International Journal of Bio-resource and Stress Management https://pphouse.org/ijbsm.php

Crossref

IJBSM February 2022, 13(2):197-204 Print ISSN 0976-3988 Online ISSN 0976-4038 **Article** AR2704

Research Article *Stress Management*

DOI: HTTPS://DOI.ORG/10.23910/1.2022.2704

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

Y. Hari, K. N. Yamini, K. Rukmini Devi, B. Satish Chandra, V. Venkanna, S. Malathi, A. Venkat Reddy, R. Shravan Kumar, N. Lingaiah, C. Cheralu, Ch. V. Durga Rani, A. Srividya , K. Rajendra Prasad, P.Raghu Rami Reddy, P. Jagan Mohan Rao, R. Uma Reddy and U. Nagabhushanam

Regional Agricultural Research Station, Prof. Jayashankar Telangana State Agricultural University (PJTSAU), Warangal, Telangana State (506 007), India

Open Access

Corresponding itsyhari@yahoo.co.in

0000-0001-8005-4815

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 F_1, BC_1F_1, BC_2F_1 and BC_3F_1 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 BC_3F_6, 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

***Citation* (VANCOUVER):** Hari et al, Marker Assisted Introgression of Gall Midge (*Gm4*) and Bacterial Blight (*xa13*) Resistant Genes in to Tellahamsa Rice Cultivar. *International Journal of Bio-resource and Stress Management*, 2022; 13(2), 197-204. HTTPS://DOI.ORG/10.23910/1.2022.2704.

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.

Conflict of interests: The authors have declared that no conflict of interest exists.

RECEIVED on 31st October 2021 RECEIVED in revised form on 02nd February 2022 ACCEPTED in final form on 25th February 2022 PUBLISHED on 28th February 2022

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 *Gm*1-11, 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 F_1s 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 F_1 plants were used as male parents and backcrossed to Tellahamsa (C10754) to generate BC_1F_1 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 BC_3 generation. The selected BC_3F_1 plants were selfed to generate BC_3F_2, which were then screened with the gene linked markers to identify the plants which are homozygous for *Gm4* and *xa13* genes. The homozygous BC_3F_2 plants were then selfed to generate BC_3F_4, BC_3F_5 and BC_3F_6 generations and at each generation the improved lines were selected based on high gall midge and BB resistance, fine-

grain type (i.e. long-slender grain type) and yield through phenotype based selection coupled with marker assisted selection for gall midge and BB resistance.

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 15×20 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) reistance 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_1s 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_1s showing heterozygous amplification pattern. Out of 115 F_1s, 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_1F_1 plants. Out of 693 BC_1F_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_1F_1

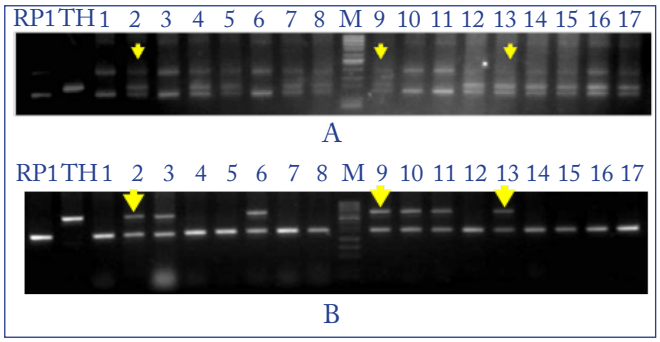

Figure 1: Foreground selection of BC_1F_1 plants for presence of target traits by using gene linked/functional markers; A: *Gm4* gene by using RM547; B: *xa13* gene by using functional marker *xa13* promoter

plants (which were heterozygous for *xa13* and *Gm4*) were then subjected for background selection using 123 parental polymorphic SSR markers and a single 'positive' BC_1F_1 plant # RPT 9 possessing maximum recovery of recurrent parent genome (73%) was selected and then backcrossed with Tellahamsa to generate BC_2F_1 plants. A similar marker-assisted selection procedure was followed for selection of BC_2F_1 and BC_3F_1 plants and a total of six BC_3F_1 plants (which were heterozygous for *xa13* and *Gm4*) were identified. The selected double positive BC_3F_1 plants were then subjected to background selection and a single 'positive' BC_3F_1 plant # RPT 9-143-32 possessing maximum recovery of recurrent parent genome (98%) was selected and selfed to generate BC_3F_2 plants. Out of 1365 BC_3F_2 plants, 175 plants were observed to be homozygous for both *xa13* and *Gm4* genes. These 175 BC_3F_2 plants were then advanced from BC_3F_2 to BC_3F_6 generations by following pedigree based method. At BC_3F_6 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 (IIRR, 2018, progress report 2017, Volume 2, Entomology) prevalent in Warangal conditions of Telangana State, India. Earlier, Pushparajan et al. (2011) carried out

