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MOLECULAR SCREENING OF F_1 PLANTS FOR THREE GALL MIDGE RESISTANCE GENES IN THE ELITE RICE CULTIVAR TELANGANA SONA

G. Rakesh[1]*, B. Vidhyadhar[2], Y. Chandra Mohan[3], Yadla Hari[4], R. Shravan Kumar[5], P. Gonya Naik[6]

[1] Department of Plant Breeding, PJTAU – Agricultural College, Aswaraopet- 507 301, Bhadradri Kothagudem Dist., Telangana- India.
[2] Department of Plant Breeding, PJTAU – Agricultural College, Warangal-506142, Telangana, India.
[3] Director, ARI; Principal Scientist, Rajendranagar-500030, Hyderabad, India.
[4] Agricultural Biotechnology, PJTAU – Institute of Biotechanology, Rajendranagar-500030, Hyderabad, India..
[5] Department of Entomology, PJTAU – Regional Agricultural Research Station, Warangal-506142, Telangana, India.
[6] Department of Plant Breeding, PJTAU – Regional Agricultural Research Station, Warangal-506142, Telangana, India.
***Corresponding author**: G. Rakesh; E-mail: gj.raki27@gmail.com

ABSTRACT
Telangana Sona is a popular rice cultivar known for its super fine, short-slender grains and low glycemic index (GI = 51.72). However, its susceptibility to the Asian rice gall midge (*Orseolia oryzae* Wood-Mason) poses a constraint to stable production. Marker-assisted introgression of complementary resistance genes offers a promising approach to improve gall midge resistance while retaining the desirable characteristics of this elite cultivar. In the present study, Telangana Sona and the donor line RMSGm3 were molecularly characterized using gene-specific functional markers targeting the gall midge resistance genes *gm3*, *Gm4* and *Gm8*. The markers gm3del3, LRR-del and PRP distinguished the donor and recurrent parents based on their allele sizes. RMSGm3 exhibited resistance-associated alleles of approximately 250, 350 and 300 bp, respectively, whereas Telangana Sona carried the corresponding susceptible alleles of approximately 550, 600 and 350 bp. A total of 150 F_1 seeds were obtained from the cross Telangana Sona × RMSGm3, of which 120 F_1 plants were screened during *rabi* 2024–25. Thirteen plants exhibited the expected heterozygous allelic patterns at all three target loci and were identified as triple-positive F_1 plants, accounting for 10.83% of the screened population. The selected plants retained the characteristic short-slender, super fine grain phenotype of Telangana Sona, while their plant height and heading time were broadly comparable to those of the recurrent parent. These findings establish a molecularly confirmed F_1 population carrying alleles associated with all three target resistance loci, providing a foundation for subsequent marker-assisted backcrossing and gene pyramiding in the Telangana Sona genetic background. However, molecular confirmation of the target loci alone does not establish effective or durable resistance. Further evaluation of advanced progenies, including confirmation of gene inheritance and resistance screening against diverse gall midge populations, is necessary to validate the resistance potential of the developed material.

KEYWORDS: *Oryza sativa*; *Orseolia oryzae*; Telangana Sona; *Molecular Markers, gm3*; *Gm4*; *Gm8*

1. INTRODUCTION
Rice (*Oryza sativa* L.) is a cornerstone of global food security and supplies a major share of the caloric intake of the world's population (Khush, 2013). India is a major rice producing country, and rice production is central to food and nutritional security across diverse production environments (Mondal *et al.,* 2022). Indian rice production reached approximately 152 million metric tonnes in 2025-26 (Abadam *et al.,* 2026). Within India, Telangana has emerged as an important rice growing state and is the production base of the elite cultivar RNR 15048, popularly known as Telangana Sona. Telangana Sona is valued for its high yield, super fine short slender grain and low glycemic index, and is extensively grown across Telangana; these attributes have made it popular among farmers and consumers alike.

