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SHORT RESEARCH ARTICLE

Identification of sex linked molecular markers in spine gourd (*Momordica dioica* Roxb.)

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Abstract

The major drawback of a dioecious spine gourd (*Momordica dioica* Roxb.) is distinguishing whether the plant is male or female at an earlier development stage. An investigation was aimed to eliminate aforesaid limitation by identifying and validating sex linked molecular marker. Twenty six male and female genotypes of each sex were screened with 12 specific SSR primers. Only two SSR primers, MdSSR 5 (MADS-box gene of spine gourd) and CMbSSR 6 (MADS-box gene of cucumber) showed amplification and polymorphism in female genotypes only. These specific female linked markers manifest existent of non-epigenetic factors entailed in gender development in the spine gourd.

Keywords: DNA extraction, *Momordica dioica,* PCR amplification, simple sequence repeat (SSR)

The vegetable plant, *M. dioica* Roxb. (2n = 28), belonging to cucurbitaceae, is known varyingly as spine gourd, akakara, bodakakara, kakor, teasle gourd, kantola, kakrol, parora, kheksa, kankoda, golbandra, dharkarela, and batkarila (Bawara et al. 2010). It is predominant in distribution worldwide, mainly in India, Sri Lanka, Myanmar, and Bangladesh. In India, it is disseminated in all the states except in north-east (Bharathi et al. 2011), but generally confined in the states like Orissa, Bihar, Uttar Pradesh and West Bengal. *M. dioica* is raised effectively in hot and humid areas and almost seems to be day-neutral (Bharathi et al. 2013). The nature of this vegetable is perennial, rhizomatous and climbs up to 3-10 m height with tapering root, which gets bulged and elongated with subsequent years. Nutritionally and medicinally, it is a highly valuable crop, and the tender green fruits are mainly used for cooking purposes (Salvi et al. 2015).

A single factor controls the sex in this vegetable, female being homozygous recessive and male being heterozygous (Sanwal et al. 2011). In male plants, the first flower produced reaches full bloom 35 days after emergence from the soil; those produced by female plants require an average of 45 days. These days, areas that are to be allocated for research work relevant to a great agricultural interest for commerce are recognition of gender at seedling stage by the external morphology of their embryogenic and juvenile forms, which is difficult prior to flowering in such cases economically vital dioecious crop. Therefore, an early diagnosis of gender is of great importance in such commercial crops, as it helps reduce the effort of breeders and cultivators in saving field space, time, and other useful resources that get wasted in maintaining undesired plants till flowering. Recently, molecular markers have made a ubiquitous and reliable rapid impact in genetic relationship, marker-trait association and detection of genetic variation at DNA level precisely in organisms, including many cucurbitaceous plants. The present study aimed to identify a genetic sex marker for the spine gourd (*M. dioica* Roxb.) to allow gender determination at any stage in the life cycle.

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The experimental plant material comprised of 25 accessions, namely, PK-5, Ambika 13-6, Ambika 13-5, RMDSG-2, RMDSG-3, AJSG-2, AJSG-1, ASG 18-1, ASG 18-2, ASG 18-3, ASG 18-4, RMDSG-1, Ambika K 12-1, Krishnapur, RMD 15-1, RMD 15-2, RMD 15-3, RMD 15-4, RMD 15-5 and Indira Kankoda-1from different locations in Chhattisgarh, three accessions each from Faizabad, Uttar Pradesh viz., NDM -1, NDM -2 and NDM -4 and Rahuri, Maharashta, Phule MD 5-1RMFG-49 and RMF-39. The 26 genotypes are unbruised, soft with young leaves of male and a female spine gourd originating from different agro-ecological regions of the country landraces, collected from the experimental farm All India Coordinated Research Network on Potential Crops, Ambikapur. Laboratory work was performed at Plant Molecular Biology and Biotechnology Laboratory, section of Genetics and Plant Breeding, RMD CARS, Ambikapur, India. Bulk segregant analysis was done by using two bulks of DNA samples of male and female were separately prepared by pooling an equal quantity of DNA from individuals of both 26 male and female spine gourds. The two bulks along with parents were amplified with 12 SSR primers using standard PCR protocol.

