Marker Assisted Introgression of Gall Midge (Gm4) and Bacterial Blight (xa13) Resistant Genes in to Tellahamsa Rice Cultivar


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ABSTRACT

Tellahamsa is a high yielding, long-slender (LS) grain type rice variety with 120 days of duration. However, it is highly susceptible to rice Gall Midge (GM) and Bacterial Blight (BB). In order to improve Tellahamsa for resistance against Gm and BB, a gene each conferring resistance against Gm (i.e. Gm4) and BB (i.e. xa13) was introgressed into Tellahamsa. An introgression line of Samba Mahsuri (RP1) possessing Gm4 and xa13 genes and with fine-grain type was used as donor parent in a backcross breeding strategy for targeted introgression of the resistance genes. PCR based molecular markers RM547, RM22554 and LRR-del for Gm4 and xa13 promoter for xa13 genes were used for foreground selection of target genes in F1, BC1F1, BC2F1 and BC3F1 generations, while 123 rice microsatellite markers polymorphic between the donor and recurrent parent were used to identify the best backcross plants, which not only possess the two target genes, but also have maximum recovery of recurrent parent genome at each generation. At BC3F6, four backcross derived line viz., WGL-1145, WGL-1146, WGL-1147 and WGL-1150 possessing Gm4 and xa13 genes, high yield, long-slender grain type, recurrent parent genome recovery ranging from 88.8-98.6% and closely resembling Tellahamsa were selected and advanced for further evaluation.

KEYWORDS: Bacterial blight, Gall midge resistance, MAS, Tellahamsa


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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

Rice (Oryza sativa L.) is an important staple food crop of India and is cultivated under different situations. The crop is affected by several biotic and abiotic stresses leading to instability in yields. The Asian rice gall midge (RGM), Orseolia oryzae (Wood-Mason) (Diptera: Cecidomyiidae), is a major pest of rice and widely spread in Asia, causing significant yield losses (Widowsky and O’Toole, 1996). Since the larvae of the insect feed inside the plant and remain enclosed within the galls, chemical control is not very effective. The exploitation of host plant resistance to RGM is an economical and environment-friendly approach to manage the pest (Khush, 1997). To date, eleven major resistance genes designated Gm1-11, that confer resistance to RGM populations have been identified, mostly in South Asia (Bentur et al., 2016). Of the eleven resistance genes, nine genes (Gm1, Gm2, gm3, Gm4, Gm5, Gm6, Gm7, Gm8, and Gm11) have been mapped to rice chromosomes (Yasala et al., 2012). Four of these genes, Gm2 (NBARC), gm3 (NB-ARC), Gm4 (NB-LRR), and Gm8 (PRP), have been functionally validated (Khush, 1997; Sama et al., 2014; Divya et al., 2018). Among the gall midge resistance genes, Gm4, on chromosome 8 from Abhaya (Mohan et al., 1997) is a major, dominant resistance gene conferring broad spectrum resistance against many GM biotypes 1, 2, 3, 4 and 4M of the insect pest existing in India (Vijayalakshmi et al., 2006; Dutta et al., 2014). Earlier, Divya et al. (2015) identified a gene encoding a leucine-rich repeat (LRR) domain containing protein was observed to be candidate for the Gm4 gene and a functional marker, LRR-del was developed for the detection of the gene. Many earlier studies have shown that through marker assisted breeding, Gm4 can be introgressed into elite rice varieties (Balachiranjeevi et al., 2015b).

Similarly, Bacterial Blight (BB) disease caused by a bacterium, Xanthomonas oryzae pv. oryzae is one of the most devastating diseases in rice and causes yield losses ranging from 74% to 81% based on severity of the disease (Mew, 1987; Srinivasan and Gnanamanickam, 2005). This disease primarily occurs in epidemic proportions in monsoon (wet) season, particularly in irrigated and rain-fed lowland ecosystems (Laha et al., 2009). Analyses of disease survey data from the past 34 years in several rice growing regions of India indicate that the disease has increased in both intensity and geographical distribution, as exemplified by several reports of BB occurrence in recent years in epidemic form (Laha et al., 2016). Chemical control against this disease has not been very successful in spite of extensive evaluation of several chemicals and antibiotics (Laha et al., 2009). Therefore, major emphasis is placed on the development and deployment of BB-resistant rice varieties (Khush et al., 1989). To date, at least 46 BB resistance genes have been identified and some of them viz., Xa4, xa5, xa13, Xa21 have been extensively used for development of BB resistant rice varieties and these provide abundant genetic resources for BB resistance breeding (Hutin et al., 2015; Balachiranjeevi et al., 2018; Yugander et al., 2018; Neelam et al., 2019; Chukwu et al., 2020). Among the BB resistance genes, xa13 has been tagged, mapped and cloned and a PCR-based functional marker xa13 Pro, (Hajira et al., 2016). Many earlier studies have shown that through marker-assisted breeding, xa13 can be introgressed into elite rice varieties (Sundaram et al., 2008; 2009). Considering all these points, the present study is aimed for introgression of gall midge (Gm4) and bacterial blight (xa13) resistance genes in to the genetic background of Tellahamsa.

