Int.J.Curr.Microbiol.App.Sci (2018) Special Issue-7: 535-543

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Special Issue-7 pp. 535-543**
Journal homepage: http://www.ijcmas.com

Original Research Article

Molecular Characterization of Fertility Restorer Genes for Milo and Maldandi Cytoplasm from Minicore Collection of Rabi Sorghum

B. Hari Vara Prasad* **and B. D. Biradar**

Department of Genetics and plant breeding, University of Agricultural sciences, Dharwad, Karnataka, India
**Corresponding author*

A B S T R A C T

Xtxp250 marker linked to Rf_1 gene clearly distinguished B-lines and R-lines based on the presence or absence of gene. The R-lines with Rf_1 gene showed band size of 1200bp and the B-lines without Rf_1 gene showed band size of 220bp. Moreover xtxp250 marker showed presence of Rf_1 gene in genotypes which were showing fertility restoration above 60 per cent. Findings from the present study revealed that the xtxp250 marker can be effectively used in selecting R-lines with > 60 per cent restoring ability. This xtxp250 marker was not able to distinguish restorer lines of milo and maldandi cytoplasms separately which means the same Rf_1 gene shows restoration on both cytoplasms. This marker can be utilized in selection of plants with Rf_1 gene in segregating populations at the early seedling stage itself.

Keywords

Cytoplasm, Gene, Marker and Restoration

Introduction

Sorghum is an important crop where exploitation of heterosis led to the green revolution in dryland areas in 1970's. Commercial exploitation of heterosis was possible owing to the availability of a stable and heritable CMS mechanism (Stephens and Holland, 1954), inspite of small bisexual flowers. Male fertility for an individual cytoplasm can be restored by a series of *Rf* genes encoded in the nucleus. The *Rf* genes block or compensate for mitochondrial dysfunctions that are phenotypically expressed during pollen development (Honma *et al.*, 2014). Little is known about the mechanism of fertility restoration in even the best characterized systems. Molecular markers have several applications in sorghum breeding programmes particularly for genes like restorer genes which can restore fertility, so that such identified genes can be transferred into a elite genetic background to exploit heterosis. Molecular tags for *Rf* loci have been identified in sorghum and other crops also (Yao *et al.*, 1997; Borner *et al.*, 1998). So far, in sorghum, different fertility restoration genes (Rf_1, Rf_2, Rf_4 and Rf_5) have been identified and recognized as members of the pentatricopeptide repeat (PPR) gene family (Praveen *et al.*, 2015). The molecular markers with respect to Rf loci will give an idea in finding out the restorer genes which shows exclusively fertility restoration on each individual cytoplasm for commercial exploitation of heterosis. In the present study, with the available information on markers linked to different Rf loci an attempt was made to identify genotypes with

Rf genes which differentially shows fertility restoration on milo and maldandi cytoplasm.

Materials and Methods

The male sterile lines utilized as testers in this study were 104A and M31-2A. 104A is based on milo cytoplasmic source and has the distinction of being used in several commercial hybrids. Male sterile line, M31-2A, representing Maldandi cytoplasm has very good grain quality traits like bold size seed, luster and corneous endosperm and resistant to biotic and abiotic stresses. Genotypes from minicore collection were used to identify restorers and maintainers on diverse cytoplasms. All the male parents were crossed to two testers and developed 2 x 168 F_1 bulked seed of every cross (F_1) was planted in a row of 4m length. About 3 heads from each row were bagged 3 days before stigma emergence. The F_1 hybrids were evaluated at botanical garden, Department of genetics and plant breeding, UAS, Dharwad in *rabi* 2015-16. All the recommended agronomic practices were followed to raise a good crop. At maturity, number of seeds were counted out of total number of spikelets per ear head and seedset percentage was calculated.

$$\text{Seed set \%} = \frac{\text{Total number of seeds}}{\text{Total number of spikelets}} \times 100$$

(Kishan and borikar, 1989)

Isolation of Genomic DNA and Quantification

The genotypes from minicore collection which were tested for fertility restoration were also genotyped to detect Rf genes using following procedure.

