Research Article

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MOLECULAR TOOL FOR SEX IDENTIFICATION (FEMALE) IN *MOMORDICA DIOICA* ROXB WITH REFERENCE TO MEDICINAL VALUES

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ABSTRACT

Spine gourd (*Momordica dioica* Roxb.) is an important vegetable with high food value and sex specific versatile medicinal value. *Momordica dioica*, a perennial, rhizomatous, distinctly dioecious climber belongs to family cucurbitaceae. As there are no distinguished morphological markers to identify sex, an easy, rapid and reliable molecular method for female plant identification at pre-flowering stage in *Momordica dioica* is reported in this paper. Molecular tool like Random amplification of polymorphic DNA was used to identify female plants before pre-flowering stage. A total of 50 random decamer primers were used for screening of specific Random amplification of polymorphic DNA markers in male and female populations. Only one primer OPA-15 amplified genomic DNA in different patterns in male and female genotypes. This sex specific band OPA-15₉₀₀ was identified only in female lines but not in male lines. This marker may be efficiently used as effective, convenient and reliable molecular markers for female identification in *Momordica dioica* at pre-flowering stages so that it can be cultivated and utilized for its medicinal purpose.

Keywords: *Momordica dioica*, Sex determination, Molecular tool, RAPD, SCAR marker.

INTRODUCTION

Momordica dioica is a semi wild, dioecious member of Cucurbitaceae. This plant is commonly known as spine gourd. It is also called as teasle gourd, Kakrol, Kantoli and in Sanskrit as Karkotak. It is a tuberous climber found in Western Ghats of Maharashtra, India and in other regions of India to Ceylon, ascending to 5000 ft. in the Himalaya, Malaya, Bangladesh and China¹⁻⁴. In Karnataka it is distributed frequently in wet forests of Western Ghats and Deccan (Belgaum, Chikmagalur, Dharwad, Dakshina and Uttar Kannada, Hassan, Mysore, Shimoga)⁵. *Momordica dioica* is propagated vegetatively through tuberous roots which are under cultivation at very few regions of India and Bangladesh⁶. *Momordica dioica* is a perennial, with tuberous roots; stem is slender, branched, furrowed, glabrous and shining. Tendrils are simple, elongate, striate and glabrous. Leaves are membranous, broadly ovate in outline, variable, cordate at base, more or less deeply 3-5 lobed, distinctly denticulate. Male flowers- peduncle solitary, slender, usually pubescent near the top, bract cuculate, strongly nerved, calyx-lobes distinct, linear lanceolate, petals wholly yellow; Female flowers- peduncles nearly as long as males, usually with a small bract near the base, ovary clothed with long soft papillate. Fruit long, ellipsoid, shortly beaked, densely echinate with soft spines. Seeds are slightly compressed, enclosed in a red pulp. *Momordica dioica* is an important vegetable with high food value, containing good amount of carotene (162 mg /100 g) and protein (3.1 g /100 g) amongst all Cucurbitaceous vegetables⁷. Both male and female *Momordica dioica* has sex specific medicinal properties^{8,9}. The leaves of female *Momordica dioica* are used as an aphrodisiac, to eradicate

intestinal parasites and to cure fever, consumption, asthma, hiccups and piles. The root of female *Momordica dioica* is used in the form of a medicinal paste to heal bleeding piles, also used with benefit in head troubles, kidney stones and jaundice. The fruit is considered pungent, bitter, hot, stomachic and laxative and plays a role in cures for biliousness, asthma, leprosy, bronchitis, fever, tumors, urinary discharges, excessive salivation and heart disease. Juice of the fruit is a domestic remedy for inflammation⁹. Powder obtained from dried fruit is used to induce sneezing, leading to nasal clearing. Ethanol extracts of fruits has showed nephroprotective activity¹⁰. The fruit pulp is proved for its hypoglycemic¹¹, hepatoprotective activity¹², anti inflammatory and analgesic activity¹³ and antibacterial¹⁴, antifeedant properties¹⁵. Karyomorphological studies¹⁶ reveal that male and female *Momordica dioica* exhibit no morphological markers and possess homomorphic chromosomes which cannot be used for sex screening. In the present study, *Momordica dioica* plant was investigated for the molecular basis of genotypic differentiation between male and female plants using molecular tool called randomly amplified polymorphic DNA (RAPD) technique. Identification of sex through RAPD markers have been reported in *Salix viminalis*¹⁷, *Carica papaya* and *Cycas circinalis*¹⁸. Genetic marker system based on direct analysis of the genomic DNA have been used widely for genetic mapping, disease diagnostics and evolutionary studies and they could prove very useful in the study of sexual determination in dioecious plants such as pistachio¹⁹ and hemp²⁰. An ideal tool to determine the sex of the plant before flowering would facilitate breeding and selection by enabling screening for gender at

the seedling stage simplifying the breeding of male and female plants for different objectives, thereby saving time and economic resources.

