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Research Article

Identification of gall midge resistant parental lines and validation of fertility restoration linked markers for hybrid rice technology

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Abstract
The success of hybrid rice technology depends on the extent of heterosis realized, pest resistance and the grain quality of rice. To identify gall midge resistant, non aromatic maintainers and restorers, 114 germplasm lines were crossed with six CMS lines (2 indigenous and four exotic) to get one hundred fifty five hybrids (Rabi, 2012-13) and evaluated with 10 checks (Kharif, 2013) at Rice Research Station, Regional Agricultural Research Station, Jagtial, Andhra Pradesh. Based on pollen sterility and spikelet fertility studies, 49 maintainers and 31 restorers were identified. Screening hybrids and their parental lines for gall midge incidence indicated the involvement of dominant genes for gall midge resistance. Forty three hybrids, eleven maintainers and eighteen restorers were resistant to gall midge biotype 3. Gall midge resistant maintainers and restorers will be used for new CMS line and hybrid development respectively. The conventional method of restore line identification among rice germplasm pool is time consuming and labor intensive. Molecular mapping of fertility restorer genes in rice have yielded several closely linked DNA markers that can be used in identifying restorer lines. In order to utilize this available information effectively in marker assisted restorer line identification, validation of reported Rf3 and Rf4 gene linked DNA markers was carried out in this study. A total of seven DNA markers reported to be closely linked with two Rf genes of wild abortive CMS (WA-cms) were chosen. These markers were screened among twenty identified restorers and five maintainer lines. The genotypic data set was generated based on the specific PCR product size. Two DNA markers in combination (RM10313 and RM6100) showed 100% selection efficiency in identifying restorers in the germplasm and 90% selection efficiency in differentiating maintainers from restorers. These validated molecular markers linked to Rf genes would save time and money besides adding accuracy in identification of restorers.

Key words:
Rice, non aromatic maintainers, fertility restoration, SSR markers, Gall midge resistance, restorers

Introduction:

Rice (*Oryza sativa* L.) is one of the most important food crops and primary source of food for more than half the world's population (Hashemi *et. al*., 2009). Over 75% of the world supply is consumed by people in Asian countries and thus rice is of immense importance to the food and lively hood security of Asia. Rice production must be increased to meet the requirement of increasing population. Since the yield of high yielding varieties (HYVs) of rice has plateaued, it is rather difficult to achieve this target with the present day inbred varieties. Hybrid rice technology is the only proven technology currently available for stepping up rice production significantly and is considered as one of the promising, practical, sustainable and eco-friendly options to break the yield ceiling in rice (Sheeba *et al.,* 2009). First hybrid was released in 1975 in China; up to 30 percent heterosis is recorded with rice hybrids. In India first hybrid was released for cultivation in 1994. Since then 59 hybrids have been released in India (DRR, 2013).

Combination of a CMS line, a maintainer line and a restorer line carrying the restorer gene (*Rf*) to restore fertility is indispensable for the development of hybrid varieties in three line system (Virmani *et al*., 2003). Presently hybrids occupy around five percent of 44 million hectares of rice area. Major reasons for slow rate of adoption of hybrid rice in India are undesirable grain quality (stickiness and mild aroma) and susceptibility to insect pests (gall midge and stem borer). Development of parental lines with desirable traits like non aromatic and gall midge resistance is a prerequisite for development of gall midge resistant hybrids with higher yields. Rice gall infestation is a serious rice problem caused by a dipterans insect pest known as gall midge (*Orseolia oryzae* (Wood-Mason)). The annual yield losses due to rice gall midge have been calculated to vary from 20% to 30% or even higher. So far, 11 gall midge resistance genes have been characterized in rice (Himabindu *et al*., 2010), and seven biotypes of the pest have been reported (Vijayalakshmi *et al.,* 2006). At present, chemicals and pesticides are less effective to control gall midge and more application of chemicals and pesticides leads to environment pollution. The best logical approach to overcome this problem is to breed new cultivars with high resistance to rice gall midge.

In hybrid rice technology maintainer and restorer lines will be identified by crossing germplasm lines with a CMS line and evaluating the F_1 for

pollen and spikelet fertility. This system of restorer identification is time consuming and labour intensive. CMS can be restored by nuclear genes governing fertility restoration (*Rf*) (Nematzadeh and Kiani, 2010). Among five fertility restorer genes identified for WA (wild abortive) cytoplasm (Rf), *Rf3* and *Rf4* genes reported to be of more value for identification of restorers (Revathi *et al.*, 2013) . Several DNA markers closely linked to *Rf* genes have been reported (Ahmadakhah *et. al.*, 2007, Bazrkar *et. al*., 2008, Alavi *et. al.*, 2009, Neeraja *et al*, 2009, Sheeba *et. al.,* 2009 and Grishma Shah *et. al.,* 2012) which are useful in marker assisted identification of restorers in rice germplasm and further use in hybrid breeding program. In order to utilize this information in normal breeding the reported markers need to be validated. Hence, present study was undertaken to identify gall midge resistant, non aromatic hybrid rice parental lines and validate markers linked to fertility restoration.