However, the cultivar is vulnerable to biotic stresses, including the Asian rice gall midge (*Orseolia oryzae* Wood Mason). Larvae feed within the developing shoot apex and induce a characteristic sterile tubular gall, or "silver shoot." Infested tillers often fail to produce normal panicles, and severe infestation can therefore cause substantial yield losses; losses of 10-100% have been reported in endemic regions of southern India (Vijaykumar *et al.,* 2022). Management is complicated by the concealed feeding habit of the larva, making host plant resistance an important component of sustainable management.

Gall midge resistance is genetically diverse, but shifts in pest virulence can erode the effectiveness of individual resistance genes, providing a rationale for combining resistance loci rather than relying on a single gene (Bentur *et al.,* 2008). Marker-assisted selection offers an efficient means of tracking resistance associated alleles during breeding, and functional markers are particularly attractive because they target polymorphisms associated with a resistance locus or candidate gene rather than relying solely on linked markers (Venkanna et al 2018; Salgotra & Neal Stewart, 2020).

The present study focuses on three gall midge resistance loci gm3, *Gm4* and *Gm8* using the gene specific markers gm3del3, LRR-del and PRP, respectively. Previous studies have associated *gm3* with a candidate NB-ARC region, *Gm4* with an LRR candidate gene and *Gm8* with a proline-rich protein (Sama et al., 2024; Divya et al., 2015 and Divya et al., 2013). Before a marker assisted backcrossing programme can be advanced efficiently, the recurrent and donor parents must be polymorphic at the target loci, and true F_1 plants must be distinguished from selfed or otherwise non hybrid progeny. This is especially important in a pyramiding programme, because the F_1 generation provides the starting material from which the desired resistance associated alleles can be recovered and fixed in subsequent generations.

Accordingly, the present study was undertaken to: (i) molecularly characterize Telangana Sona and RMSGm3 using functional markers associated with *gm3*, *Gm4* and *Gm8*; (ii) establish parental polymorphism and marker diagnosticity; (iii) generate and molecularly validate F_1 progeny carrying the resistance-associated alleles at all three target loci; and (iv) document the preliminary agronomic and grain phenotype of the validated F_1 plants as a basis for subsequent marker assisted backcrossing and gene pyramiding.

2. MATERIALS AND METHODS

2.1 Experimental site and crop management

Hybridization and development of the F_1 population were initiated during *Kharif* 2024 at the Institute of Rice Research, Rajendranagar, Hyderabad. The soil was sandy clay loam with a pH of 7.82 (slightly alkaline), an electrical conductivity of 0.03 dS m^{-1} and an organic carbon content of 0.73%. Available soil nutrients were N 270 kg ha^{-1}, P 50 kg ha^{-1} and K 254 kg ha^{-1}. Fertilizer was applied at 120:60:40 kg N, P_2O_5 and K_2O ha^{-1}, respectively. Nitrogen was applied in three equal doses, whereas P_2O_5 and K_2O were applied as a basal dose.

2.2 Plant materials

Telangana Sona, an elite rice cultivar developed by Professor Jayashankar Telangana State Agricultural University, was used as the recurrent female parent. It is a semi tall, short duration cultivar of approximately 125 days, characterized by super fine grain and a reported low glycemic index of 51.72, but is highly susceptible to gall midge.

RMSGm3, a stable homozygous line in the Akshaydhan background obtained from ICAR IIRR, was used as the donor parent; it carries the resistance-associated alleles detected by the three target markers.

2.3 Staggered sowing and hybridization

Staggered sowings were used to synchronize flowering of the parental lines. Telangana Sona was sown on 5th, 12^{th,} and 19th July 2024 (Table 1). Hybridization was performed using the clipping method of emasculation. Florets expected to open the following day were clipped at the upper portion, and all six anthers were removed with fine forceps during the evening. Emasculated panicles were protected with butter-paper bags. The following morning, between 09:00 and 11:00 h, fresh pollen from RMSGm3 was applied to receptive stigmas. Crossed seeds were harvested 24 days after pollination, sun dried and the plant material are processed for molecular validation.

