic Screening of Warangal Rice Varieties against Gall Midge (*Orseolia oryzae*)

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## ABSTRACT

Nineteen rice varieties released from Regional Agricultural Research Station (RARS), Warangal, Professor Jayashankar Telangana State Agricultural University (PJTSAU), Telangana, India along with one susceptible check (TN1) were screened for gall midge resistance at both phenotypic and genotypic conditions during *kharif* 2016 (July- November). Among the 19 rice varieties screened, Sheetal had showed highly resistance reaction to gall midge at field level and also at genotypic level by possessing three gall midge genes like *gm3* (*Gm3del3*), *Gm*$^{4}$ (*Gm4* LRR) and *Gm8* (PRP). The varieties like Orugallu, Bhadrakali, Shiva, Kesava and Ramappa were showed moderate level of resistance reaction to gall midge in the field, and also possessing only *Gm3* gene, while one rice variety like WGL-915 had showed moderate level of resistance to gall midge in the field by possessing only *Gm4* gene.

**Keywords** Gall midge, Molecular markers, Rice, Screening, Phenotypic, Genotypic.

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## INTRODUCTION

Rice (*Oryza sativa* L.) is an important staple food crop and is a major source of livelihood for more than half of the world population, around 9% of the earth's arable land is under rice production. World-wide, rice is cultivated in 165 million hectares with an annual production of 500.82 million tonnes (FAO 2017-18). Of the several pests causing significant yield loss, the Asian rice gall midge [*Orseolia oryzae* (Wood-Mason)], an endemic dipteran pest of rice, causes an annual loss of about US$80 million (Krishnaiah and Varma 2011). Two species of the rice gall

**Table 1.** The details of rice varieties used in present study.

| Sl. No. | Varieties | Characteristics |
|---|---|---|
| 1 | Kakatiya (WGL-13801) | A high yielding medium slender (MS) grain type rice variety with 120 days of duration, of released in the year 1974 and it is derived from the cross between IR8 and W1263 and is resistant to gall midge |
| 2 | Surekha (WGL-13400) | A high yielding long slender (LS) grain type rice variety with 130–135 days duration of releazed in the year 1976 and it is derived from the cross between IR8 and Sayam 29 and is resistant to gall midge |
| 3 | Pothana (WGL-22245) | A high yielding long slender (LS) grain type rice variety with 125 days duration of released in the year 1976 and it is derived from the cross between IR579 and W12708 and is resistant to gall midge |
| 4 | Kavya (WGL-48684) | A high yielding medium slender (MS) grain type rice variety with 135 days duration of released in the year 1991 and it is derived from the cross between WGL-27120/WGL17672 and Mayuri and Surekha and is resistant to gall midge |
| 5 | Erramallelu (WGL-20471) | A high yielding long slender (LS) grain type rice variety with 120-125 days duration of released in the year 1991 and it is derived from the cross between BC5-55 and W12708 and is resistant to gall midge |
| 6 | Orugallu (WGL-47970) | A high yielding medium slender (MS) grain type rice variety with 145days duration of released in the year 1993and it is derived from the cross between OBS677 and IR 2070-423-2-5resistant to gall midge |
| 7 | Bhadrakali (WGL-3962) | A high yielding long slender (LS) grain type rice variety with 135 days duration of releazed in the year 1994 and it is derived from the cross between palguna and IR36 is resistant to gall midge |
| 8 | Shiva (WGL-39430) | A high yielding long slender (LS) grain type rice variety with 130–135 days duration of released in the year 1996 and it is derived from the cross between Palguna and IR 50 is resistant to gall midge |
| 9 | Keshava (WGL-3825) | A high yielding long slender (LS) grain type rice variety with 125 days duration of released in the year 1996 and it is derived from the cross between WGL 28712 IR36 is resistant to gall midge |
| 10 | Varalu (WGL-14377) | A high yielding long slender (LS) grain type rice variety with 90–105 days duration of released in the year 2002 and it is derived from the cross between WGL 20471 and CR544-1-2 and is resistant to gall midge |
| 11 | WGL-14 (Warangal Samba) | A high yielding medium slender (MS) grain type rice variety with 135 -140 days duration of released in the year 2005 and it is derived from the cross between B.P.T5204 and IRC5984/I.BPT3291 is resistant to gall midge |
| 12 | WGL-32100 (Warangal Sannalu) | A high yielding long slender (LS) grain type rice variety with 135 days duration of released in the year 2006 and it is derived from the cross between Divya and BPT5204 is resistant to gall midge |
| 13 | Ramappa (WGL-23985) | A high yielding medium slender (MS) grain type rice variety with 125–130 days duration of released in the year 2009 and it is derived from the cross between. Kavya and AC 20 and is resistant to gall midge |
| 14 | Sheethal (WGL-283) | A high yielding long slender grain (LS) type rice variety with 125–130 days duration of released in the year 2013 and it is derived from the cross between Chathanya and The llahamsa and is resistant to gall midge |
| 15 | Siddi (WG-44) | High yielding medium slender (MS) grain type rice variety with 140-145 days duration of released in the year 2013 and it is derived from the cross between. B.P.T5240/ARC 5984) and Kavya /(kavya/BPT5204 is resistant to gall midge |
| 16 | WGL-347 (Somanath) | A high yielding medium slender (MS) grain type rice variety with 130-135 days duration of released in the year 2015 and it is derived from the cross between NLR-145 and Kavya is resistant to gall midge |
| 17 | WGL-401 | A high yielding Potential cold tolerant medium slender (MS) grain type rice variety with 125–130 days duration of it is derived from the cross between. BPT 5204/Bhadrakali) and suitable for *kharif* and *rabi* is resistant to gall midge |
| 18 | WGL-505 | A high yielding medium slender (MS) grain type rice variety with 125–130 days duration of it is derived from the cross between. BPT 5204/RYP1 and suitable for *kharif* and is resistant to gall midge |