High molecular weight genomic DNA was extracted for molecular biology work including marker detection technology. With the help of anion detergent (SDS), genomic DNA was extracted from fresh seedlings. SDS method described by Dellaporta *et al.*, (1989) was used for DNA isolation. Qualitative analysis was done by agarose gel electrophoresis. For that 1 per cent agarose gel was prepared and electrophoresis was done at 50 volts for 3-4 h. After that gel was visualized on an UV transilluminator.

For PCR amplification, a master mix without DNA template was prepared for different tubes to reduce pipetting error. The master mix was then redistributed in each PCR tube (24 ml each) and finally 1.0 ml of different DNA template was added in each tube. PCR amplification was performed in a final volume of 25 ml reaction set up containing 1 ml of DNA, 2 ml of dNTPs, 2.5 ml PCR buffer, 2.5 ml of forward primer, 2.5 ml of reverse primers and 0.4 ml of Taq DNA polymerase.

The reaction conditions were as follows: initial denaturation (94°C for 5 min) followed by 35 cycles of denaturation (94°C for 1 min), annealing at 55°C for 2 min (temperature reduced by 1°C for each cycle) and primer extension (72°C for 2 min).

This step was followed by final cycle of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 7 min. PCR amplified DNA fragments were resolved by submerged horizontal electrophoresis in 1 per cent agarose gel and visualized by staining with Ethidium bromide. After completion of electrophoresis, image of the gel was viewed and saved in a gel documentation system (Alpha Imager EC). SSR primers were selected on the basis of their close association with fertility restoration in sorghum genotypes as proved in previous studies. The details of primers are given in following table

Results and Discussion

Validation of linked marker analysis

To assess validity and to estimate selection efficiency of the Rf linked marker in identifying restorers, A_1 and A_4 CMS lines were cross-pollinated with 168 lines on milo (A_1) to develop 168 F_1's and 137 lines on maldandi (A_4) to develop 137 F_1's. The F_1's were evaluated during postrainy season, 2015-16 to assess the fertility restoration ability of pollinators. These lines were also genotyped with markers linked with different 'Rf' genes identified.

The markers which linked to the Rf loci were used to study polymorphism between restorer lines, maintainer lines and CMS lines. A grain sorghum genotype (RLT-2), which had been previously identified with the restorer gene and B-lines (104B and M31-2B) of grain sorghum were used as checks. The fertility restoration pattern in minicore collection of sorghum were not previously known, and they were selected for the experiment to identify restorer and non-restorer lines from them.

Marker analysis

For validation of linked markers to Rf locus, a set of SSRs of Xtxp, Xgap, Xcup, SB series covering Rf loci already mapped in sorghum were selected. The genotypes of minicore collection were screened for the presence or absence of male fertility gene by using a total of 9 markers.

Marker linked to Rf locus

Of the several Rf gene linked markers tested, xtxp250 marker was polymorphic between B-lines and R-lines. The phenotypic assessment of 168 F_1's on milo and 137 F_1's on maldandi identified 43 restorers and 22 maintainers on milo cytoplasm while 19 restorers and 37 maintainers on maldandi CMS. All restorers on maldandi also restored fertility on milo cytoplasm (Table 1 &2). Line RLT-2, a restorer on milo was a restorer on maldandi cytoplasm also. In sorghum, it is generally observed that a line which restores fertility on maldandi typically also restorers on milo, while lines that restores fertility on milo cytoplasm may or may not acts as restorers on maldandi cytoplasm. In order to assess validity and to estimate selection accuracy of the Rf linked marker xtxp250 for its use in marker-assisted selection, we further genotyped the above genotypes with the marker.

All the restorer lines showed a band identical to the restorer RLT-2 (fertile on A_1) and maintainers on milo and maldandi respectively showed band similar to milo (104B) and (M 31-2B) maintainer lines. Thus, xtxp250 marker helps in for selecting a restorer gene among a set of germplasm lines on milo (A_1) and maldandi cytoplasm (A_4). The marker xtxp250 also identified all the maintainers correctly. This indicated a good correspondence between the phenotypic assessment and the Rf linked maker analysis in classifying restorers and non-restorers.