Material and Methods

The present investigation was conducted at Research farm of Regional Agricultural Research Station, Jagtial, Karimnagar, Andhra Pradesh, India during Rabi, 2012-13 and *Kharif*, 2013. The experimental material comprised 114 non aromatic germplasm lines and six Cytoplasmic Male Sterile (CMS) lines from two sources *viz.,* indigenous (JMS1 and JMS11 developed at RARS) and exotic (CMS11A(IR68902A), CMS14A(IR69628A), CMS23A(IR72081A) and CMS46A(IR80559A) developed at International Rice Research Institute, IRRI, Manila, Philippines). A total of 155 hybrids were produced by crossing germplasm lines with CMS lines during rabi 2012-13. Twenty five days old seedlings of both hybrids and male parents were planted in augmented design, with checks repeating after every ten hybrids. Ten popular high yielding varieties (JGL11470, JGL18047, MTU1010, JGL20171, JGL19621, JGL3844 & Badri) and hybrids (27P31, 27P64, JKRH401) of different maturity and duration were used as checks in the experiment. Each entry was planted in a single row of 20 plants with standard spacing 20x20cm. Recommended package of practices were followed during crop growth period. Observations were recorded on five randomly selected plants in each treatment for gall midge incidence, pollen sterility and spikelet fertility. To get more gall midge incidence susceptible check (BPT5204) was planted around and in-between the experiment plot. Gall midge incidence percentage was recorded in each entry 30 days after transplanting, by counting number of hills with silver shoots. Entries with less than 10 percent of hills with silver shoots were considered as "resistant" and others grouped as "susceptible" (SES, 2002). Jagtial is known to be hot spot for gall midge screening and more than 98 percent of gall midge incidence was observed in susceptible check BPT5204.

Pollen sterility and Spikelet fertility:Pollen and spikelet fertility percentages were assessed during flowering and harvesting stage respectively (Yui *et al.,* 2003). To estimate pollen sterility, samples are collected from at least ten florets from individual plants at flowering stage and fixed in 70% alcohol. Two to three anthers are extracted from five of the florets on a glass slide and pollen are squeezed out with a spear-shaped needle in a drop of 2% IKI (Iodine-Potassium Iodide) solution. Removed anthers were crushed and visualized under microscope at 100X magnification. At least three microscopic fields are used to count sterile pollen grains, the unstained, irregular pollen grains were recorded as sterile and completely stained, round pollen grains as fertile. Finally, the percentage pollen sterility was calculated based on the number of sterile pollens over the total number of pollen grains analyzed. Entries with more than 95% pollen sterility were considered as "sterile"; 70% to 95% as "partially sterile"; 40% to 69.9% as "partially fertile" and less than 40% as "Fertile". Spikelet fertility was estimated by bagging three panicles per plant with selfing covers during flowering stage and at harvesting stage, bagged panicles were collected, sun dried and spikelet's were counted for the number of filled and unfilled grains. The percentage spikelet fertility was calculated by considering the number of unfilled/filled grains over total number of spikelet per panicle. Classified the hybrids based on spikelet fertility as Fertile (>75%), Partial (10% - 75%), sterile (<10%) (SES, 2002). The criteria for classifying the parental lines as maintainers and restorers were used as proposed by Virmani *et al*. (1997).

Genotyping: Molecular markers reported for two fertility restorer (*Rf*) genes (*Rf3* & *Rf4*) of WA cytoplasm was studied. Based on the centi Morgan (cM) distance between gene and the marker, closely linked markers were identified and used for polymerase chain reaction (PCR) analysis (Table 1). DNA was isolated using mini-preparation method (Thippeswamy, 2007) by collecting young leaves in 1.5 micro-centrifuge tubes, labeled and placed the tubes on ice during transit. Leaf samples were cut in to small pieces and grinded by adding 800 µL extraction buffer. 400 µL of chloroform was added, mix well and spun for 5 min at 12,000 rpm, top aqueous phase is transferred into another tube. 800 µL of chilled absolute ethanol was added, mixed gently and spun for 5 min in a micro-centrifuge at 12,000rpm, supernatant was decanted. DNA pellet was washed with 70% ethanol and air-dried the DNA and suspended the DNA in 50 µL of TE buffer. The integrity of DNA was judged through gel analysis by casting 0.8% agarose gel in 1X TBE (Tris Borate EDTA) buffer containing 3 µL of Ethidium bromide at 100 Volts.

PCR amplification was carried out in 20 ul reaction volume containing 20 ng genomic DNA, 1X PCR buffer (Tris with 1.5 mM $MgCl_2$), 50 uM dNTP (2.5mM each dNTP), 5pM of each forward and reverse primer, 0.5 units of *taq* polymerase enzyme. Amplification was performed in a thermal cycler (Eppendorf, USA) and the PCR performed with a program of initial denaturation at 94°C for 5 minutes, cyclic denaturation at 94°C for 2 minute, primer annealing at 50°-54°C (vary from marker to marker) for 1 minute and primer extension at 72°C for 2 minute. The cycle was repeated 40 times and ended with the final extension at 72°C for 10 minutes. The amplified PCR products were resolved in gel electrophoresis on 3.0% Seakem®LE agarose gel (Lonza, USA) along with 50 bp molecular marker (Bangalore Genie, India), stained with ethidium bromide and documented using gel documentation system (Alpha Innotech, USA). The genotypic dataset was generated based on the PCR amplification profile by scoring presence and absence of specific allele with specific base pair (bp) size for all the samples. Validation of linked markers was carried out using 20 identified restorers and five maintainers.