**Table 1.** Continued.

| Sl. No. | Varieties | Characteristics |
|---|---|---|
| 19 | WGL-915 | A high yielding long bold (LB) grain type rice variety with 130–135 days duration of the cross between (SN22 and IRBBN39) and suitable for *kharif* and *rabi* is resistant to gall midge |
| 20 | TN1 (susceptible check) | A high yielding bold grain type rice variety with 120 days duration of released in the year and TN1 is susceptible to all the resistant gall midge biotypes |

midge have been identified so far, i.e., Asian rice gall midge, *Orseolia oryzae* Wood- Mason and African rice gall midge, *O. oryzivora*. Both species belong to the family Cecidomyiidae of the order Diptera. The larvae of the pest feeds on the apical meristem causing formation of tubular sheath called 'silver shoot' in place of normal inflorescence. During wet season, chemical control of pest is inefficient due to its internal feeding habit, hydrological and edaphological condition. The best way to manage the pest is the cultivation of resistant varieties. A vast majority of high yielding rice varieties are prone to gall midge attack, but few cultivars and landraces are immune to it (Bentur *et al.* 2016).Till date, 11 GM resistance genes (*Gm1* to *Gm11*) (Dutta *et al.* 2014, Das and Rao 2015, Hasan *et al*. 2015 ; Bentur *et al.* 2016, Hari *et al.* 2022) were identified, out of which only eight GM resistance genes (*Gm1, Gm2, gm3, Gm4, Gm6, Gm7, Gm8* and *Gm11*) have been tagged and mapped on to different chromosomes (Nair *et al.* 2011) with the exception of *Gm5* only being tagged but not mapped (Yasala *et al.* 2012). Seven biotypes (GMB1 to GMB6 and GMB4M) of GM have been identified in rice (Vijayalakshmi *et al*. 2006 and Himabindu *et al.* 2010). Of the 11 genes identified, $gm^3$ is the only recessive gene identified so far (Bentur *et al.* 2016) and only two genes, *Gm1* and *Gm8* confer resistance without the expression of hypersensitive reaction (HR –ve type). All the other genes confer resistance with HR (HR +ve type) (Bentur *et al.* 2003). Sama *et al* (2014) reported the recessive gene *gm3* imparts resistance to biotype 1, 2, 3, 4 and 7. It has been mapped within 0.56 Mb region on chromosome 4 between SSR markers RM 17480 and RM 22685. *Gm4* encodes wide range of resistance containing F-box family proteins, NBS-LRR (Nucleotide Binding Site-Leucine-Rich Repeat) regions suggesting their involvement in HR+ mediated gall midge resistance in rice (Mohapatra *et al.* 2014). This gene has been mapped on the short arm of chromosome 8 between two microsatellite markers, RM547 and RM22555 on one side while two microsatellite markers, RM22550 and RM 2551 on other side of the gene in the cultivar PTB 10 (Nanda *et al.* 2010). A LRR gene, suspected to be a candidate gene at *Gm4* locus has been identified and a candidate gene based marker for this gene has also been developed (Dutta *et al.* 2014, Divya *et al.* 2015). Developing rice varieties possessing two or more resistance gene (s) against gall midge through molecular breeding is the most effective way for enhancing the durability of resistance. Considering this, the present study was conducted and aimed at, phenotypic and genotypic confirmation of gall midge resistance in the rice varieties released from Regional Agricultural Research Station, Warangal.