2.4 DNA isolation and quality assessment

Genomic DNA was extracted from 4 week old seedlings using a modified cetyl trimethylammonium bromide procedure (Zhang et al., 1996) with few modifications. DNA quantity and integrity were determined by electrophoresis using a lambda DNA standard and agarose gels. Samples were normalized to approximately 50 ng μL^{-1} for PCR.

2.5 Functional markers and parental polymorphism

Three gene specific functional markers were used for foreground selection: gm3del3 for *gm3*, LRR-del for *Gm4* and PRP for *Gm8*. The candidate regions/proteins are the NB-ARC domain for *gm3*, an NB-LRR protein for *Gm4* and a proline-rich protein for *Gm8*. Telangana Sona, RMSGm3 and the resistant checks were screened to establish marker-specific allelic patterns. Marker information is summarized in Table 2 and Figure 1.

2.6 PCR amplification and product resolution

PCR reactions were performed in 10 µL volumes containing 1.0 µL template DNA (approximately 50 ng), 0.3 µL each of forward and reverse primers (2.5 pmol), 1.0 µL dNTPs (2.5 mM), 1.0 µL 10× Taq buffer and 0.2 µL Taq DNA polymerase (5 U μL^{-1}). The thermal profile consisted of initial denaturation at 94 °C for 5 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 1 min; followed by a final extension at 72 °C for 7 min. Products generated with gm3del3 and LRR-del were separated on 1.5% agarose gels, whereas the PRP product was resolved on 3.0% agarose. Electrophoresis was conducted at 90 V for 90 min, and products were visualized under UV illumination using a 100 bp DNA ladder for size estimation.

2.7 Molecular validation of F_1 plants

F_1 plants were screened with the three target markers. A plant was considered a true F_1 carrying all three target alleles when it showed the expected heterozygous pattern that is, simultaneous detection of the recurrent parent susceptible allele and the donor resistance associated allele for *gm3*, *Gm4* and *Gm8*. The expected combinations were approximately 250/550 bp for *gm3*, 350/600 bp for *Gm4* and 300/350 bp for *Gm8*.

2.8 Agro morphological and grain assessment

Validated F_1 plants were examined for days to 50% flowering, plant height and grain phenotype. Days to 50% flowering was defined as the number of days from sowing to panicle emergence in 50% of the plants. Plant height was measured from the plant base to the tip of the main panicle at maturity. Grain length and breadth

were measured on 10 randomly selected grains per plant, and the length to breadth ratio was calculated following the Standard Evaluation System for Rice.

3. RESULTS

3.1 Molecular differentiation of the recurrent and donor parents

The success of marker assisted introgression depends on diagnostic polymorphism between the recurrent and donor parents. Telangana Sona and RMSGm3 were therefore evaluated using the three gene specific markers together with locus specific resistant checks. Clear differences in allele size were observed for all three target loci (Table 3; Figure 2). For *gm3*, gm3del3 produced an approximately 250 bp resistance associated band in RMSGm3 and in the check 1-RP-4516-3-6, whereas Telangana Sona produced an approximately 550 bp susceptible band. For *Gm4*, LRR-del identified an approximately 350 bp resistance associated allele in RMSGm3 and the check DRR 2313, while Telangana Sona showed an approximately 600 bp susceptible allele. For *Gm8*, the PRP assay produced an approximately 300 bp resistance associated allele in RMSGm3 and the check Aganni, whereas Telangana Sona produced an approximately 350 bp susceptible allele. These results established a clear molecular contrast between the recurrent and donor lines at all three target loci.

3.2 Development and identification of triple positive F_1 plants

The Telangana Sona × RMSGm3 cross yielded 150 F_1 seeds during *Kharif* 2024. Of these, 120 F_1 plants were raised and screened during *Rabi* 2024-25. The screening simultaneously assessed hybridity and the presence of donor resistance associated alleles at the three target loci. Thirteen plants displayed the expected heterozygous allelic pattern for *gm3*, *Gm4* and *Gm8* and were therefore classified as true triple positive F_1 plants (Table 4 and Figures 3, 4 and 5). The selected plants were F_1-10, F_1-18, F_1-26, F_1-33, F_1-48, F_1-55, F_1-58, F_1-72, F_1-81, F_1-96, F_1-112, F_1-118 and F_1-120. Thus, 13 of the 120 screened plants were triple-positive, corresponding to 10.83% of the screened F_1 population.