2. MATERIALS AND METHODS

The present study was initiated during kharif, 2010 with the objective to improve gall midge and BB resistance of Tellahamsa through marker-assisted backcross breeding coupled with phenotypic selection for agro-morphological traits.

2.1. Plant material

An introgression line of Samba Mahsuri (i.e. RP1=B95-1×Abhaya) possessing xa13 and Gm4 genes in homozygous condition was used as the donor parent for BB and gall midge resistance, while a well-adapted popular rice variety, Tellahamsa (C10754; Parentage: HR 12×TN-1) released in 1968 from Acharya NG Ranga Agricultural University (ANGRAU), Rajendranagar, Hyderabad, Telengana State, India was chosen as the recurrent parent. Taichung Native 1 (TN1) was used as a susceptible check, while screening the backcross derived lines for gall midge and BB resistance.

2.2. Crossing scheme

RP 1 (i.e. B95-1×Abhaya) was used as the male parent and crossed with Tellahamsa (C10754) during kharif, 2010. The F1's were screened with PCR based molecular markers linked to the target genes for selection of plants possessing the resistance allele of Gm4 and xa13 genes in heterozygous condition. The selected F1 plants were used as male parents and backcrossed to Tellahamsa (C10754) to generate BC1F1 plants, which were then screened with the gene linked markers to identify the plants which are heterozygous for Gm4 and xa13 genes. The process of marker assisted backcross breeding strategy was continued till BC4 generation. The selected BC1, BC2, BC3 and BC4 plants were selfed to generate BC1F2, which were then screened with the gene linked markers to identify the plants which are homozygous for Gm4 and xa13 genes. The homozygous BC1F2 plants were then selfed to generate BC1F3, BC1F4, and BC1F5 generations and at each generation the improved lines were selected based on high gall midge and BB resistance, fine-
2.3. Screening for Gall midge resistance

For Phenotypic screening of Gall midge resistance, backcross derived lines of Tellahamsa along with parents and susceptible check (TN1) were raised under field conditions. All the recommended agronomic practices for rice cultivation were followed except application of any insecticide throughout the crop growth during kharif, 2018. Symptoms on plants were scored on 30 and 50 days after transplanting based on percent of silver shoot damage. Test entries with nil damage and up to 5% silver shoot damage were considered as resistant while others were grouped as susceptible (Vijaya Lakshmi et al., 2006). Scoring was done as per Standard Evaluation System (SES) (Anonymous, 1996).

2.4. Screening for BB resistance

Donor and recurrent parents along with backcross derived lines of Tellahamsa were screened for their bacterial blight resistance under field conditions by inoculating plants with Xoo isolate (DX002) at maximum tillering stage (Kauffman et al., 1973) and measurement of BB lesion length, the disease score was also calculated as per IRRI-standard evaluation system (IRRI-SES) scale Anonymous, 2014.

2.5. Marker assisted selection for Gall midge and BB resistance

For targeted introgression of Gm4 and xa13 into Tellahamsa, a marker-assisted backcross breeding program was adopted. Backcrossing was done till BC$_3$, generation, after which the plants were advanced through pedigree method. DNA was isolated from the parents and backcross progenies by following the protocol of Zheng et al., (1995). The PCR based SSR marker xa13-Prom (Chu et al., 2006; Hajira et al., 2016) for xa13 gene and SSR markers like RM547 (Himabindu, 2010), RM22554 and LRR-del (Divya et al., 2015) for Gm4 gene were used to identify the allelic status with respect to xa13 and Gm4 genes at F$_1$, and subsequent backcross generations. PCR was performed using 1 U of Taq DNA polymerase (Fermentas, Lithuania) and 1x 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 30 s, annealing at 55°C for 30 s, extension at 72°C for 1 min and a final extension of 7 min at 72°C. The amplified product of xa13-Prom and LRR-del were electrophoretically resolved on a 1.2% Seakem LE® agarose gel (Lonza, USA), while the amplicons of RM547 and RM22554 (Gm4) and parental polymorphic markers used for background selection were resolved on a 3.5% Seakem LE® Agarose gels containing 0.5 mg ml$^{-1}$ of ethidium bromide in 0.5x TBE buffer and visualized under UV.