**Table 2.** Standard evaluation system scale for scoring the reaction against gall midge.

| Per cent damage | Score | Reaction |
|---|---|---|
| Based on per cent silver shoots | | |
| 0 | 0 | Highly resistant |
| < 1 | 1 | Resistant |
| 1–5 | 3 | Moderately resistant |
| 6–10 | 5 | Moderately susceptible |
| 11–25 | 7 | Susceptible |
| > 25 | 9 | Highly susceptible |
| Based on per cent plant/hill damage | | |
| 0–10 | | Resistant |
| >10 | | Susceptible |

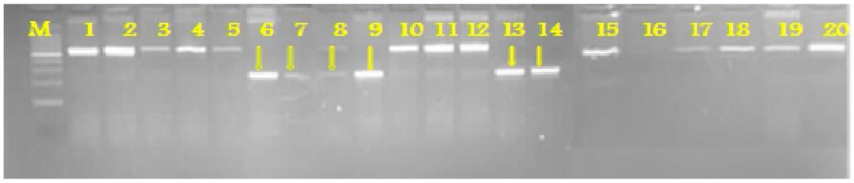


**Fig. 1.** Genotyping of rice varieties released from Warangal research station for presence of gm3 gene by using functional marker *Gm3del3*. The lane shows on the top of the gel representa M : 100 bp ladder, the lane numbers 1 to 20 represents list of rice varieties (Table 2) released from war Warangal research station.

## MATERIALS AND METHODS

The experiment was carried out at Regional Agricultural Research Station, Warangal, Professor Jayashankar Telangana State Agricultural University (PJTSAU), Telangana State, India during rainy season (*kharif*) 2016 (July-November). Nineteen rice varieties (Table 1) along with one susceptible check (TN1) were screened for gall midge resistance at both phenotypic and genotypic levels.

Sowing was intentionally delayed by about 4 weeks when compared to normal sowings for enhancing the population of target pest i.e., gall midge in the field. All the recommended agronomical measures were adopted to conduct the experiment. No plant protection measures were followed against insect pests. Galls which are symptoms of gall midge damage or level of infestation were counted on all the twenty plants in each row at 41 and 59 days after transplanting. Percentage hill and tiller infestations were computed using the following formula.

$$\text{Percent Plant/hill Damage (PD\%)} = \frac{\text{Number of plants with silver shoots}}{\text{Total number of plants}} \times 100$$

$$\text{Percent Silver Shoots (SS \%)} = \frac{\text{Mean number of silver shoots per plant}}{\text{Mean number of tillers per plant}} \times 100$$

Tiller damage levels were expressed as scores between the values of 0 and 9 (Table 2) according to Standard Evaluation System, International Rice Research Institute (IRRI) for gall midge (IRRI 2013).

### Marker assisted selection for gall midge resistance

Young leaf samples were collected from Nineteen

**Table 3.** List of primers used in the study.

| Sl. No. | Gene | Chromosome no. | Primer | Forward sequence information | Reverse sequence information | Reference |
|---|---|---|---|---|---|---|
| 1 | *gm3* | 4 | *Gm3del3* | CTGCCAGAGAT GGGCCTTCCA | CGTACAAATTCCT GTACCACTC | Sama *et al*. (2014) |
| 2 | *Gm4* | 8 | *Gm4LRR* | GTGGATCGAGA GAAGACAAG | CTTGAGGACGATA TTCAAGC | Divya *et al.* (2013) |
| 3 | *Gm8* | 8 | *PRP* | TCATGTTGCAGA TCAACC | AGCCATATGAAAAC CACCAA | Divya *et al*. (2013) |