Results and discussion

Identification of restorers and maintainers in the existing germplasm is the most quick and simple approach for exploitation of hybrid vigor in rice. One hundred and fifty five hybrids were produced and evaluated by crossing 114 non-aromatic germplasm lines with six female lines *viz.*, JMS1A (64 hybrids), JMS11A (11 hybrids), CMS11A (13 hybrids), CMS14A (4 hybrids), CMS23A (36 hybrids), CMS46A (27 hybrids). Sixty one hybrids showed fertile reaction in pollen studies, out of which 38 were classified as fertile and 23 were partials in field spikelet fertility studies. Eight hybrids having partially fertile pollen were having fertile spikelet fertility and most of the hybrids (28) with partial pollen sterility recorded either partial (18) or sterile (10) spikelet fertility (Table2). All 58 hybrids having sterile pollen grains had sterile spikelet fertility. Thirty one genotypes were considered as restorers, which showed fertile pollen and spikelet fertility when crossed with different CMS lines. Among 114 lines used, forty nine genotypes were identified as maintainers which were showing hybrid sterility. In the present study 43% of total germplasm constituted of maintainers and 27% of restorers. Higher frequency of maintainers than restorers was also found by Ali and Khan (1996), Sabar and Akhter (2003), Virmani and Kumar (2004) and Akhter *et al*. (2008). The new non aromatic maintainer lines (Table 3) will be used to develop locally adapted CMS lines through recurrent backcrossing. Three genotypes (JGL21823, JGL21851 and JGL22311) behaved as a restorer for the one CMS line and as maintainer for other CMS line (Table 2 & 3). The variations in behavior of fertility restoration indicate that their fertility restorer genes interact differently with nuclear genes of various maintainers. Similar results have been reported by Hemareddy *et al*. (2000), Gannamani (2001) and Bisne and Motiramani (2005) and Sri Krishnalatha and Deepak Sharma (2012). A total of 77 non aromatic maintainers and restorers identified in the present study form a new genetic pool for exploitation of hybrid vigor and diversification of hybrid rice parental lines (Table 3).

Gall midge screening results revealed that the forty three hybrids, eleven maintainers and eighteen restorers are resistant to gall midge (Table2 & 3). All six female lines used have shown varying levels of gall midge susceptibility (21% to 64%) and most of the hybrids are resistant whenever male parent is resistant. This indicates the dominant gene inheritance of gall midge resistance. This finding is in line with the dominant gene inheritance of so far reported 11 gall midge resistance genes except *gm3* (Bentur *et. al.,* 2003, Thippeswamy *et.al.,* 2006 and Sama *et.al.,* 2014). Seven hybrids (TCN385, TCN412, TCN449, TCN455, TCN487, TCN508 and TCN509) showed resistance though both the parents are susceptible; this may be due to involvement of multiple genes with minor effects. Biotype3 is most virulent and largely prevalent in India and same biotype is reported at Jagtial (DRR, 2014). Hence, these resistant hybrids will be having wider adaptability and reduce the average annual yield loss of US$ 80 million (Widowsky and O'Toole, 1996) due to gall midge in India.

Validation of *Rf* gene linked DNA markers: A total of seven DNA markers reported to be linked with two fertility restorer genes (*Rf3* and *Rf4*) of WA cytoplasm among twenty restorer lines and five maintainer lines. Genotypic data showed number of alleles identified per marker was variable. Only those alleles reported to be linked to *Rf* genes were scored and efficiency of each marker to identify restorer and maintainer is calculated (Table4). Nas *et al*. (2003) demonstrated for the first time use of molecular markers for restorer line identification and reported that PCR based marker RG140STS exhibited 83% efficiency in identifying putative restorers. Efficiency of linked markers to identify restorer vary from 0 (RM3873) to 90 (RM10318). PCR based markers RM10313 and RM6100 linked to *Rf3* and *Rf4* respectively, found to be more accurate compared to other linked markers in identifying restorers and differentiating maintainers from others. These two DNA markers in combination (RM10313 and RM6100) are 100% efficient in identifying restorers in the germplasm and 90% efficient in differentiating maintainers from restorers (Table4, Figure1 & 2). These results are in close confirmation earlier reports. Revathi *et al*. (2013) found PCR based markers RM6100 and

RM10313 exhibiting 80 to 85% efficiency in restorer identification and Singh *et al*. (2005) reported that usefulness of RM6100 in marker aided selection of restorer with selection accuracy of 97%. RM6100 amplified the Rf4 linked allele in a majority of the restorers with a selection accuracy of 94.87% (Sheeba *et al*., 2009).

Alavi *et al*. (2009) reported that RM1 and RM3873 primers are having 89 and 74% efficiency in MAS for fertility restoration trait. However, when they are used together, their efficiency would be 99% in identification of restorers. In present study RM1 and RM3873 showed around 35% efficiency in restorer identification whereas non-restorers also identified with higher selection accuracy in comparison with pollen and spikelet fertility. Map distance between *Rf3* gene and the molecular markers RM1, RM3233, and RM3873 is 5.6cM, 17cM and 14cM respectively. As these primers are not closely linked with *Rf* genes, they are not able to differentiate putative restorers and non-restorers. Hence, these primers are not useful in marker assisted selection of restorer lines. Two other primers (RM258 and RM10318) linked to *Rf4* gene were 50% efficient in restorer identification. Identification of candidate gene based marker for fertility restoration trait would be very useful in distinguishing restorers from non-restorers. Recently candidate gene based marker for fertility restoration trait has been reported (Ngangkham et. al., 2010). These genic markers are based on pentatricopeptide repeat (PPR) motif containing genes on chromosome 10. But further experiment is required to validate the identified candidate PPR genes to establish its precise role in restoration of fertility of WA-CMS. The present study indicates that molecular screening with RM6100 and RM10313 for fertility restoration can be a useful tool for identifying restorers from breeding lines of unknown restoration status with 100% efficiency without making and evaluating large number of test crosses. But identified restorers based on molecular screening must be test crossed with appropriate CMS lines to confirm higher level of heterosis. Thus use of molecular markers linked to *Rf* genes would save time and money besides adding accuracy in identification of restorers. These markers are useful in marker assisted identification of *Rf* genes in back cross breeding program to develop near isogenic lines with multiple *Rf* genes towards the development of superior restorer lines. The identified gall midge resistant restorers are being used for development of high yielding and wide adoptable rice hybrids. Maintainers with gall midge resistance and good grain quality were used in back cross breeding program for development of new CMS lines.