3.3 Preliminary agro morphological and grain phenotype of validated F_1 plants

The 13 molecularly validated F_1 plants were evaluated for selected agro morphological and grain traits. All selected plants retained the short slender, super fine grain phenotype characteristic of Telangana Sona. Plant height and heading time were broadly comparable with the recurrent parent. These observations indicate that, at the F_1 stage and for the traits examined, the cross did not produce an obvious departure from the elite recurrent parent phenotype.

4. DISCUSSION

The principal outcome of this study is the molecular establishment of a triple positive F_1 population from the cross Telangana Sona × RMSGm3. The recurrent parent is an elite, quality oriented rice cultivar whose susceptibility to gall midge limits production stability, whereas the donor provides resistance associated alleles at three target loci. The availability of F_1 plants carrying the donor allele at all three loci provides a defined starting population for subsequent marker assisted backcrossing.

The parental polymorphism observed with gm3del3, LRR-del and PRP was central to this process. The results obtained in the present investigation are in agreement with the findings of Sama *et al.* (2014), Divya *et al.* (2013; 2015), who reported the successful use of gm3del3, LRR-del and PRP functional markers for the detection of the *gm3*, *Gm4* and *Gm8* resistance genes, respectively. The recurrent and donor alleles were distinguishable at each locus; the markers could be used to identify heterozygous progeny directly. Functional markers are particularly useful for early generation foreground selection when the objective is to assemble specific resistance associated alleles (Salgotra & Neal Stewart, 2020). Similar observations were also reported by Bentur 2015), Venkanna *et al.* (2018), Hari et al. (2022), Sravani et al. (2026) who demonstrated the effectiveness of these functional markers in foreground selection and gene pyramiding of gall midge resistance genes. Therefore, the functional markers employed in the present study were found to be highly reliable for confirming the presence of the target resistance genes as well as establishing polymorphism between the donor and recurrent parent, thereby providing a strong foundation for marker-assisted backcross breeding.

The identification of 13 triple positive plants among 120 screened F_1 plants demonstrates the feasibility of simultaneously tracking the three target loci. The validated plants carried the expected recurrent and donor derived alleles at all three loci and therefore met the molecular criterion used in this study for a true tri gene F_1. Similar marker assisted selection strategies have been successfully employed for the introgression and pyramiding of gall midge resistance genes in rice. Venkanna *et al.* (2018) used the functional marker gm3del3, along with RM219 and RM22685, for foreground selection of *gm3*, *Gm1*, and *Gm8* genes, respectively, to identify true F_1 plants and subsequently select homozygous individuals carrying all three gall midge resistance genes during pedigree breeding of the rice variety Kavya. Similarly, Hari *et al.* (2022) employed marker-assisted backcross breeding for the introgression of *Gm4* and *xa13* genes into the rice cultivar Tellahamsa. Foreground selection was carried out in the F_1, BC_1F_1, BC_2F_1 and BC_3F_1 generations using the functional marker LRR-del for *Gm4* and the gene-specific marker xa13 promoter for *xa13* gene, enabling the identification of plants carrying the target genes in the heterozygous condition before advancing them to subsequent generations. Likewise, Janaki Ramayya *et al.* (2021) integrated marker assisted forward breeding and marker assisted backcross breeding for the improvement of the elite rice variety Naveen by pyramiding multiple stress resistance genes, including the gall midge resistance gene *Gm8*. The authors performed foreground selection at

every generation using the gene-linked PRP marker for *Gm8*, thereby ensuring the transmission of the target allele throughout the breeding programme. Further in the present study the retention of the characteristic short slender, super fine grain phenotype supports their use as starting material for subsequent breeding generations.