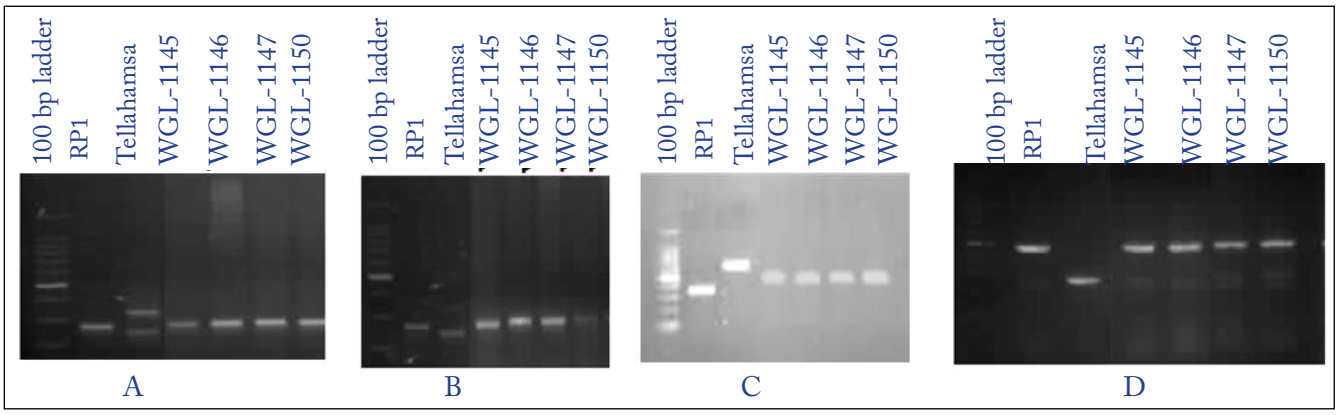

Figure 2: Genotype of BC3F6 plants for presence of target traits by using gene linked/ functional markers; Figure A: *Gm4* gene by using RM547; B: *Gm4* gene by using RM22554; C: *Gm4* gene by using LRR-del; D: *xa13* gene by using *xa13* promoter

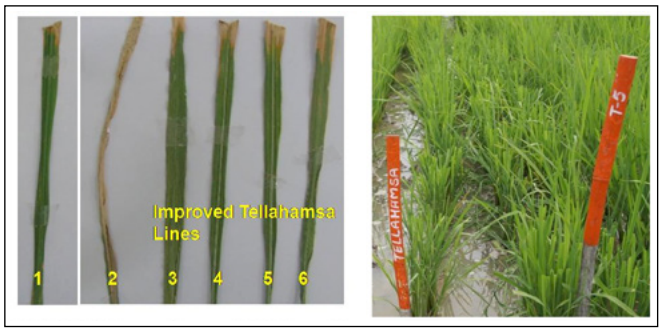

Figure 3: Inoculation of *Xoo* isolate (DX002) under field conditions; 1: RP1; 2: Tellahamsa; Improved Tellahamsa lines viz., 3: WGL-1145; 4: WGL-1146; 5: WGL-1147; 6: WGL-1150; Leaf clipping method of inoculation developed by Kauffmann et al., 1973

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 BC_3F_6 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

Table 1: Phenotypic screening of BC_3F_6 lines for gall midge resistance during *kharif*, 2018 at RARS, Warangal