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Table 1. Fertility restorer (Rf) genes and linked markers used for validation

Sl. No.	Linked gene	DNA Marker	Genetic Distance (cM)	Forward sequence(5' - 3')	Reverse sequence (5' - 3')	Chromosome location	Allele size in restorer (bp)	Reference
1	***Rf3***	RM1	5.6	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGACCTGAC	1	115	Ahmadakhah *et. al.*, (2007)
2	***Rf3***	RM3873	14	GCTATAGACGCCTCCTCCTTATCC	AAAGCTAGCTAGGACCGACATGC	1	210	Alavi *et. al.*, (2009)
3	***Rf3***	RM3233	16.9	GAAATTCGAAATGGAGGGAGAGC	GGTGAGTAAACAGTGGTGGTGAGC	1	140	Alavi *et. al.*, (2009)
4	***Rf3***	RM10313	4	ACTTACACAAGGCCGGGAAAGG	TGGTAGTGGTAACTCTACCGATGG	1	188	Neeraja *et al., (*2009)
5	***Rf4***	RM258	4.4	TGCTGTATGTAGCTCGCACC	TGGCCTTTAAAGCTGTCGC	10	140	Bazrkar *et. al*., (2008)
6	***Rf4***	RM10318	5	TGTCTCACACATTGCACACTTACC	GGCCTAACCCAACACATGTCC	10	187	Grishma shah *et. al.*, (2012)
7	***Rf4***	RM6100	1.2	TTCCCTGCAAGATTCTAGCTACACC	TGTTCGTCGACCAAGAACTCAGG	10	185	Sheeba *et. al., (*2009)

Table2. Pollen sterility & spikelet fertility of hybrids and gall midge incidence of hybrids and their parents

S.No.	Hybrid	Female	Male	Pollen sterility	Spikelet fertility	Gall midge incidence*			S.No.	Hybrid	Female	Male	Pollen sterility	Spikelet fertility	Gall midge incidence*		
						Hybrid	Male	Female							Hybrid	Male	Female
1	**TCN 347**	CMS 46A	JGL402	S	S	32	40	64	79	**TCN 432**	CMS23 A	JGL2114 6	F	P	65	80	32
2	**TCN 348**	CMS 46A	JGL1834	F	P	80	90	64	80	**TCN 433**	CMS23 A	JGL2115 2	F	F	0	0	32
3	**TCN 349**	CMS 23A	JGL2114	PS	P	16	10	32	81	**TCN 435**	CMS23 A	JGL2115 9	PS	P	0	0	32
4	**TCN 350**	CMS 46A	JGL5614	F	F	61	70	64	82	**TCN 436**	CMS23 A	JGL2116 3	PS	P	0	0	32
5	**TCN 351**	CMS4 6A	JGL5868	F	F	95	50	64	83	**TCN 437**	CMS23 A	JGL2116 4	F	P	35	80	32
6	**TCN 352**	CMS4 6A	JGL13392	F	F	0	0	64	84	**TCN 438**	CMS23 A	JGL2177 5	F	P	45	50	32
7	**TCN 353**	CMS4 6A	JGL13546	S	S	0	0	64	85	**TCN 439**	CMS23 A	JGL2177 9	F	F	30	70	32
8	**TCN 354**	CMS4 6A	JGL15230	F	P	0	0	64	86	**TCN 441**	JMS1A	JGL2177 9	F	F	65	40	21
9	**TCN 355**	CMS1 4A	JGL15246	S	S	60	80	41	87	**TCN 442**	JMS1A	JGL2178 9	S	S	20	0	21
10	**TCN 356**	CMS4 6A	JGL15324	F	P	55	60	64	88	**TCN 444**	JMS1A	JGL2179 4	S	S	25	10	21
11	**TCN 357**	JMS1 A	JGL17194	S	S	25	0	21	89	**TCN 445**	CMS11 A	JGL2179 7	S	S	0	0	50
12	**TCN 358**	CMS4 6A	JGL17574	S	S	0	0	64	90	**TCN 446**	CMS23 A	JGL2179 7	PS	S	0	0	32
13	**TCN 359**	CMS1 4A	JGL17653	F	F	35	60	41	91	**TCN 447**	JMS1A	JGL2179 7	S	S	20	0	21

Table2. Contd..

S.No.	Hybrid	Female	Male	Pollen sterility	Spikelet fertility	Gall midge incidence*			S.No.	Hybrid	Female	Male	Pollen sterility	Spikelet fertility	Gall midge incidence*		
14	**TCN 360**	CMS46A	JGL17653	F	F	85	60	64	92	**TCN 448**	JMS1A	JGL21800	F	F	30	10	21
15	**TCN 361**	CMS11A	JGL17758	S	S	47	80	50	93	**TCN 449**	JMS1A	JGL21803	F	P	0	20	21
16	**TCN 362**	JMS1A	JGL17758	S	S	70	90	21	94	**TCN 450**	CMS23A	JGL21806	S	S	0	0	32
17	**TCN 363**	CMS14A	JGL17777	PS	P	15	0	41	95	**TCN 452**	CMS46A	JGL21806	S	S	30	0	64
18	**TCN 364**	CMS46A	JGL17777	PS	P	0	0	64	96	**TCN 453**	CMS23A	JGL21812	S	S	20	20	32
19	**TCN 365**	CMS46A	JGL17782	S	S	90	100	64	97	**TCN 455**	JMS1A	JGL21812	S	S	0	20	21
20	**TCN 366**	JMS1A	JGL17970	S	S	0	0	21	98	**TCN 457**	CMS23A	JGL21814	F	P	30	50	32
21	**TCN 367**	CMS46A	JGL18000	PS	P	80	10	64	99	**TCN 458**	JMS1A	JGL21814	PS	P	85	70	21
22	**TCN 368**	JMS1A	JGL18045	S	S	63	10	21	100	**TCN 459**	CMS11A	JGL21815	F	F	75	80	50
23	**TCN 369**	CMS46A	JGL18065	F	P	100	100	64	101	**TCN 460**	CMS23A	JGL21815	F	F	70	100	32
24	**TCN 370**	JMS1A	JGL18079	S	S	80	100	21	102	**TCN 461**	JMS1A	JGL21815	F	F	45	100	21
25	**TCN 372**	CMS46A	JGL18203	PF	F	95	100	64	103	**TCN 464**	JMS1A	JGL21819	F	F	0	0	21
26	**TCN 373**	CMS46A	JGL18213	F	F	0	0	64	104	**TCN 465**	JMS1A	JGL21820	PS	P	0	0	21
27	**TCN 374**	CMS46A	JGL18215	S	S	10	20	64	105	**TCN 466**	JMS1A	JGL21820	F	F	0	0	21
28	**TCN 376**	CMS46A	JGL18222	S	S	16	20	64	106	**TCN 467**	CMS23A	JGL21823	F	F	0	0	32
29	**TCN 377**	CMS46A	JGL18230	PF	F	0	0	64	107	**TCN 469**	JMS1A	JGL21823	S	S	0	0	21
30	**TCN 378**	CMS46A	JGL18256	F	P	10	30	64	108	**TCN 471**	CMS23A	JGL21828	F	F	0	0	32
31	**TCN 379**	CMS46A	JGL18262	S	S	15	30	64	109	**TCN 472**	JMS1A	JGL21831	F	P	0	0	21
32	**TCN 380**	CMS23A	JGL18621	F	P	63	100	32	110	**TCN 473**	JMS11A	JGL21836	F	F	75	80	33
33	**TCN 381**	CMS46A	JGL18624	F	F	0	0	64	111	**TCN 474**	CMS23A	JGL21845	S	S	0	0	32