Restorer lines produced a band size of 1200 bp of xtxp250 marker (Fig.1-3) flanking the male fertile restorer gene *Rf_1*. Maintainer lines showed band size of 220 bp with respective to the standard maintainer lines 104B and M31-2B. The band size was similar in CMS lines and its maintainer lines which shows lack of restorer gene. The results indicated that the marker could identify correctly the sorghum genotypes which have male restorer gene. The rest of the markers didn't detect Rf gene across the genotypes.

The linked markers have potential use in screening germplasm lines to identify promising fertility restorers on milo and maldandi and to develop common restorers on both the CMS systems.

Among the different markers screened for different 'Rf' loci in minicore collection of sorghum, the SSR marker xtxp250 showed polymorphism between B and R-lines. The marker xtxp250 was very tightly linked with Rf_1 locus showed banding pattern of 1200 bp for R-lines indicating presence of Rf gene and 220 bp for B-lines indicating absence of Rf gene. In the present study, male sterile, maintainer lines produced PCR bands of similar sizes which indicates absence of restorer gene in its nuclear background. Klein *et al.*, (2001) reported that xtxp250 primer was linked with QTLs for fertility restoration gene, Rf_1 in milo based R-lines.

Other SSR markers viz., Drenshsbm-95, Xcup05, Xtxp250, TS304T, TS050 and Xtxp18 which were linked to Rf_1 loci did not show any polymorphism between B-lines and R-lines. While markers linked to Rf_4 loci (LW7, LW8 and LW9) did not showed amplification.

Table.1 Classification of restoration based on mean seed set percentage

Restoration class	Seed set %	Diverse sources of cytoplasm			
		Milo		Maldandi	
		No. of restorers	Mean seed set %	No. of restorers	Mean seed set %
Strong restoration	>90 %	43	94.34	19	94.47
High restoration	80 to 90 %	1	81.23	4	82.96
Moderate restoration	60 to 80 %	7	73.76	15	69.44
Partial restoration	10 to 60 %	78	28.26	39	26.05
Low restoration	<10 %	17	6.39	23	7.04
No seed set	0 %	22	0.00	37	0.00

Classification of genotypes in minicore collection was done by following procedure described by Biradar *et al.*, (1996) below

Category	Seed set%
Strong restoration	>90 %
High restoration	80 to 90 %
Moderate restoration	60 to 80 %
Partial restoration	10 to 60 %
Low restoration	<10 %
No seed set	0 %

List of markers used for characterization of restorer and non-restorer lines in minicore collection of sorghum for different 'Rf' loci

S. No	SSR-markers	Forward primer	Reverse primer	loci	T_m
1	Drenshsbm-95	GTGGTTTGTTCCAGCCTTTG	GGGGGAGATGTGTTTCTACG	Rf1	55
2	Xcup05	GGAAGGTTTGCAAGAACAGG	CCAGCCCAACAAGTGCTATC	Rf1	55
3	Xtxp250	GCACATCCTCTAAAACTACTTAGT	GAACAGGACGATGTGATAGAT	Rf1	55
4	TS304T	ACATAAAAGCCCCTCTTC	CTTTCACACCCTTTATTCA	Rf1	55
5	TS050	TCGTGGATTTGCATTCCTTGAA	GAATGTGCCTTGTTTCTGTGCG	Rf1	55
6	Xtxp18	ACTGTCTAGAACAAGCTGCG	TTGCTCTAGCTAGGCATTTC	Rf1	55
7	LW7	GATCTACATATGTGGAGCTC	AGATCTCGGCGCTGCGGAGCAGAA	Rf4	60
8	LW8	AGATGGAAAGCTCTGTGCTG	AACACTCCACTAGGTGGGTCA	Rf4	58
9	LW9	ACCTTCGACGGAATGTTTCAGG	TTCGTCCGTTTGTGGTGAGTTG	Rf4	58

Fig.1 Molecular characterization of R-lines with > 60 per cent seedset in F_1's on both *milo* and *maldandi* cytoplasms for Rf_1 gene using xtxp250 marker