2.6. Evaluation of agro morphological characters

Thirty-day-old seedlings of the selected backcross derived lines were transplanted in the main field at a spacing of 15x20 cm$^2$ along with the donor and recurrent parents. Standard agronomic practices were followed to raise a healthy crop, which were evaluated during the wet season (June–November) in 2018. Data were recorded for the agronomic traits, viz., days to 50% flowering (DFF), mean days to maturity, mean plant height (cm), number of productive panicles plant$^{-1}$, panicle weight (g), spikelet fertility (%), panicle length (cm), grain yield plant$^{-1}$ (g), 1000 seed weight (g) and grain type.

3. RESULTS AND DISCUSSION

Earlier, Gopalakrishnan et al. (2008), Basavaraj et al. (2010), Hari et al. (2011), Balachiranjeevi et al. (2015a) developed an improved version of an elite Basmati rice variety, Pusa Basmati 1, Pusa RH10, KMR-3R and DRR 17B respectively, for BB resistance through MAS coupled with phenotypic selection for agro-morphological traits using a strategy similar to that of ours. For gall midge resistance use of host plant resistance is the most effective way of control and thus gall midge resistance breeding has taken priority in rice improvement programs (Bentur et al., 2003). The PCR based DNA markers used in the present study (i.e. xa13 promoter and RM547, RM22554 and LRR-del) were tightly linked to xa13 and Gm4 genes (Chu et al., 2006; Hajira et al., 2016; Himabindu, 2010; Divya et al., 2015), respectively and hence able to identify the double positive plants precisely without any false positives at any stage of MABB. Similar to our study, earlier, Balachiranjeevi et al. (2015b) improved an elite maintainer line of DRR 17B for bacterial blight (Xa21) and gall midge (Gm4) resistance through marker assisted selection. Recently, Jamaloddin et al. (2020) improved Tellahamsa for BB (Xa21+xa13 genes) and Blast (Pi54+Pi1 genes) resistance through Marker Assisted Backcross Breeding (MABB), but in the present study we report the improvement of Tellahamsa for Gall midge (Gm4) and BB (xa13) resistance through MABB.

The F$_s$ generated from the cross, RP1/Tellahamsa were screened for presence of the target resistance genes Gm4 and xa13 using the gene-linked molecular markers RM547 and xa13 promoter, respectively to identify the ‘true’ F$_s$ showing heterozygous amplification pattern. Out of 115 F$_s$, eight plants were observed to possess both the target resistance genes, i.e. Gm4 and xa13 in heterozygous condition and these were then used as male parent and backcrossed to Tellahamsa to generate BC$_1$F$_1$ plants. Out of 693 BC$_1$F$_1$ plants, a total of 341 were identified to be positive for xa13, 292 were positive for Gm4 and 11 were identified to be double positive for both Gm4 and xa13 genes (Figure 1A and 1B) using the gene-linked markers. The 11 BC$_1$F$_1$
Figure 1: Foreground selection of BC1F1 plants for presence of target traits by using gene linked/functional markers; A: Gm4 gene by using RM547; B: xa13 gene by using functional marker xa13 promoter