Nucleotide sequence of *M. dioica* and other cucurbitaceous crops *viz*., cucumber and watermelon from NCBI site were searched for development of sex-specific microsatellite markers in spine gourd. The specific sequences information for MADS box gene, which is responsible for flowering in spine gourd, cucumber and watermelon were searched. It is one of the genes playing a necessary role in the floral formation and further evaluation of homeotic mutants in cucumber, which has divulged that MADS-box genes are not sex-determining but rather likely to act as regulators of the processes directing to the development of definite sex (Kater et al. 2001). It even manifests specific expression (Perl-Treves et al. 1998) in the stamens and carpels. The gene participating in ethylene biosynthesis was also taken for primer designing. By undertaking the above-mentioned nucleotide sequences information, FASTA sequences were chosen to design the SSR primers through primer designing program i.e., Batchprimer 3, then from any primer design servers by choosing SSR screening and primers type primers were picked and forward and reverse sequences were obtained. Standard criteria for selecting SSR primer from FASTA sequence were applied during the designing of SSR primers by Batchprimer 3.

Male plant

Female plant

SSR markers identification and validation

In the current study annealing temperature used is quite distinct and low, and the variables such as dNTPs, buffer, Mg^{2+}, Taq polymerase were decided empirically. All the synthesized 12 SSR primers were screened in a single genotype of *M. dioica* for checking their quality and annealing temperature. Based on this experiment, a total 10 SSR primers, namely, MdSSR1, MdSSR2, MdSSR3, CMbSSR7, CMbSSR8, CEbSSR9, CEbSSR10, and WMSSR11 were monomorphic annealing temperature (51 to 57°C), two, MdSSR5 and CMbSSR6 were polymorphic, and remaining two, MdSSR4 and WMSSR11 markers were not amplified at annealing temperature 50°C and 51°C, respectively. Markers, MdSSR5 and CMbSSR6 showed polymorphism and amplified bands (~500bp and ~700bp) obtained were specific to only female genotypes of spine gourd.

In order to validate those obtained specific markers, an attempt was made in which the specific polymorphic SSR markers MdSSR 5 and CMbSSR 6 of band size 500 and 700 base pairs respectively were taken, which was obtained when screened with single genotypes of both male and female spine gourd. For validation, screening of the above-mentioned polymorphic markers with all the 26 male and female genotypes was done. The result obtained after screening proved that those specific polymorphic markers are present only in female genotypes and are of true type represented below in Fig. 1.

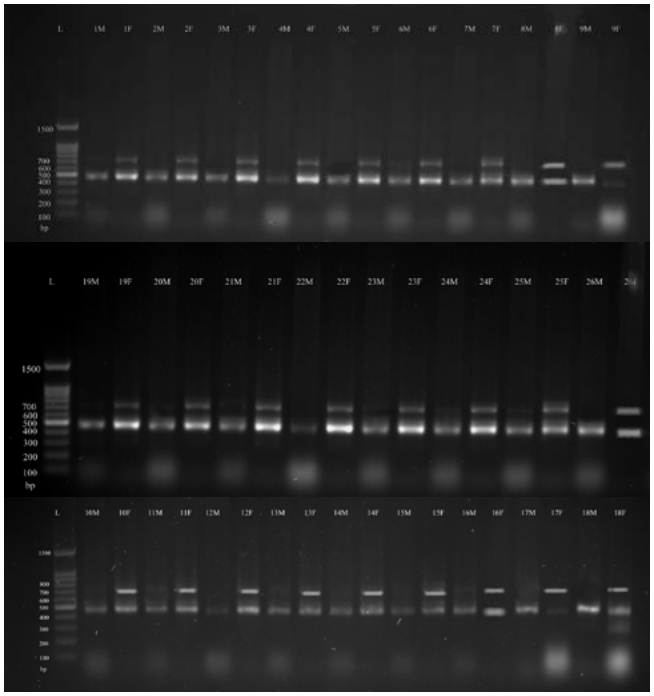

Fig. 1. PCR amplification using primer CMbSSR 6 in 26 male and 26 female genotypes of spine gourd
L= Ladder, M= Male, F= Female and bp= Base pairs

Similar result findings were made by Adhikari et al. (2014) in *Trichosanthes dioica* was he identified and validated a male sex-specific new ISSR marker of 550 base pairs when screening the genomic DNA with forty two ISSR markers through touch-down polymerase chain reactions. In papaya, Gangopadhyay et al. (2007) developed a bisexual and female-specific marker using $(GACA)_4$. Baratakke et al. (2009) found male specific RAPD marker OPA-15.

Authors' contribution

Conceptualization of research (JKT); Designing of the experiments (JKT); Contribution of experimental materials (JKT); Execution of field/lab experiments and data collection (GA, JK, VPS); Analysis of data and interpretation (JKT); Preparation of the manuscript (JKT, BKD, GA, VPS);

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