Table2. Contd..

S.No.	Hybrid	Female	Male	Pollen sterility	Spikelet fertility	Gall midge incidence*			S.No.	Hybrid	Female	Male	Pollen sterility	Spikelet fertility	Gall midge incidence*		
34	**TCN 382**	JMS1A	JGL18778	S	S	0	0	21	112	**TCN 475**	JMS11A	JGL21845	PS	S	0	0	33
35	**TCN 383**	JMS1A	JGL18779	PF	F	85	0	21	113	**TCN 476**	JMS1A	JGL21845	S	S	0	0	21
36	**TCN 384**	CMS11A	JGL18801	F	F	0	0	50	114	**TCN 477**	CMS23A	JGL21851	S	S	0	0	32
37	**TCN 385**	CMS23A	JGL19605	F	F	0	20	32	115	**TCN 479**	JMS11A	JGL21851	F	F	25	0	33
38	**TCN 386**	JMS1A	JGL20122	S	S	35	0	21	116	**TCN 480**	JMS1A	JGL21851	F	F	0	0	21
39	**TCN 387**	JMS1A	JGL20184	PS	S	75	100	21	117	**TCN 482**	JMS1A	JGL21857	S	S	11	20	21
40	**TCN 388**	JMS1A	JGL20218	S	S	75	30	21	118	**TCN 484**	JMS1A	JGL21861	S	S	0	0	21
41	**TCN 389**	JMS1A	JGL20232	S	S	75	60	21	119	**TCN 485**	CMS23A	JGL21862	PF	F	25	0	32
42	**TCN 390**	JMS1A	JGL20621	S	S	100	90	21	120	**TCN 486**	CMS23A	JGL21864	F	F	10	95	32
43	**TCN 391**	JMS1A	JGL20624	F	P	67	50	21	121	**TCN 487**	JMS11A	JGL21867	F	F	0	95	33
44	**TCN 393**	JMS1A	JGL20644	F	P	100	90	21	122	**TCN 488**	JMS1A	JGL21868	S	S	100	80	21
45	**TCN 394**	JMS1A	JGL20649	F	F	65	80	21	123	**TCN 490**	CMS23A	JGL21870	S	S	40	50	32
46	**TCN 395**	CMS11A	JGL20670	F	F	40	40	50	124	**TCN 492**	JMS1A	JGL21878	PS	P	60	10	21
47	**TCN 396**	JMS1A	JGL20670	PF	F	75	30	21	125	**TCN 493**	CMS23A	JGL21881	F	F	53	80	32
48	**TCN 397**	CMS11A	JGL20769	F	F	79	45	50	126	**TCN 495**	JMS1A	JGL21881	PS	P	85	80	21
49	**TCN 398**	JMS1A	JGL20769	PF	F	95	60	21	127	**TCN 496**	JMS1A	JGL21883	S	S	85	80	21
50	**TCN 400**	JMS1A	JGL20770	F	P	71	90	21	128	**TCN 497**	JMS1A	JGL21884	PS	S	35	20	21
51	**TCN 401**	JMS1A	JGL20777	F	P	15	20	21	129	**TCN 498**	JMS11A	JGL22244	S	S	75	80	33
52	**TCN 402**	CMS11A	JGL20779	F	F	65	100	50	130	**TCN 499**	JMS1A	JGL22244	PS	S	95	80	21
53	**TCN 403**	CMS23A	JGL20779	F	F	60	80	32	131	**TCN 500**	JMS1A	JGL22248	S	S	20	20	21
54	**TCN 404**	JMS1A	JGL20779	F	P	95	70	21	132	**TCN 501**	JMS11A	JGL22249	S	S	20	50	33

Table2. Contd..