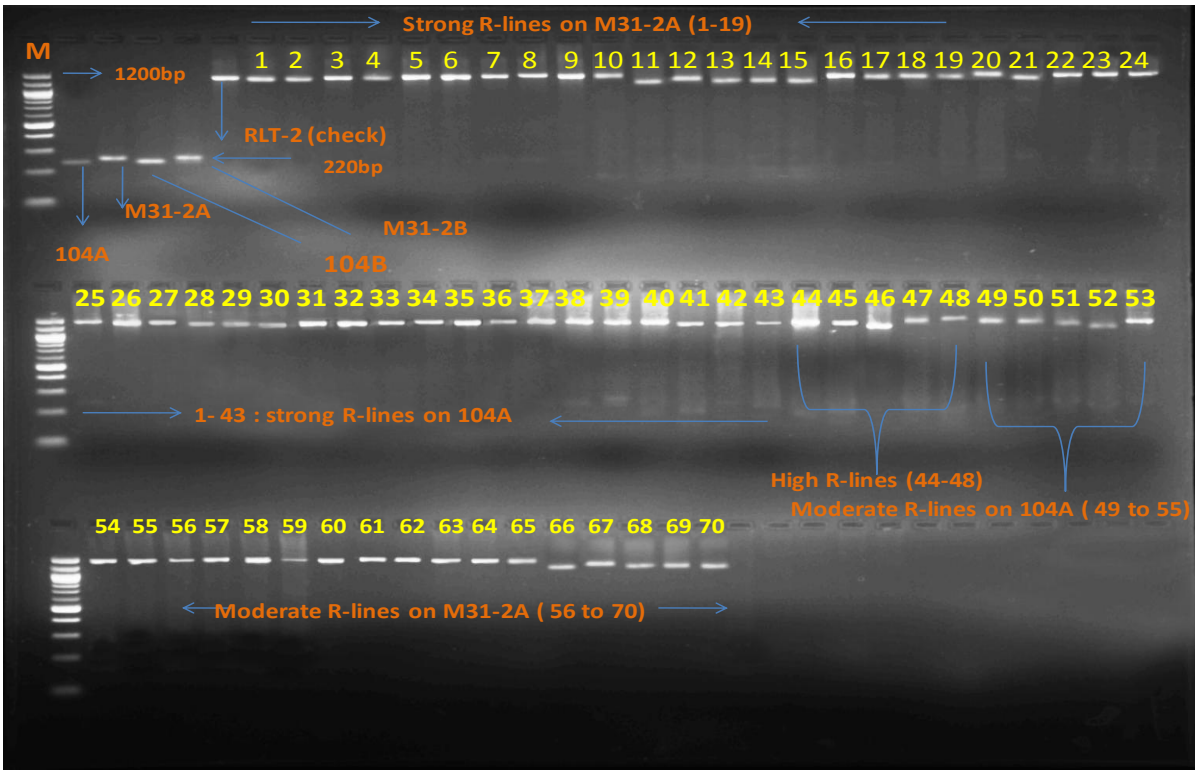


Fig.2 Molecular characterization of R-lines with 10-60 per cent seedset in F_1's on both *milo* and *maldandi* cytoplasms for Rf_1 gene using xtxp250 marker