An important distinction, however, must be maintained between molecular genotype and phenotypic resistance. The present study establishes the presence of resistance associated alleles in heterozygous F_1 plants; it does not demonstrate broad spectrum or durable gall midge resistance. This is particularly important for the interpretation of *gm3*, because the resistance locus has been reported to behave recessively (Sathishkumar et al., 2025). Heterozygous F_1 plants should therefore not automatically be assumed to express the same resistance phenotype as homozygous resistant progeny. Phenotypic evaluation should be performed in appropriate advanced generations after fixation of the target alleles.

The same caution applies to claims concerning resistance breadth. Gall midge virulence can change among pest populations, and the performance of resistance genes may vary among biotypes (Vijaykumar et al., 2022) (Bentur et al., 2008). The present study therefore provides the molecular foundation for testing whether the three locus combination broadens resistance; it does not itself establish that outcome.

The preliminary retention of a Telangana Sona like grain type is nevertheless relevant for breeding. An improved line is valuable only if resistance is accompanied by preservation of the characteristics that make the recurrent parent desirable. The present observations are encouraging, but quantitative evaluation of grain length, breadth, length to breadth ratio, milling quality, cooking quality and other commercially relevant traits in replicated advanced generations will be required to establish quality retention and exclude undesirable donor effects.

Marker assisted backcrossing can be strengthened by background selection. Foreground selection identifies the target loci, whereas background selection reduces unnecessary donor genome and accelerates recovery of the recurrent parent genetic background (Collard & Mackill, 2008). Genome-wide SNP, GBS or sufficiently dense SSR analysis would therefore be a logical next step for estimating recurrent parent genome recovery and donor segment size around the target loci. The target loci also warrant careful monitoring in later generations, because the marker framework used in this study places *Gm4* and *Gm8* on chromosome 8; their joint recovery and recombination should be evaluated using informative flanking markers. Such analysis would help optimize pyramiding and minimize linkage drag.

5. CONCLUSION

Overall, the present work represents the molecular validation stage of a precision breeding programme. Its strongest demonstrated contribution is the establishment of diagnostic parental polymorphism and 13 molecularly validated tri gene F_1 plants. Advanced generation fixation, direct gall midge challenge, testing against diverse pest populations, quantitative field evaluation and grain quality analysis are required to establish the performance of the resulting Telangana Sona derived lines.

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DECLARATIONS

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Author contributions. G. Rakesh, the first author and Ph.D. research scholar, conceptualized and conducted the research as part of his doctoral thesis, carried out the experiments, performed molecular screening and data analysis, interpreted the results, and prepared the original manuscript draft. B. Vidhyadhar, Y. Chandra Mohan, Yadla Hari, R. Shravan Kumar, and P. Gonya Naik, as members of the doctoral research advisory committee, provided scientific guidance, contributed to the research methodology and experimental planning, reviewed and critically revised the manuscript, and supervised the research work in their respective areas of expertise. All authors read and approved the final version of the manuscript.

Data availability. All data generated or analysed during this study are included in the published article. Additional information may be obtained from the corresponding author upon reasonable request.

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Table 1. Details of staggered sowings used for the crossing programme.

Sowing	Date	Female parent
I	05.07.2024	Telangana Sona
II	12.07.2024	Telangana Sona
III	19.07.2024	Telangana Sona

Table 2. Functional markers used for foreground selection.

Gene	Chromosome	Marker	Candidate region/protein	Reference
gm3	4	gm3del3	NB-ARC domain	Sama et al., (2014)
Gm4	8	LRR-del	NB-LRR/LRR protein	Divya et al., (2015)
Gm8	8	PRP	Proline-rich protein	Divya et al., (2013)

S = susceptible allele; R = resistance associated allele; – = not tested.

Table 3. Genotyping results of recurrent, donor and resistant check genotypes using foreground markers.