S.No.	Hybrid	Female	Male	Pollen sterility	Spikelet fertility	Gall midge incidence*			S.No.	Hybrid	Female	Male	Pollen sterility	Spikelet fertility	Gall midge incidence*		
55	**TCN 405**	CMS11A	JGL21002	F	P	60	100	50	133	**TCN 502**	JMS1A	JGL22249	S	S	20	40	21
56	**TCN 406**	JMS1A	JGL21002	S	S	90	100	21	134	**TCN 504**	CMS23A	JGL22250	S	S	50	100	32
57	**TCN 407**	JMS1A	JGL21005	F	F	100	90	21	135	**TCN 505**	JMS11A	JGL22256	S	S	5	30	33
58	**TCN 408**	JMS1A	JGL21005	F	F	100	90	21	136	**TCN 506**	JMS1A	JGL22256	S	S	5	30	21
59	**TCN 409**	CMS11A	JGL21034	F	P	70	100	50	137	**TCN 507**	JMS1A	JGL22268	S	S	90	100	21
60	**TCN 411**	CMS11A	JGL21046	F	P	65	50	50	138	**TCN 508**	JMS11A	JGL22277	S	S	0	20	33
61	**TCN 412**	CMS11A	JGL21046	PS	S	0	50	50	139	**TCN 509**	JMS1A	JGL22277	S	S	0	20	21
62	**TCN 413**	JMS1A	JGL21046	S	S	50	40	21	140	**TCN 510**	CMS23A	JGL22281	F	F	50	100	32
63	**TCN 414**	JMS1A	JGL21046	S	S	73	20	21	141	**TCN 511**	CMS46A	JGL22281	PS	P	78	90	64
64	**TCN 415**	CMS11A	JGL21051	F	P	30	70	50	142	**TCN 512**	JMS1A	JGL22284	S	S	11	0	21
65	**TCN 416**	CMS23A	JGL21053	S	S	0	0	32	143	**TCN 513**	CMS23A	JGL22285	S	S	0	0	32
66	**TCN 419**	JMS1A	JGL21067	F	F	30	10	21	144	**TCN 514**	CMS46A	JGL22285	PS	P	32	20	64
67	**TCN 420**	JMS1A	JGL21071	F	F	90	80	21	145	**TCN 515**	CMS14A	JGL22297	PS	P	50	90	41
68	**TCN 421**	JMS1A	JGL21073	S	S	75	70	21	146	**TCN 516**	CMS23A	JGL22297	PS	P	100	100	32
69	**TCN 422**	CMS11A	JGL21097	F	P	65	100	50	147	**TCN 517**	CMS46A	JGL22297	S	S	89	100	64
70	**TCN 423**	CMS23A	JGL21097	PS	S	63	100	32	148	**TCN 518**	JMS11A	JGL22311	F	F	0	0	33
71	**TCN 424**	JMS1A	JGL21097	S	S	80	100	21	149	**TCN 519**	JMS1A	JGL22311	S	S	0	0	21
72	**TCN 425**	CMS23A	JGL21101	F	F	15	100	32	150	**TCN 520**	JMS11A	JGL22316	PF	F	10	20	33
73	**TCN 426**	JMS1A	JGL21101	F	P	5	20	21	151	**TCN 521**	JMS1A	JGL22316	PS	P	10	30	21
74	**TCN 427**	CMS23A	JGL21122	PS	P	40	10	32	152	**TCN 522**	JMS11A	JGL22318	PF	F	26	9	33
75	**TCN 428**	CMS23A	JGL21129	PS	S	75	100	32	153	**TCN 523**	CMS46A	JGL22333	PS	P	85	100	64

Table2. Contd..

S.No.	Hybrid	Female	Male	Pollen sterility	Spikelet fertility	Gall midge incidence*			S.No.	Hybrid	Female	Male	Pollen sterility	Spikelet fertility	Gall midge incidence*		
76	**TCN 429**	CMS23A	JGL21133	PS	S	73	60	32	154	**TCN 525**	JMS1A	Jaisriam	PS	P	76	40	21
77	**TCN 430**	JMS1A	JGL21133	S	S	100	90	21	155	**TCN 526**	CMS23A	JGL21849	S	S	0	0	32
78	**TCN 431**	CMS23A	JGL21143	PS	S	0	0	32									

* Gall midge incidence percentage

Table3. List of non aromatic maintainers and restorers along with gall midge incidence reaction

Sl. No.	**Genotype**	**Pedigree**	**Pollen sterility of hybrid**	**Spikelet fertility of hybrid**	**Parental type**	**GMIP**	**RGM**	**Sl. No.**	**Genotype**	**Pedigree**	**Pollen sterility of hybrid**	**Spikelet fertility of hybrid**	**Parental type**	**GMIP**	**RGM**
1	**JGL402**	BPT5204 X WGL48684	S	S	Maintainer	40	Suceptible	40	**JGL21133**	MTU1010 X JGL3855	S	S	Maintainer	90	Suceptible
2	**JGL5614**	JGL1798 X Betagamblin	F	F	Restorer	70	Suceptible	41	**JGL21152**	MTU1010 X JGL3855	F	F	Restorer	0	Resistant
3	**JGL5868**	JGL245 X Gedozipeton	F	F	Restorer	50	Suceptible	42	**JGL21779**	IET20473 X JGL11118	F	F	Restorer	70	Suceptible
4	**JGL13392**	JGL420 X Vijetha	F	F	Restorer	0	Resistant	43	**JGL21789**	MTU1010 X JGL11118	S	S	Maintainer	0	Resistant
5	**JGL13546**	MTU4870 X Godavari Isukalu	S	S	Maintainer	0	Resistant	44	**JGL21794**	MTU1010 X JGL11118	S	S	Maintainer	10	Suceptible
6	**JGL15246**	JGL1798 X Godavari Isukalu	S	S	Maintainer	80	Suceptible	45	**JGL21797**	MTU1010 X JGL11118	S	S	Maintainer	0	Resistant
7	**JGL17194**	JGL402 X MTU1010	S	S	Maintainer	0	Resistant	46	**JGL21800**	MTU1010 X JGL1118	F	F	Restorer	10	Suceptible
8	**JGL17574**	JGL3844 X JGL7046	S	S	Maintainer	0	Resistant	47	**JGL21806**	JGL11727 X JGL11470	S	S	Maintainer	0	Resistant
9	**JGL17653**	JGL3828 X OR1032-5-2	F	F	Restorer	60	Suceptible	48	**JGL21812**	JGL11727 X JGL11470	S	S	Maintainer	20	Suceptible
10	**JGL17758**	JGL7046 X NLR34452 // WGL14377	S	S	Maintainer	80	Suceptible	49	**JGL21815**	JGL13595 X JGL11470	F	F	Restorer	80	Suceptible
11	**JGL17782**	MTU4870 X White ponny//JGL3855	S	S	Maintainer	100	Suceptible	50	**JGL21819**	JGL13595 X JGL11470	F	F	Restorer	0	Resistant
12	**JGL17970**	MTU1001 X JGL11470	S	S	Maintainer	0	Resistant	51	**JGL21820**	JGL13595 X JGL11470	F	F	Restorer	0	Resistant

Table3. Contd..