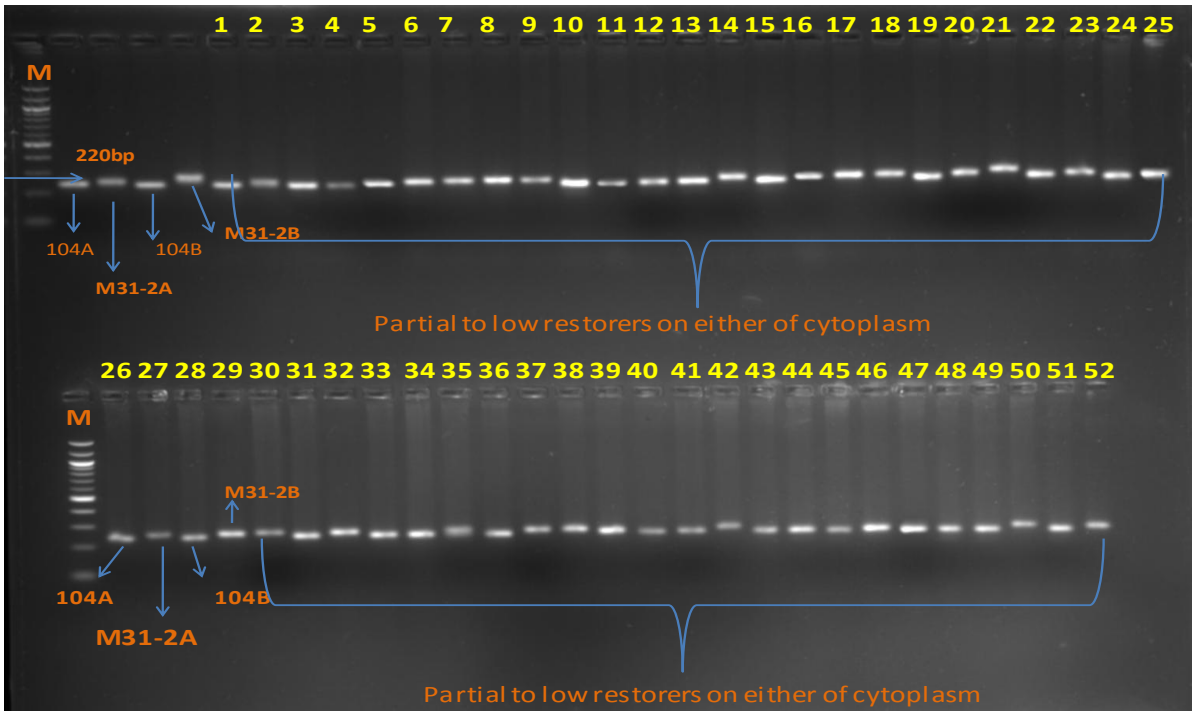
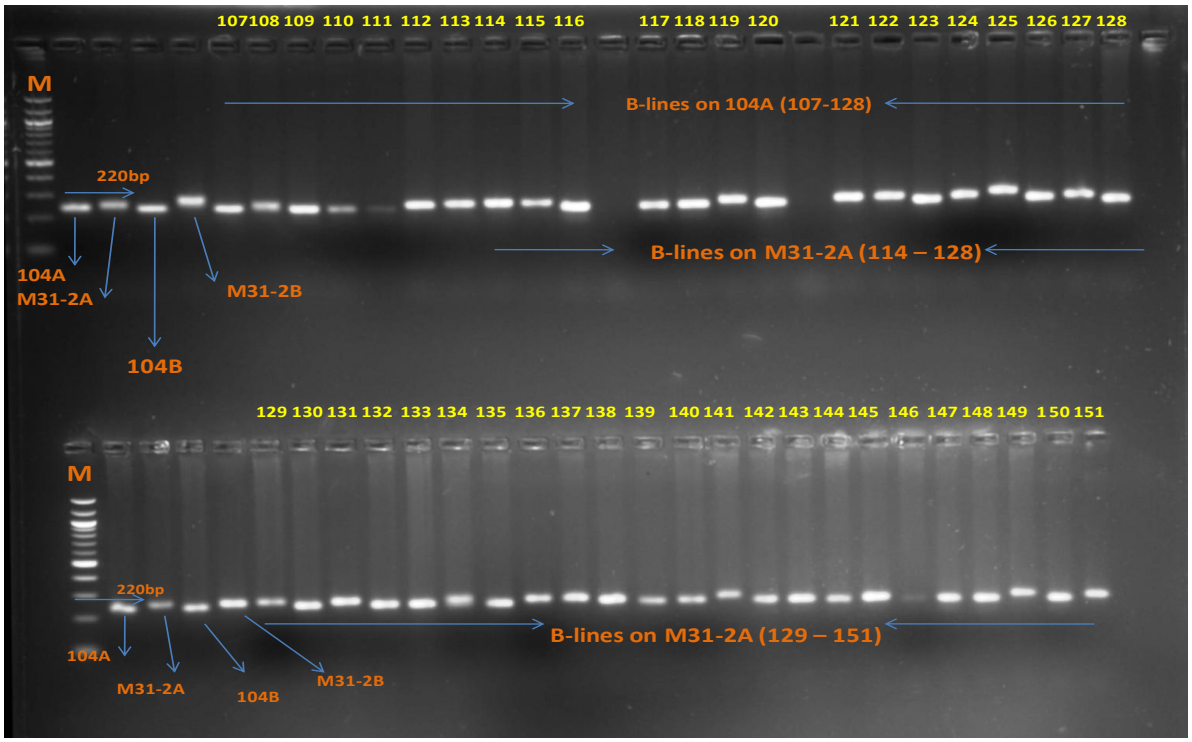