**Table 4.** Phenotypic and Genotypic Screening of rice varieties against Gall midge during *kharif*, 2016 at RARS, Warangal. HS-Highly susceptible, MR-Moderately resistant, R-Resistant, S-Susceptible, rr- susceptible allele, RR-Resistant allele.

| Sl. No. | Designation | 41 DAT | | 59 DAT | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | % galls on hill basis | % galls on tiller basis | % galls on hill basis | % galls on tiller basis | Damage score | Phenotypic reaction | Allelic status of Gm genes *gm3* | *Gm4* |
| 1 | Kakatiya | 25 | 2.06 | 90 | 24.9 | 7 | S | rr | rr |
| 2 | Surekha | 45 | 4.38 | 100 | 43.3 | 9 | HS | rr | rr |
| 3 | Pothana | 50 | 4.55 | 100 | 39.2 | 9 | HS | rr | rr |
| 4 | Kavya | 10 | 0.71 | 100 | 19.72 | 7 | S | rr | rr |
| 5 | Erramallelu | 15 | 0.96 | 60 | 9.18 | 5 | MS | rr | rr |
| 6 | Orugallu | 15 | 1.01 | 75 | 3.50 | 3 | MR | RR | rr |
| 7 | Bhadrakali | 30 | 1.05 | 55 | 3.69 | 3 | MR | RR | rr |
| 8 | Shiva | 40 | 2.27 | 75 | 4.81 | 3 | MR | RR | rr |
| 9 | Keshava | 15 | 0.02 | 65 | 2.36 | 3 | MR | RR | rr |
| 10 | Varalu | 35 | 2.4 | 65 | 8.57 | 5 | MS | rr | rr |
| 11 | WGL-14 | 35 | 2.37 | 100 | 28.39 | 9 | HS | rr | rr |
| 12 | WGL-32100 | 20 | 1.37 | 95 | 26.13 | 9 | HS | rr | rr |
| 13 | Ramappa | 10 | 0.88 | 40 | 4.37 | 3 | MR | RR | rr |
| 14 | Sheethal | 00 | 0.00 | 00 | 0.00 | 0 | HR | RR | RR |
| 15 | Siddi | 45 | 8.77 | 90 | 28.87 | 9 | HS | rr | rr |
| 16 | WGL-347 | 45 | 4.18 | 90 | 22.48 | 9 | S | rr | rr |
| 17 | WGL-401 | 35 | 3.27 | 95 | 30.86 | 9 | HS | rr | rr |
| 18 | WGL-505 | 15 | 1.15 | 75 | 9.88 | 5 | MS | rr | rr |
| 19 | WGL-915 | 25 | 1.05 | 70 | 3.87 | 3 | MR | rr | RR |
| 20 | TN1 | 50 | 4.52 | 95 | 27.34 | 9 | HS | rr | rr |

rice varieties (Table 1) along with susceptible check (TN1) and isolated the genomic DNA by following the protocol of Zheng *et al* (1995). PCR was performed using 1 U of Taq DNA polymerase (Fermentas, Lithuania) and 1 × PCR buffer (Genei, India) in 10-µl reaction volume with a thermal profile of 94°C for 5 min (initial denaturation), followed by 35 cycles of denaturation at 94°C for 30s, annealing at 55°C for 30s, extension at 72°C for 1 min and a final extension of 7 min at 72°C. The amplified product was electrophoretically resolved on a 1.5% Seakem LE® agarose gel (Lonza, USA), containing 0.5 mg/ml of ethidium bromide in 0.5 × TBE buffer and visualized under UV.

Three primers i.e, *Gm3 del3*, *Gm4 LRR* and *PRP* (Table 3) were used for screening of Nineteen rice varieties along with the susceptible check (TN1) (Tables 1 and 4) for the presence or absence of gall midge resistance genes.