Sl. No.	Entry no.	30 DAT		50 DAT	
		% Damage on hill basis	% Galls on tiller basis	% Damage on hill basis	% Galls on tiller basis
1.	Tellahamsa (recurrent parent)	50	16.99	80	21.27
2.	RP1 (i.e. B95-1× Abhaya) (donor parent)	0	0.00	0	0.00
3.	TN-1 (Control)	100	23.00	100	22.80
Backcross derived improved lines of Tellahamsa					
4.	WGL-1145	0	0.0	0	0.0
5.	WGL-1146	0	0.0	0	0.0
6.	WGL-1147	0	0.0	0	0.0
7.	WGL-1150	0	0.0	0	0.0

with virulent isolates of the bacterial blight pathogen, the donor parent RP1 displayed an average lesion length of 1.95 cm with a disease score of 3, while the recipient parent Tellahamsa displayed an average lesion length of 23.1 cm with a disease score of 9 and all the four backcross derived lines WGL-1145, WGL-1146, WGL-1147 and WGL-1150 (Figure 3) displayed a high level of resistance equivalent to the donor parent with average lesion lengths of 3.65 cm, with a disease score of 3 in each case.

3.2. Yield and agronomic performance of improved parental lines

The lines *viz.*, WGL-1145, WGL-1146, WGL-1147 and WGL-1150, which showed high level of resistance to gall midge and bacterial blight were evaluated for grain yield during the *kharif,* 2018. The backcross derived improved lines exhibited grain yields on par with Tellahamsa (7.21 kg 10 m^{-2}) with marginal differences (Table 2).

However, no significant variation was observed with respect to the number of productive tillers $plant^{-1}$, panicle weight, panicle length, spikelet fertility and grain yield $plant^{-1}$ among the backcross derived lines as compared to Tellahamsa (Table 2).

We resorted to phenotype based visual selection for long-slender grain type (Figure 4A and 4B), starting from BC_1F_1 generation onwards and due to a stringent selection involving screening a large number of backcross plants,

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

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

* 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

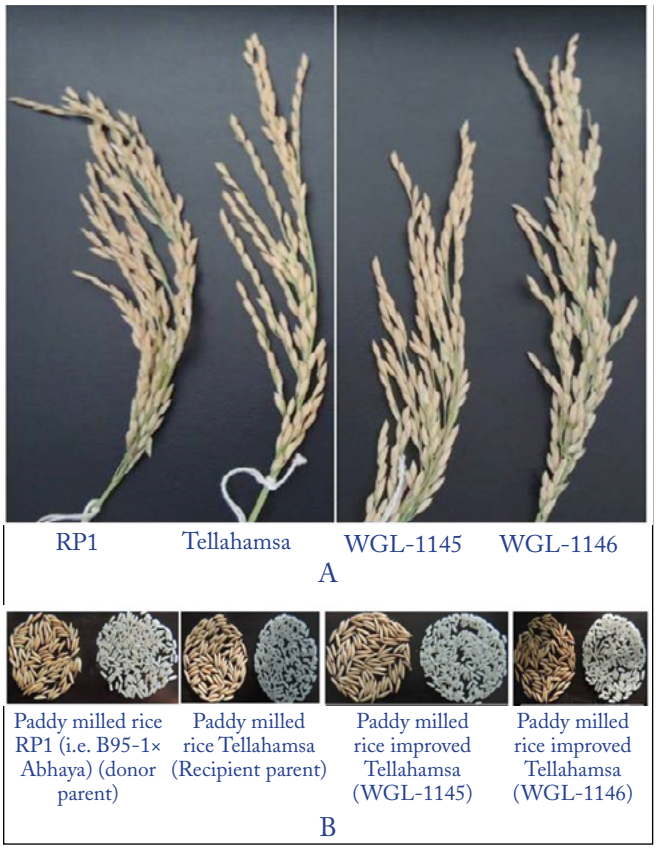

Figure 4A: Panicle types of improved Tellahamsa lines along with parents; 4B: Paddy and milled rice of improved Tellahamsa lines along with parents

successful in identifying the plants which not only possessed gall midge and BB resistance but also long-slender 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 *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

Anonymous, 1996. Standard evaluation system for rice, 4th edn. International Rice Research Institute, Manila, Philippines.

Anonymous, 2014. Standard evaluation system for rice, International Rice Research Institute, Manila, Philippines.