Fig.3 Molecular characterization of B-lines with zero per cent seed set in F_1's on both *milo* and *maldandi* cytoplasms for Rf_1 gene using xtxp250 marker



On milo cytoplasm 43 genotypes identified as strong restorers (>90 % seedset) and on maldandi cytoplasm 19 genotypes identified as strong restorers. At the molecular level this xtxp250 marker could not discriminate R-lines of milo and maldandi cytoplasm. This shows the same Rf_1 gene involved in fertility restoration on both cytoplasms. Praveen *et al.*, (2015) identified restorer gene Rf_6, which was able to restore male fertility on both A_1 and A_2 cytoplasms.

Out of 43 strong restorers 19 showed restoration on both milo and maldandi cytoplasm and remaining 24 showed restoration on milo cytoplasm only. This shows there could be more than one major Rf gene which is necessary for restoration on maldandi cytoplasm. Elkonin *et al.*, (1998) reported that fertility restoration could be because of one or more major Rf genes on different male sterile cytoplasm. The markers linked for other than Rf_1 loci did not showed amplification which means additional markers have to be developed to tag unidentified Rf gene in those genotypes which showed restoration on maldandi cytoplasm.

The xtxp250 marker which showed polymorphism between B-lines and R-lines was not able to discriminate between the strong restorer lines (> 90 %) and other classes of restorer groups (high (80-90 %) and moderate (60-80 %)) which means there was no difference in banding pattern. These differences in fertility restoration across diverse cytoplasmic sources could be due to accumulation of number of modifier genes in nuclear background of pollinator parent. Bunphan *et al.*, (2013) reported in sorghum that the fertility restoration varies with accumulation of number of minor genes along with major restorer gene. These findings shows that this xtxp250 marker can be used to identify an individual with atleast

of 60 per cent fertility restoration which is practically beneficial. The restorer groups like partial (10-60 % seedset), low (<10 % seedset) with less than 60 per cent seedset indicates there could be other weak restorer gene or effect of modifier genes. These findings reveal there is a scope to investigate on minor genes in nuclear background.

Since xtxp250 had shown high selection efficiency, it could be useful for screening of germplasm to identify the potential restorers on A_1 (milo) to high extent with the presence of Rf_1 gene but when it comes to maldandi cytoplasm still more progress has to be achieved by developing mapping population and tagging of 'Rf' loci. Chauhan *et al.,* (2015) reported that xtxp250 marker found to be polymorphic for Rf_1 gene on milo cytoplasm. Klein *et al.,* (2001) recorded polymorphism among QTLs for all the genotypes and concluded xtxp250 marker was very useful for the identification of Rf_1 gene in sorghum breeding programme.

The present study validated the application of a linked marker xtxp250 for identifying the restorers for Rf_1 in minicore collection, which can accelerate breeding of restorer lines and thus, enhance efficiency of hybrid breeding in sorghum. Chandrashekar *et al.,* (2013) reported in mustard that the validated markers for Rf gene can be used to test the purity of hybrids as an effective substitute to the time consuming and laborious grow-out test (GOT).

From the study the SSR marker xtxp250 can be used for primary selection at seedling stage to detect the presence of Rf gene. Hence, works required for test crosses by breeders would be reduced. Further studies are needed to confirm the efficiency of identified marker by transferring Rf_1 in to elite line using marker and such line has to be crossed with CMS lines to see fertility pattern of F_1 plants. Ayachit *et al.,* (2013) reported that the plants identified with presence of Rf gene using linked marker could be useful in transferring the fertility restorer gene in to elite parental lines with an ease. Bunphan *et al.,* (2013) reported that MAS using linked markers helps in preliminary screening of individuals with Rf gene which would reduce population size for line selection.

From these findings developing a restorer line/transferring Rf gene for milo and maldandi CMS systems across genetic backgrounds with high heterotic potential by utilizing validated markers for Rf genes can enable sorghum breeders to exploit both milo and maldandi cytoplasm.

Hence, from the present study 'xtxp250' marker will be useful in identifying sorghum individuals with 'Rf_1' loci which can accelerate breeding of restorer lines.