Sl. No.	Genotype	Pedigree	Pollen sterility of hybrid	Spikelet fertility of hybrid	Parental type	GMIP	RGM	Sl. No.	Genotype	Pedigree	Pollen sterility of hybrid	Spikelet fertility of hybrid	Parental type	GMIP	RGM
13	**JGL18045**	MTU1010 X JGL13595	S	S	Maintainer	10	Suceptible	52	**JGL21823**	JGL13595 X JGL11470	S/F	S/F	Maintainer & Restorer	0	Resistant
14	**JGL18079**	MTU1010 X JGL13595	S	S	Maintainer	100	Suceptible	53	**JGL21828**	JGL13595 X JGL11470	F	F	Restorer	0	Resistant
15	**JGL18213**	WGL32100 X JGL3855	F	F	Restorer	0	Resistant	54	**JGL21836**	JGL11118 X JGL11727	F	F	Restorer	80	Suceptible
16	**JGL18215**	WGL32100 X JGL3855	S	S	Maintainer	20	Suceptible	55	**JGL21845**	JGL11118 X JGL11727	S	S	Maintainer	0	Resistant
17	**JGL18222**	JGL3855 X JGL7046	S	S	Maintainer	20	Suceptible	56	**JGL21851**	JGL11118 X JGL11727	S/F	S/F	Maintainer & Restorer	0	Resistant
18	**JGL18262**	MTU4870 X NLR34452//JGL3855	S	S	Maintainer	30	Suceptible	57	**JGL21857**	JGL11118 X JGL11727	S	S	Maintainer	20	Suceptible
19	**JGL18624**	JGL7046 X NLR34452//WGL14377	F	F	Restorer	0	Resistant	58	**JGL21861**	MTU110 X JGL11727	S	S	Maintainer	0	Resistant
20	**JGL18778**	MTU1001 X JGL11470	S	S	Maintainer	0	Resistant	59	**JGL21864**	MTU110 X JGL11727	F	F	Restorer	95	Suceptible
21	**JGL18801**	MTU1010 X JGL13595	F	F	Restorer	0	Resistant	60	**JGL21867**	MTU110 X JGL11727	F	F	Restorer	95	Suceptible
22	**JGL19605**	JGL11470 X T1477	F	F	Restorer	20	Suceptible	61	**JGL21868**	MTU110 X JGL11727	S	S	Maintainer	80	Suceptible
23	**JGL20122**	MTU1010 X JGL11727	S	S	Maintainer	0	Resistant	62	**JGL21870**	MTU110 X JGL11727	S	S	Maintainer	50	Suceptible
24	**JGL20218**	MTU1010 X JGL3855	S	S	Maintainer	30	Suceptible	63	**JGL21881**	MTU1010 X JGL11470	F	F	Restorer	80	Suceptible
25	**JGL20232**	MTU1010 X JGL3855	S	S	Maintainer	60	Suceptible	64	**JGL21883**	MTU1010 X JGL11470	S	S	Maintainer	80	Suceptible
26	**JGL20621**	KrishnaHamsa X JGL17970	S	S	Maintainer	90	Suceptible	65	**JGL22244**	KrishnaHamsa X JGL3844	S	S	Maintainer	80	Suceptible
27	**JGL20649**	MTU1010 X JGL11118	F	F	Restorer	80	Suceptible	66	**JGL22248**	KrishnaHamsa X JGL3844	S	S	Maintainer	20	Suceptible
28	**JGL20670**	JGL11727 X JGL11470	F	F	Restorer	40	Suceptible	67	**JGL22249**	KrishnaHamsa X JGL3844	S	S	Maintainer	50	Suceptible
29	**JGL20769**	MTU1010 X JGL13595	F	F	Restorer	45	Suceptible	68	**JGL22250**	KrishnaHamsa X JGL3844	S	S	Maintainer	100	Suceptible

Table3. Contd..

Sl. No.	Genotype	Pedigree	Pollen sterility of hybrid	Spikelet fertility of hybrid	Parental type	GMIP	RGM	Sl. No.	Genotype	Pedigree	Pollen sterility of hybrid	Spikelet fertility of hybrid	Parental type	GMIP	RGM
30	**JGL20779**	MTU1010 X JGL13595	F	F	Restorer	100	Suceptible	69	**JGL22256**	KrishnaHamsa X JGL3844	S	S	Maintainer	30	Suceptible
31	**JGL21002**	MTU1010 X JGL11727	S	S	Maintainer	100	Suceptible	70	**JGL22268**	KrishnaHamsa X JGL3844	S	S	Maintainer	100	Suceptible
32	**JGL21005**	MTU1010 X JGL11727	F	F	Restorer	90	Suceptible	71	**JGL22277**	KrishnaHamsa X JGL3844	S	S	Maintainer	20	Suceptible
33	**JGL21046**	MTU1010 X JGL11727	S	S	Maintainer	40	Suceptible	72	**JGL22281**	KrishnaHamsa X JGL3844	F	F	Restorer	100	Suceptible
34	**JGL21053**	MTU1010 X JGL11470	S	S	Maintainer	0	Resistant	73	**JGL22284**	KrishnaHamsa X JGL3844	S	S	Maintainer	0	Resistant
35	**JGL21067**	MTU1010 X JGL11470	F	F	Restorer	10	Suceptible	74	**JGL22285**	KrishnaHamsa X JGL3844	S	S	Maintainer	0	Resistant
36	**JGL21071**	MTU1010 X JGL11470	F	F	Restorer	80	Suceptible	75	**JGL22297**	KrishnaHamsa X JGL3844	S	S	Maintainer	100	Suceptible
37	**JGL21073**	MTU1010 X JGL11470	S	S	Maintainer	70	Suceptible	76	**JGL22311**	IR64 X JGL 3844	S/F	S/F	Maintainer & Restorer	0	Resistant
38	**JGL21097**	MTU1010 X JGL3844	S	S	Maintainer	100	Suceptible	77	**JGL21849**	JGL11118 X JGL11727	S	S	Maintainer	0	Resistant
39	**JGL21101**	MTU1010 X JGL3844	F	F	Restorer	100	Suceptible	GMIP=Gall midge incidence percentage; RGM=Reaction to gall midge							