plants (which were heterozygous for xa13 and Gm4) were then subjected for background selection using 123 parental polymorphic SSR markers and a single ‘positive’ BC1F1 plant # RPT 9 possessing maximum recovery of recurrent parent genome (73%) was selected and then backcrossed with Tellahamsa to generate BC2F1 plants. A similar marker-assisted selection procedure was followed for selection of BC2F1 and BC3F1 plants and a total of six BC3F1 plants (which were heterozygous for xa13 and Gm4) were identified. The selected double positive BC3F1 plants were then subjected to background selection and a single ‘positive’ BC3F1 plant # RPT 9-143-32 possessing maximum recovery of recurrent parent genome (98%) was selected and selfed to generate BC3F2 plants. Out of 1365 BC3F2 plants, 175 plants were observed to be homozygous for both xa13 and Gm4 genes. These 175 BC3F2 plants were then advanced from BC3F2 to BC3F6 generations by following pedigree based method. At BC3F6 generation we identified four backcross derived improved lines namely i.e. WGL1145, WGL1146, WGL1147 and WGL1150 (Figure 2) displayed high level of gall midge and BB resistance (Figure 3) on par with donor parent and high yield (Table 1) as compared to the original recurrent parent, while one improved line namely WGL1145 displayed durable resistance to Gall midge biotypes (IRR, 2018, progress report 2017, Volume 2, Entomology) prevalent in Warangal conditions of Telangana State, India. Earlier, Pushparajan et al. (2011) carried out association mapping for salinity tolerance in rice by using molecular markers.

3.1. Gall midge and BB resistance reaction of the selected backcross derived lines of Tellahamsa

All the four selected double positive BC3F6 lines along with donor and recipient parents were phenotypically screened for Gall midge and BB resistance. The donor parent RP1, which possesses Gm4 gene showed high level of resistance to rice Gall midge with ‘0’ % galls on tiller basis and the recurrent parent Tellahamsa, showed presence of 21.3 % galls on tiller basis (Table 1), while all the four backcross derived lines viz., WGL-1145, WGL-1146, WGL-1147 and WGL-1150, displayed a high level of resistance to gall midge without any galls on their leaves with a score of ‘0’% galls on tiller basis (Table 1). Similarly, when phenotypically screened

Figure 2: Genotype of BC3F6 plants for presence of target traits by using gene linked/functional markers; Figure A: Gm4 gene by using RM547; B: Gm4 gene by using RM22554; C: Gm4 gene by using LRR-del; D: xa13 gene by using xa13 promoter

Figure 3: Inoculation of Xoo isolate (DX002) under field conditions; 1: RP1; 2: Tellahamsa; Improved Tellahamsa lines viz., 3: WGL-1145; 4: WGL-1146; 5: WGL-1147; 6: WGL-1150; Leaf clipping method of inoculation developed by Kauflmann et al., 1973
Table 1: Phenotypic screening of BC₁F₆ lines for gall midge resistance during kharif, 2018 at RARS, Warangal

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Entry no.</th>
<th>30 DAT</th>
<th>50 DAT</th>
<th>30 DAT</th>
<th>50 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% Damage on hill basis</td>
<td>% Galls on tiller basis</td>
<td>% Damage on hill basis</td>
<td>% Galls on tiller basis</td>
</tr>
<tr>
<td>1.</td>
<td>Tellahamsa (recurrent parent)</td>
<td>50</td>
<td>16.99</td>
<td>80</td>
<td>21.27</td>
</tr>
<tr>
<td>2.</td>
<td>RP1 (i.e. B95-1× Abhaya) (donor parent)</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>3.</td>
<td>TN-1 (Control)</td>
<td>100</td>
<td>23.00</td>
<td>100</td>
<td>22.80</td>
</tr>
<tr>
<td>4.</td>
<td>WGL-1145</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>5.</td>
<td>WGL-1146</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>6.</td>
<td>WGL-1147</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>7.</td>
<td>WGL-1150</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Backcross derived improved lines of Tellahamsa

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Entry no.</th>
<th>30 DAT</th>
<th>50 DAT</th>
<th>30 DAT</th>
<th>50 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.</td>
<td>WGL-1145</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>5.</td>
<td>WGL-1146</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>6.</td>
<td>WGL-1147</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>7.</td>
<td>WGL-1150</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Table 2: Yield and agronomic performance of the parents, improved lines of Tellahamsa under field conditions without gall midge and BB incidence