Xtxp250 marker linked to Rf_1 gene clearly distinguished B-lines and R-lines based on the presence or absence of gene. This xtxp250 marker was not able to distinguish restorer lines of milo and maldandi cytoplasms which means there could be other Rf gene for restoration on maldandi cytoplasm which was not detected by markers used in the present study. Findings from the present study revealed the xtxp250 marker can be effectively used in selecting R-lines with > 60 per cent restoring ability. This marker can be utilized in selection of plants with Rf_1 gene in segregating populations at the early seedling stage itself.

References

Ayachit, R. S., Patel, D. H., Patel, M. M. and Kumar, V., 2013. Identification of fertility restorer genes in cotton

through DNA markers. *Ind. J. Plant Physiol*., 18(1): 71–72.

Biradar, B. D., Parameshwarappa, R., Patil, S. S. and Goud, J. V., 1996. Heterosis studies involving diverse sources of cytoplasmic genetic male sterility system in sorghum. *Karnataka J. Agric. Sci.,* 9: 627-634.

Borner, A., Korzun, V., Polley, A., Malyshev, S. and Malz, G., 1998. Genetics and molecular mapping of a male fertility restoration locus (*Rfg1*) in rye (*Secale cereale* L.). *Theor. Appl Genet.,* 97: 99–102.

Bunphan, D., Jaisil, P. and Sanitchon, J., 2013. Application of LW7 marker for identification of progenies with male sterility gene in sweet sorghum population. *Crop Breed. Appl. Biotech.*, 13: 59-66.

Chandrashekar, U. S., Dadlani, M., Vishwanath, K., Manjunath Prasad, CT. and Chakrabarty, S. K., 2013. Validation of molecular markers linked to sterility and fertility restorer genes in *Brassica juncea* (Linn) Czern and Coss. *Ann. Pl. Sci.,* 2 (3): 96-99.

Chauhan, P., Pandey, P. K., Shrotria, P. K., Singh, N. K., Pandey, G. and Singh, S., 2015. Allele specific diversity using fertility restoration related SSR markers in sorghum [*Sorghum bicolor* (L.) Moench]. *Forage Res.,* 40 (4): 222-227.

Elkonin, L. A., Kozhemyakin, V. V. and Ishin A. G., 1998. Nuclear-cytoplasmic interactions in restoration of male fertility in the '9E' and A4 CMS-inducing cytoplasms of sorghum. *Theor. Appl. Genet*., 97: 626–632.

Honma, Y., Taguchi, K., Hiyama, H., Kurino, R. Y., Mikami, T. and Kubo, T., 2014. Molecular mapping of *restorer- of- fertility 2* gene identified from a sugar beet (*Beta vulgaris* L. ssp. *vulgaris*) homozygous for the non-restoring *restorer- of- fertility 1* allele. *Theor. Appl. Genet.,* 127: 2567–2574.

Kishan, A. G. and Borikar, S. T., 1989. Genetic relationship between some cytoplasmic male sterility systems in sorghum. *Euphytica,* 42: 259-269.

Klein, R.R., Klein, P.E., Chhabra, A.K., Dong, J., Pammi, S., Childs, K.L., Mullet, J.E., Rooney, W.L. and Schertz, K.F., 2001. Molecular mapping of the rf1 gene for pollen fertility restoration in sorghum (*Sorghum bicolor* L.). *Theor. Appl. Genet*., 102:1206–1212.

Praveen, M., Anurag Uttam, G., Suneetha, N., Umakanth, A., Patil, J.V. and Madhusudhana, R., 2015. Inheritance and molecular mapping of Rf6 locus with pollen fertility restoration ability on A_1 and A_2 cytoplasms in sorghum. *Pl. Sci.*, 238: 73–80.

Stephens, J. C. and Holland R. F., 1954. Cytoplasmic male sterility for hybrid sorghum seed production. *Agronomy J.*, 46: 20-23.

Yao, F.Y., Xu, C.G., Yu, S.B., Li, J.X., Gao, Y.J., Li, X.H. and Zhang, Q., 1997. Mapping and genetic analysis of two fertility restorer loci in the wild-abortive cytoplasmic male sterility system of rice (*Oryza sativa* L). *Euphytica,* 98: 183–187.