Table 4. Fertility restoration linked marker validation results in restorer and maintainer lines

Sl.No.	Genotypes	RM1 (115bp)	RM3233 (140bp)	RM10313 (188bp)	RM3873 (210bp)	RM10318 (187bp)	RM6100 (185bp)	RM258 (140bp)
1	JGL5868	0	P	P	0	P	P	0
2	JGL13392	0	0	0	0	0	P	0
3	JGL17653	0	P	P	0	P	P	P
4	JGL18203	P	P	0	0	P	P	0
5	JGL18213	P	0	P	0	P	P	0
6	JGL18624	P	P	P	0	P	0	P
7	JGL20769	P	P	P	0	P	P	P
8	JGL20779	0	P	P	0	P	P	0
9	JGL21005	P	0	P	0	P	0	0
10	JGL21067	P	0	P	0	0	P	0
11	JGL21071	P	P	0	0	P	P	P
12	JGL21101	0	P	P	0	P	P	0
13	JGL21779	P	0	P	0	P	0	P
14	JGL21820	0	P	P	0	P	P	0
15	JGL21823	0	P	P	0	P	P	0
16	JGL21823	0	P	P	0	P	0	P
17	JGL21828	0	P	0	0	P	P	0
18	JGL21836	0	0	P	0	P	0	0
19	JGL21851	P	0	P	0	P	P	0
20	JGL22311	0	P	0	0	P	P	P
Efficiency (%)		**45**	**65**	**75**	**0**	**90**	**75**	**35**

Note: **P**=Presence of linked allele; **0**= Absence of linked allele

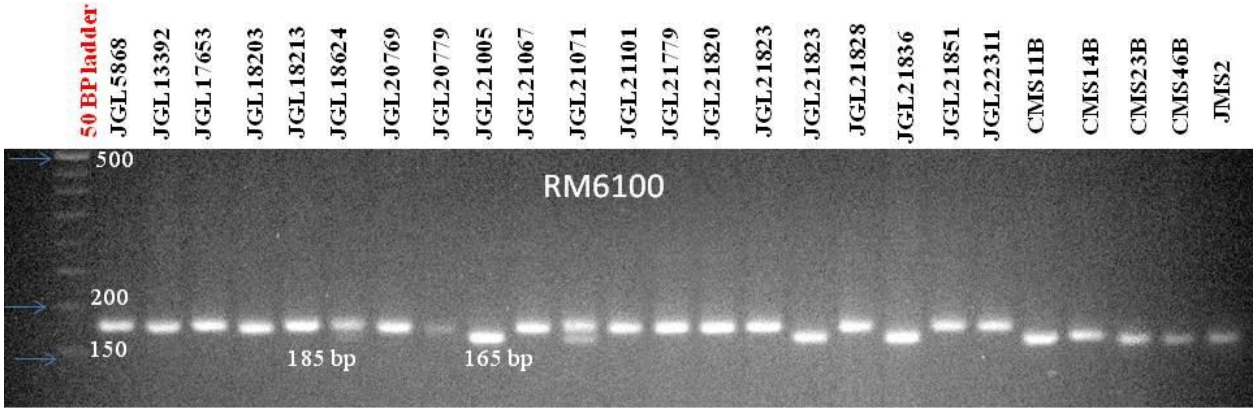

Figure 1. Amplification pattern of RM6100 linked to fertility restorer (*Rf4*) gene

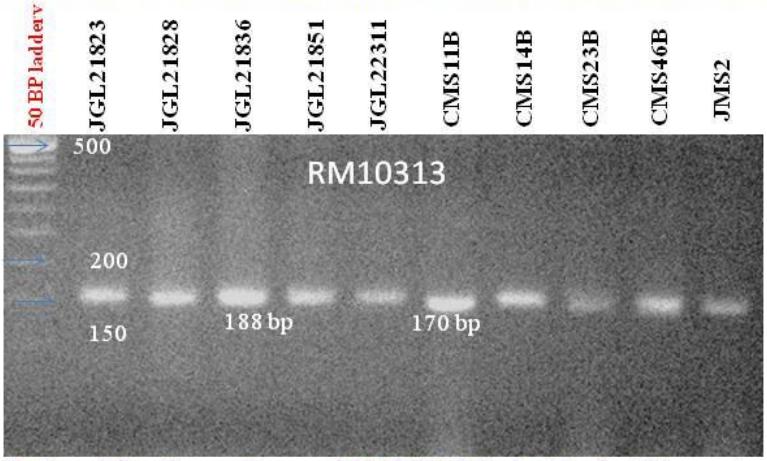

Figure 2. Amplification pattern of RM10313 linked to fertility restorer (*Rf3*) gene