Balachiranjeevi, C.H., Bhaskar Naik, S., Abhilash, V., Akanksha, S., Viraktamath, B.C., Madhav, M.S., Hariprasad, A.S., Laha, G.S., Prasad, M.S., Balachandran, S.M., Neeraja, C.N., Satendra Kumar M., Senguttuvel, P., Kemparaju, K.B., Bhadana, V.P., Ram, T., Harika, G., Mahadeva swamy, H.K., Hajira, S., Yugander, A., Pranathi, K., Anila, M., Rekha, G., Kousik, M.B.V.N., Dilipkumar, T., Swapnil, R.K., Giri, A., Sundaram, R.M., 2015a. Marker-assisted introgression of bacterial blight and blast resistance into DRR17B, an elite, fine-grain type maintainer line of rice. Molecular Breeding 35(151), 1–12.

Balachiranjeevi, C.H., Bhaskar Naik, S., Abhilash, V., Harika, G., Mahadev Swamy, H.K., Hajira, S., Dilip Kumar, T., Anila, M., Kale, R.R., Yugender, A., Pranathi, K., Koushik, M.B.V.N., Suneetha, K., Bhadana, V.P., Hariprasad, A.S., Laha, G.S., Rekha, G., Balachandran, S.M., Madhav, M.S., Senguttuvel, P., Fiyaz, A.R., Viraktamath, B.C., Giri, A., Swamy, B.P. M., Ali, J.,Sundaram, R.M., 2018. Marker-assisted pyramiding of two major, broad-spectrum

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

Balachiranjeevi, C.H., Bhaskar Naik, S., Abhilash, V., Mahadeva Swamy, H.K., Harika, G., Hajira, S., Pranathi, K., Anila, M., Dilipkumar, T., Rekha, G., Hari Prasad, A.S., Laha, G.S., Madav, M.S., Giri. A., Sundaram, R.M., 2015b. Improvement of an elite maintainer line DRR17B for bacterial blight and Gall midge resistance through marker assisted selection. Annals of Plant and Soil Research 17, 605–608.

Basavaraj, S.H., Singh, V.K., Singh, A., Singh, A., Singh, A., Anand, D., Yadav, S., Ellur, R.K., Singh, D., Gopala Krishnan, S., Nagarajan, M., Mohapatra, T., Prabhu, K.V., Singh, A.K., 2010. Marker-assisted improvement of bacterial blight resistance in parental lines of Pusa RH10, a superfine grain aromatic rice hybrid. Molecular Breeding 26, 293–305.

Bentur, J.S., Pasalu, I.C., Sarma, N.P., Prasadarao, U., Mishra, B., 2003. Gall midge resistance in rice. DRR research Paper Series 01/2003. Directorate of rice research, Hyderabad, 20.

Bentur, J.S., Rawat, N., Divya, D., Sinha, D.K., Agarrwal, R., Atray I., Nair, S., 2016. Rice gall midge interactions: battle for survival. Journal of Insect Physiology 84, 40–49.

Chen, S.C., Lin, G., Xu, X.H., Zhang, Q., 2001. Improving bacterial blight resistance of 6078, an elite restorer line of hybrid rice, by molecular marker-aided selection. Plant Breeding 120, 133–137.

Chu, Z., Yuan, M., Yao, J., Ge, , Yuan, B., Li, C., Xu, X., Li, B., Fu, Z., Bennetzen, J.L., Zhang, Q., Wang, S., 2006. Promoter mutations of an essential gene for pollen development result in disease resistance in rice. Genes and Development 20, 1250–1255.

Chukwu, S.C., Rafii, M.Y., Ramlee, S.I., Ismail, S.I., Oladosu Y., Muhammad, I.L., Ibrahim, M., Muideen, A., Jatto, M.I., Yusuf, B.R., 2020. Recovery of recurrent parent genome in a marker assisted backcrossing against rice blast and blight infections using functional markers and SSRs. Plants 9, 1–15.

Divya, D., Himabindu, K., Nair, S., Bentur, J.S., 2015. Cloning of a gene encoding LRR protein and its validation as candidate gall midge resistance gene, *Gm4*, in rice. Euphytica 203, 185–195.

Divya, D., Madhavi, K.R., Dass, M.A., Maku, R.A., Mallikarjuna, G., Sundaram, R.M., Laha, G.S., Padmakumari, A.P., Patel, H.K., Prasad, M.S., Sonti, R.V., Bentur, J.S., 2018. Expression profile of defense genes in rice lines pyramided with resistance genes against bacterial blight, fungal blast and insect gall midge. Rice 11(40), 1–13.