<table>
<thead>
<tr>
<th>Sl. no.</th>
<th>Name of the parent/ Cross</th>
<th>Days to 50% flowering (DFF)</th>
<th>Mean days to maturity</th>
<th>Mean plant height (cm)</th>
<th>No. of productive panicles plant⁻¹</th>
<th>Panicle weight (g)</th>
<th>Panicle length (cm)</th>
<th>Grain yield plant⁻¹ (g)</th>
<th>1000 seed weight (g)</th>
<th>Grain type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Tellahamsa (recurrent parent)</td>
<td>95</td>
<td>125</td>
<td>103.2</td>
<td>12</td>
<td>1.85</td>
<td>21.22</td>
<td>17.99</td>
<td>21.42</td>
<td>LS</td>
</tr>
<tr>
<td>2.</td>
<td>RP1 (i.e. B95-1× Abhaya) (donor parent)</td>
<td>112</td>
<td>142</td>
<td>98.26</td>
<td>12</td>
<td>1.83</td>
<td>19.74</td>
<td>18.24</td>
<td>12.99</td>
<td>MS</td>
</tr>
<tr>
<td>4.</td>
<td>WGL-1145</td>
<td>97</td>
<td>127</td>
<td>106.5</td>
<td>13</td>
<td>2.12</td>
<td>21.6</td>
<td>18.13</td>
<td>21.82</td>
<td>LS</td>
</tr>
<tr>
<td>5.</td>
<td>WGL-1146</td>
<td>95</td>
<td>125</td>
<td>102.4</td>
<td>12</td>
<td>2.37</td>
<td>22.5</td>
<td>18.73</td>
<td>22.01</td>
<td>LS</td>
</tr>
<tr>
<td>6.</td>
<td>WGL-1147</td>
<td>95</td>
<td>125</td>
<td>104.6</td>
<td>12</td>
<td>2.06</td>
<td>21.2</td>
<td>18.01</td>
<td>21.32</td>
<td>LS</td>
</tr>
<tr>
<td>7.</td>
<td>WGL-1150</td>
<td>98</td>
<td>128</td>
<td>105.6</td>
<td>12</td>
<td>2.03</td>
<td>21.3</td>
<td>18.04</td>
<td>21.12</td>
<td>LS</td>
</tr>
</tbody>
</table>

* MS and LS indicate medium slender and long slender, respectively; Note: RP1 (i.e. B95-1×Abhaya) donor line for bacterial blight and gall midge resistance; Tellahamsa: recipient parent; backcross derived improved Tellahamsa lines viz., WGL-1145, WGL-1146; WGL-1147 and WGL-1150
successful in identifying the plants which not only possessed gall midge and BB resistance but also long-slim grain type. Earlier, Joseph et al. (2004), Gopalakrishnan et al. (2008), Sundaram et al. (2008), Hari et al. (2011) adopted a strategy of morphology based selection for grain type coupled with marker-based selection of target trait (i.e. bacterial blight resistance) while developing improved versions of Pusa Basmati-1, Samba Mahsuri and KMR-3R. A similar approach was adopted in the present study and it was observed that the grain quality characters of the improved lines of Tellahamsa were on par with recipient parent with marginal differences. In the improved lines of Tellahamsa, no apparent yield penalty associated with presence of the BB and gall midge resistance genes \(xa13\) and \(Gm4\), respectively was noticed (Table 2).

4. CONCLUSION

All the four backcross derived lines \textit{viz.}, WGL-1145, WGL-1146, WGL-1147 and WGL-1150, displayed a high level of gall midge resistance without any galls on their leaves with a score of 0% galls on tiller basis and also displayed a high level of bacterial blight resistance equivalent to the donor parent with average lesion lengths of 3.65 cm, with a disease score of 3 in each case. The near-complete recovery of yield, grain quality characters in the improved lines of Tellahamsa lines along with bacterial blight and gall midge resistance is a significant achievement of this study.

5. FURTHER RESEARCH

Pre-breeding lines in the genetic background of Tellahamsa possessing twin characteristics of bacterial blight and gall midge resistance will be available for use as donors in conventional breeding programs. Elite breeding lines in the genetic background of Tellahamsa possessing twin characteristics of bacterial blight and gall midge resistance would replace the existing/original varieties of Tellahamsa.

6. ACKNOWLEDGEMENT

The research was conducted with the kind and a support from the Professor Jayashankar Telangana State Agricultural University (PJTSAU) is gratefully acknowledged. The donor parent seeds i.e. RP1=B95-1×Abhaya (possessing \(xa13\) and \(Gm4\) genes in homozygous condition) and Xoo isolate (DX002) for BB resistance obtained from Indian Institute of Rice Research (IIRR), Rajendranagar, Hyderabad is gratefully acknowledged.

7. REFERENCES

bacterial blight resistance genes, *Xa21* and *Xa33* into an elite maintainer line of rice, DRR17B. PLoS One 13(10), 1–16.