## RESULTS AND DISCUSSION

### Phenotypic screening for gall midge resistance

Field screening of the rice varieties for gall midge incidence at RARS, Warangal was considered as appropriate because it is one of the hot spot locations in India for gall midge incidence.The test entries were screened and damage scores were assessed at i.e 41 DAT and 59 DAT (Table 4), for their resistance against gall midge by using standard evaluation system of IRRI (2013) (Table 2). At second observation (59 DAT) the percentage of galls on tiller basis was ranged from 0.00% –43.3% and the percentage of galls on hill basis was from 0–100%. The susceptible check TN-1 showed 95% and 27.34% galls on hills and tillers respectively, with the damage score of 9 (Table 4), and in other 19 entries, only one entry was noticed with “Nil” gall midge incidence i.e. Sheetal with the damage score of “0” (Table 4) and five more entries

i.e. Orugallu, Bhadrakali, Shiva, Kesava, Ramappa and WGL-915 were showed moderate level of gall midge resistance reaction with a damage score of "3" (Table 4). Remaining 14 entries showed moderate to highly susceptible reaction with damage score of 5, 7 and 9, respectively (Table 4). However in all the moderately resistant entries, >10% hill damage was recorded (Table 4).

Dutta *et al.* (2014) considered test entries with nil damage and up to 20 % plant damage as resistant, while others were grouped as susceptible. Mohapatra *et al.* (2016) screened 48 rice genotypes at National Rice Research Institute, Cuttack during 2014-15 using the method described by Bentur and Kalode (1996). The Cuttack population of GM is considered as biotype 2. Earlier, Krishnaiah *et al.* (1983), found cultivation of gallmidge-resistant varieties such as Surekha and Phalguna in 70% of the rice growing areas in gallmidge-endemic districts in Telangana and north coastal districts in Andhra Pradesh, reduced pest incidence considerably, resulting almost 45 % increase in yield. This denotes the impact and importance of gall midge resistant varieties in reducing the gall midge incidence.

Kumar *et al.* (2020) found "Nil" damage for gall midge in phenotypic screening of IBT MRR 18, IBT MRR 23 and IBT MRR 24 with highly resistant reaction and 6 rice entries namely, IBT MRR 17, IBT MRR 19, IBT MRR 20, IBT MRR 21, IBT MRR 22 and IBT MRR 28 were found resistant to gall midge. Among 83 elite rice genotypes screened in the field by Kumar *et al* (2022), the genotypes WGL-1789, WGL-1790, WGL-1798 and WGL-1800 were found highly resistant and WGL-1767, WGL-1778, WGL-1782 and WGL- 1792 were found to be resistant to gall midge.

### Genotyping for Gall Midge Resistance

Among the nineteen rice varieties used in the present study, the rice variety Sheetal showed high level of gall midge resistance (Table 4) by possessing three gall midge genes like *gm3* (*Gm3 del3*) (Fig. 1), *Gm4* (*Gm4 LRR*) and *Gm8* (*PRP*). The varieties like Orugallu, Bhadrakali, Shiva, Kesava and Ramappa were possessing only *gm3* gene (Table 4), while one rice variety namely WGL-915 was possessing only *Gm4* gene (Table 4).

Similar to our study, earlier, Venkanna *et al.* (2018) used functional markers for genotyping of gall midge resistance and confirmed the presence of the resistant allele of *Gm1*, *gm3* and *Gm8* genes in the $F_1$ and subsequent generations. Gene expression studies carried out recently by Rawat *et al* (2010, 2012) suggested that the involvement of typical pest-induced phenyl propanoid-mediated resistance in the rice variety Suraksha carrying *Gm11* gene possessing HR+ resistance, while the genes of this pathway are not modulated in the variety Kavya possessing *Gm1* gene providing HR– type resistance. Pasalu and Rajamani, 1996 reported that *Gm4* gene showed resistance against the biotypes GMB1, GMB2, GMB3 and GMB4.

## CONCLUSION

The rice varieties like Sheetal, Orugallu, Bhadrakali, Shiva, Kesava, Ramappa and WGL-915 were promising and showed resistance to gall midge at both phenotypic and genotypic level (Table 4). Hence, they can be used as donor parents in varietal development programs for development of gall midge resistant rice varieties. The remaining 13 rice varieties (Table 4), even though they were earlier reported as resistant to gall midge, they showed susceptible reaction to gall midge in the present study because of the breakdown of resistance due to evolution of new rice biotypes.

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