Genotype	*gm3* / gm3del3	*Gm4* / LRR-del	*Gm8* / PRP
Telangana Sona	S	S	S
RMSGm3	R	R	R
DRR2313	–	R	–
Aganni	–	–	R
1-RP-4516-3-6	R	–	–

Table 4. Confirmed F1 plants carrying heterozygous alleles at all three target loci.

S.No.	Plant identity	Allelic status	Days to flowering (Days)	Plant height (cm)	Grain type
1.	Telangana Sona	*Gm3Gm3gm4 gm4gm8gm8*	85	108	Short slender super fine grain
2.	F_1-10	*Gm3gm3Gm4 gm4Gm8gm8*	82	96	Short slender super fine grain
3.	F_1-18		80	99	Short slender super fine grain
4.	F_1-26		84	98	Short slender super fine grain
5.	F_1-33		86	104	Short slender super fine grain
6.	F_1-48		89	103	Short slender super fine grain
7.	F_1-55		88	105	Short slender super fine grain
8.	F_1-58		90	110	Short slender super fine grain
9.	F_1-72		91	98	Short slender super fine grain
10.	F_1-81		85	98	Short slender super fine grain
11.	F_1-96		86	99	Short slender super fine grain
12.	F_1-112		88	101	Short slender super fine grain
13.	F_1-118		84	96	Short slender super fine grain
14.	F_1-120		83	105	Short slender super fine grain

Figures

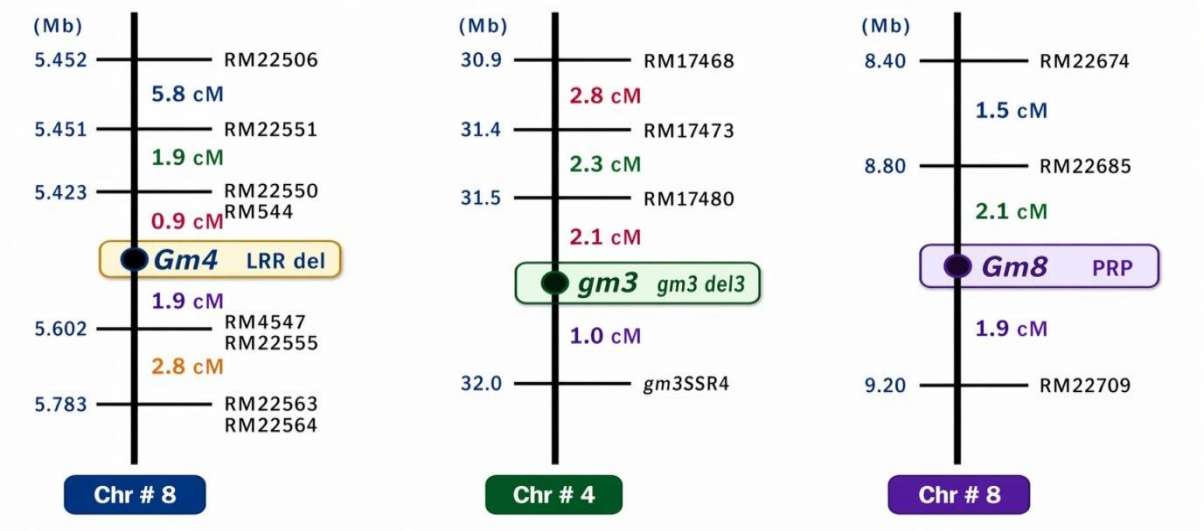


Fig. 1. Linkage map showing target gall midge resistance genes along with functional markers (modified from Bentur 2015)