Dutta, S.S., Divya, D., Durga Rani, Ch. V., Dayakar Reddy, T., Visalakshmi, V., Cheralu, C., Singh, I.K., Bentur, J.S., 2014. Characterization of gall midge resistant rice genotypes using resistance gene specific markers. Journal of Experimental Biology and Agricultural Sciences 2, 2320–8694.

Gopalakrishnan, S., Sharma, R.K., Anand Rajkumar, K., Joseph, M., Singh, V.P., Singh, A.K., Bhat, K.V., Singh, N.K., Mohapatra, T., 2008. Integrating marker assisted background analysis with foreground selection for identification of superior bacterial blight resistant recombinants in Basmati rice. Plant Breeding 127, 131–139.

Hajira, S., Sundaram, R.M., Laha, G.S., Yugander, A., Balachandran, S.M., Viraktamath, B.C., Sujatha, K., Balachiranjeevi, C.H., Pranathi, K., Anila, M., Bhaskar Naik, S., Abhilash, V., Mahadevaswamy, H.K., Kousik, M.B.V.N., Dilip Kumar, T., Harika, G., Rekha, G., 2016. A Single tube, functional marker-based multiplex PCR assay for simultaneous detection of major bacterial blight resistance genes in rice, *Xa21, xa13* and *xa5*. Rice Science 23, 1224–1227.

Hari, Y., Srinivasarao, K., Viraktamath, B.C., Hariprasad, A.S., Laha, G.S., Ilyas Ahmed, M., Natarajkumar, P., Ramesha, M.S., Neeraja, C.N., Balachandran, S.M., Shobha Rani, N., Balaji Suresh, P., Sujatha, K., Manish Pandey, M., Ashok Reddy, G., Madhav, M.S., Sundaram, R.M., 2011. Marker-assisted improvement of a stable restorer line, KMR-3R and its derived hybrid KRH2 for bacterial blight resistance and grain quality. Plant Breeding 130, 608–616.

Himabindu, K., Suneetha, K., Sama, V.S. A. K., Bentur, J.S., 2010. A new rice gall midge resistance gene in the breeding line CR57-MR1523, mapping with flanking markers and development of NILs. Euphytica 174(2), 179–187.

Hutin, M., Sabot, F., Ghesquiere, A., Koebnik, R., Szurek, B., 2015. A knowledge-based molecular screen uncovers a broad-spectrum OsSWEET14 resistance allele to bacterial blight from wild rice. Plant Journal 84, 694–703.

Indian Institute of Rice Research, 2018. Progress Report, 2017, Vol.2, Crop protection (Entomology, Plant Pathology) All India Coordinated Rice Improvement Project (ICAR) ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad – 500 030, Telangana State, India.

Jamaloddin, M., Durga Rani, C.V., Swathi, G., Anuradha, C., Vanisri, S., Rajan, C.P.D., Krishnam Raju, S., Bhuvaneshwari, V., Jagadeeswar, R., Laha, G.S., Prasad, M.S., Satyanarayana, P. V., Cheralu, C., Rajani, G., Ramprasad, E., Sravanthi, P., Arun Prem Kumar,

N., Aruna Kumari, K., Yamini K.N., Mahesh, D., Sanjeev Rao, D., Sundaram, R.M., Madhav, M.S., 2020. Marker Assisted Gene Pyramiding (MAGP) for bacterial blight and blast resistance into mega rice variety Tellahamsa PLoS ONE 15(6), 1–19.

Khush, G.S., 1997. Origin, dispersal, cultivation and variation of rice. Plant Molecular Biology 35, 25–34.

Khush, G.S., Mackill, D.J., Sidhu, G.S., 1989. Breeding rice for resistance to bacterial leaf blight. In: International Rice Research Institute (IRRI). Bacterial blight of rice. Manila, Philippines, IRRI, 207–217.

Laha, G.S., Reddy, C.S., Krishnaveni, D., Sundaram, R.M., Prasad, M.S., Ram, T., Muralidharan, K., Viraktamath, B.C., 2009. Bacterial blight of rice and its management. In: DRR Technical Bulletin No. 41. Directorate of Rice Research (ICAR), Hyderabad, India, 37.