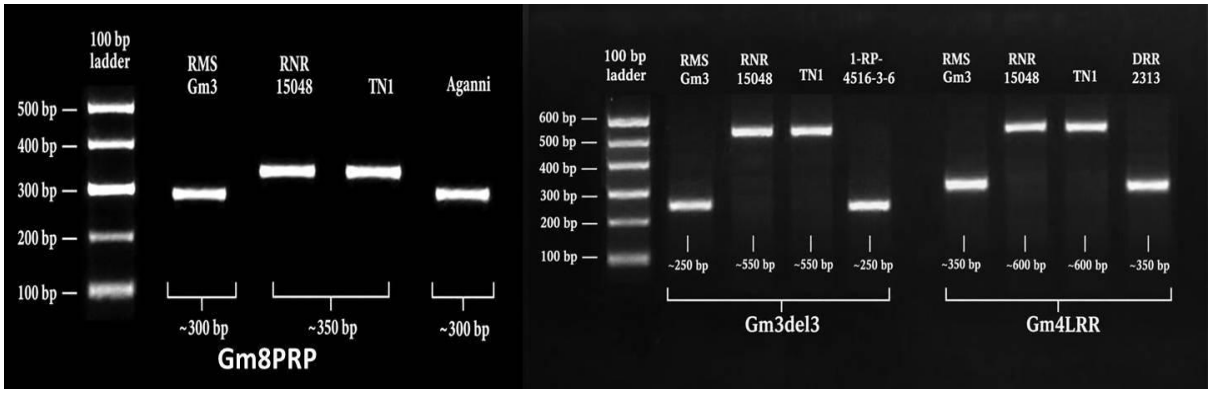


Fig. 2. Verification of parental lines, screening for the presence of *gm3*, *Gm4*, and *Gm8* resistance genes, and confirmation of parental polymorphism between the recurrent parent Telangana Sona (RNR 15048) and donor parent RMSGm3 using the gm3del3, LRR-Del, and PRP markers, respectively.

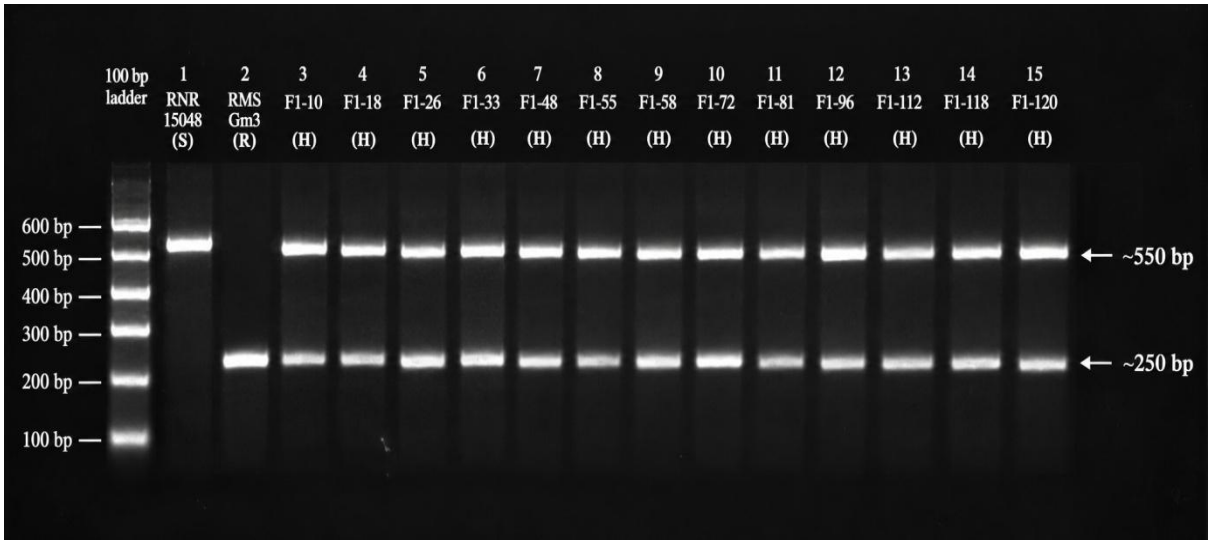


Fig. 3. Foreground analysis for confirmation of heterozygosity in F_1 plants for *gm3* gene using gm3del3 marker.