Laha, G.S., Sailaja, B., Prasad, M.S., Ladhalakshmi, D., Krishnaveni, D., Singh, R., Prakasam, V., Yugander, A., Kannan, C., Valarmathi, P., Ravindrababu, V., 2016. Changes in rice disease scenario in india: an analysis from production oriented survey. Technical Bulletin No. 91. ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad, Telangana State, India, 95.

Mew, T.W., 1987. Current status and future prospects of research on bacterial blight of rice. Annual Review Phytopathololgy 25, 359–382.

Mohan, M., Nair, S., Bhagwat, A., Krishna, T.G., Yano, M., Bhatia, C.R., Sasaki, T., 1997. Genome mapping, molecular markers and marker-assisted selection in crop. Molecular Breeding 3, 87–103.

Neelam, K., Mahajan, R., Vikas, G., Dharminder, B., Gill, B.K., Komal, R., Jagjeet Singh, L., Gurjit Singh, M., Kuldeep, S., 2020. High-resolution genetic mapping of a novel bacterial blight resistance gene *xa45*(t) identified from *Oryza glaberrima* and transferred to *Oryza sativa*. Theoretical and Applied Genetics 133(3), 689–705.

Pushparajan, N., Krishnasamy, V., Chandra Babu, R., Kannanbabu, J.R., 2011. Association mapping of salinity tolerance in rice using molecular markers. International Journal of Bio-resource and Stress Management 2(3), 307–312.

Sama, V.S.A.K., Rawat, N., Sundaram, R.M., Himabindu, K., Naik, B.S., Viraktamath, B.C., Bentur, J.S., 2014. A putative candidate for the recessive gall midge resistance gene *gm3* in rice identified and validated. Theoretical and Applied Genetics 127, 113–124.

Srinivasan, B., Gnanamanickam, S., 2005. Identification of a new source of resistance in wild rice, *Oryza rufipogon* to bacterial blight of rice caused by Indian strains of *Xanthomonas oryzae* pv. *oryzae*. Current Science 88(8), 1229–1231.

Sundaram, R.M., Vishnupriya, M.R., Biradar, S.K., Laha, G.S., Reddy, A.G., Rani, N.S., Sarma, N.P., Sonti, R.V., 2008. Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite indica rice variety. Euphytica 160, 411–422.

Sundaram, R.M., Vishnupriya, M.R., Laha, G.S., Shobha Rani, N., Srinivas Rao, P., Balachandaran, S.M., Ashok Reddy, G., Sarma, N.P., Shonti, R.V., 2009. Introduction of bacterial blight resistance into Triguna, a high yielding, mid-early duration rice variety. Biotechnology Journal 4, 400–407.

Vijayalakshmi, P., Amudhan, S., Himabindu, K., Cheralu, C., Bentur, J.S., 2006. A new biotype of the Asian rice gall midge *Orseolia oryzae* (Diptera: Cecidomyiidae) characterized from the Warangal population in Andhra Pradesh. International Journal of Tropical Insect Science 26, 207–211.

Widowsky, D.A., O'Toole, J.C., 1996. Prioritizing rice research agenda for eastern India. In: Evanson, R.E., Herdt, R.W., Hussain, M., (Eds.), Rice research in Asia: progress and priorities. International Rice Research Institute, Philippines, 109–129.

Yasala, A.K., Rawat, N., Sama, V.S.A.K., Himabindu, K., Sundaram, R.M., Bentur, J.S., 2012. In silico analysis for gene content in rice genomic regions mapped for the gall midge resistance genes. Plant Omics Journal 5, 405–413.

Yugander, A., Sundaram, R.M., Singh, K., Ladhalakshmi, D., Rao, L.V.S., Madhav, M.S., Jyothi, B., Prasad, M.S., Laha, G.S., 2018. Incorporation of the novel bacterial blight resistance gene *Xa38* into the genetic background of elite rice variety improved Samba Mahsuri. PLoS ONE 13 (5), 1–16.

Zheng, K., Subudhi, P.K., Domingo, J., Maopanty, G., Huang, N., 1995. Rapid DNA isolation for marker assisted selection in rice breeding. Rice Genetics News Letter 12, 255–258.