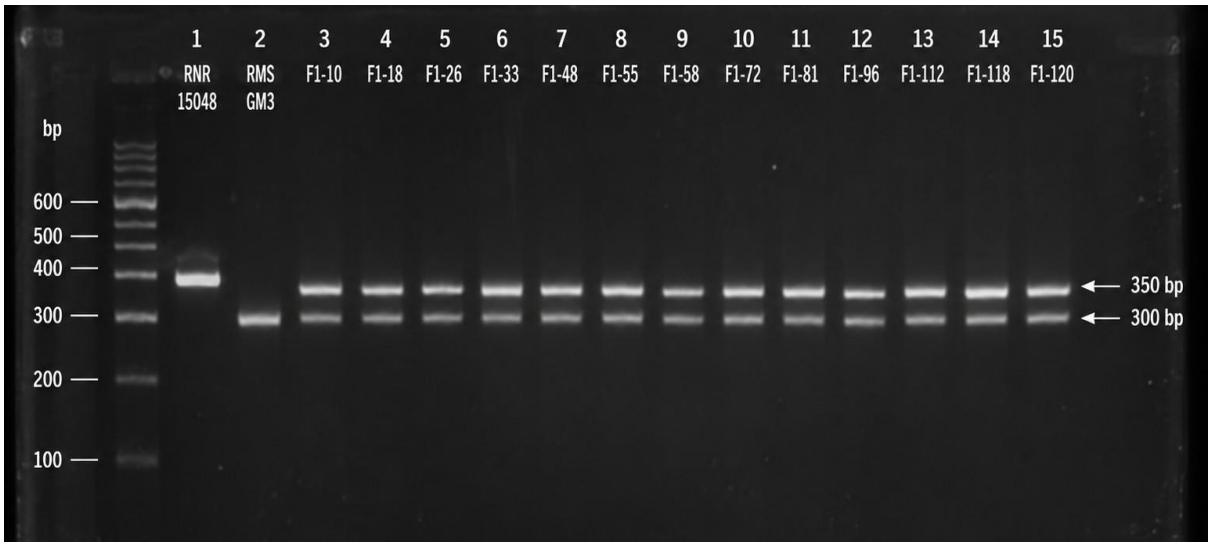


Fig. 4. Foreground analysis for confirmation of heterozygosity in F_1 plants for *Gm4* gene using LRR-Del marker.

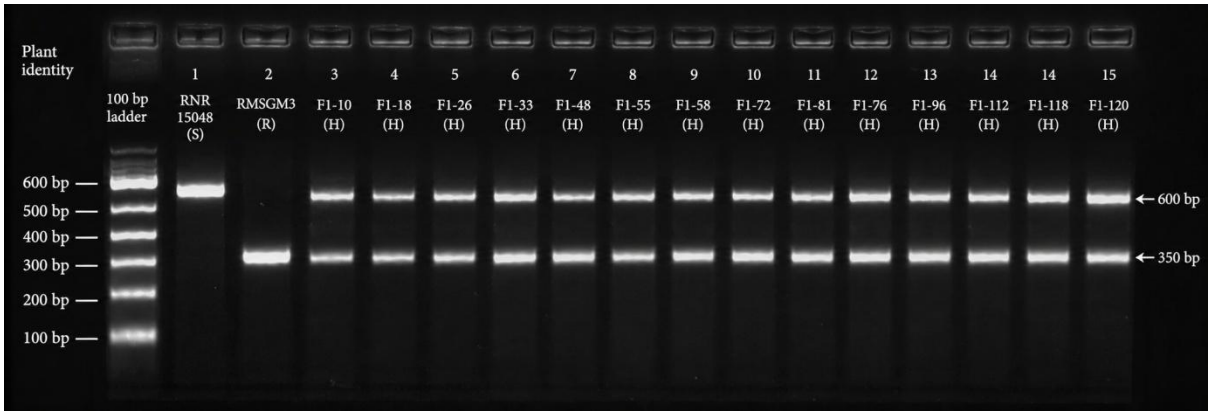


Fig. 5. Foreground analysis for confirmation of heterozygosity in F_1 plants for *Gm8* gene using PRP marker.