MATERIALS AND METHOD

Both male and female populations, with well defined sexual characters were collected from 20 different geographical locations. Total genomic DNA was isolated from young, fresh leaves of well differentiated (confirmed through flowers) male and female plants by modified CTAB method following the standard protocol²¹. Quantification and purity of isolated DNA was checked through UV-spectrophotometer. Purified genomic DNA was subjected to PCR amplification for RAPD analysis, using fifty decamer random primers (Table 1). Each 20 µl

of the PCR reaction mixture consisted of 50 ng genomic DNA, 1.5 mM MgCl₂, 200 µM each dNTP, 15 pM primer (Operon Tech., Alameda, USA) and 0.5 units of Taq DNA polymerase (Bangalore Genei, Bangalore, India). Amplifications were carried out in a Gradient Palm Cycler (Corbett Research, Australia) with initial denaturation for 4 minutes and each cycle with 15 seconds at 94°C, 15 seconds annealing at 35°C and 1.15 minutes for extension at 72°C. The reaction continued for 40 cycles followed by 7 minutes at 72°C to ensure the completeness of the primer extension. Amplified products were separated by electrophoresis on 1.2 % (w/v) agarose gels and visualized by staining with ethidium bromide and documented.

Table 1: List of Primers used for the RAPD analysis

Sl. No.	Primer	Primer sequence 5'-----3'	Melting temperature (T _m) °C	GC content (%)	Molecular weight (g/M)
1	OPA-01	CAGGCCCTTC	34.0	70	2963
2	OPA-02	TGCCGAGCTG	34.0	70	3041
3	OPA-03	AGTCAGCCAC	32.0	60	2996
4	OPA-04	AATCGGGCTG	32.0	60	3065
5	OPA-05	AGGGGTCTTG	32.0	60	3095
6	OPA-06	GGTCCCTGAC	34.0	70	3002
7	OPA-07	GAAACGGGTG	32.0	60	3113
8	OPA-08	GTGACGTAGG	32.0	60	3104
9	OPA-09	GGGTAACGCC	34.0	70	3050
10	OPA-10	GTGATCGCAG	32.0	60	3065
11	OPA-11	CAATCGCCGT	32.0	60	2987
12	OPA-12	TCGGCGATAG	32.0	60	3065
13	OPA-13	CAGCACCCAC	34.0	70	2942
14	OPA-14	TCTGTGCTGG	32.0	60	3047
15	OPA-15	TTCCGAACCC	32.0	60	2948
16	OPA-16	AGCCAGCGAA	32.0	60	3044
17	OPA-17	GACCGCTTGT	32.0	60	3017
18	OPA-18	AGGTGACCGT	32.0	60	3065
19	OPA-19	CAAACGTCGG	32.0	60	3035
20	OPA-20	GTTGCGATCC	32.0	60	3017
21	OPB-01	GTTCGCTCC	32.0	60	2969
22	OPB-02	TGATCCCTGG	32.0	60	3017
23	OPB-03	CATCCCCTG	34.0	70	2924
24	OPB-04	GGACTGGAGT	32.0	60	3104
25	OPB-05	TGCGCCCTTC	34.0	70	2954
26	OPB-06	TGCTCTGCCC	34.0	70	2954
27	OPB-07	GGTGACGCAG	34.0	70	3089
28	OPB-08	GTCCACACGG	34.0	70	3011
29	OPB-09	TGGGGGACTC	34.0	70	3080
30	OPB-10	CTGCTGGGAC	34.0	70	3041
31	OPB-11	GTAGACCGT	32.0	60	3026
32	OPB-12	CCTTGACGCA	32.0	60	2987
33	OPB-13	TTCCCCGCT	34.0	70	2915
34	OPB-14	TCCGCTCTGG	34.0	70	2993
35	OPB-15	GGAGGGTGT	32.0	60	3134
36	OPB-16	TTTGCCCGGA	32.0	60	3017
37	OPB-17	AGGGAACGAG	32.0	60	3122
38	OPB-18	CCACAGCAGT	32.0	60	2996
39	OPB-19	ACCCCCGAAG	34.0	70	2981
40	OPB-20	GGACCCTTAC	32.0	60	2987
41	OPC-01	TTCGAGCCAG	32.0	60	3026
42	OPC-02	GTGAGGCGTC	32.0	60	3080
43	OPC-03	GGGGGTCTTT	32.0	60	3086
44	OPC-04	CCGCATCTAC	32.0	60	2948
45	OPC-05	GATGACCGCC	34.0	70	3011
46	OPC-06	GAACGGACTC	32.0	60	3035
47	OPC-07	GTCCCGACGA	34.0	70	3011
48	OPC-08	TGGACCGGTG	34.0	70	3080
49	OPC-09	CTCACCGTCC	34.0	70	2924
50	OPBF-11	GACGACCGCA	34.0	70	3020

Fig. 1. Fractionation of RAPD products developed by OPA-15 primer in male and female *M. dioica* on 1.2% agarose gel. Lane M-molecular weight marker; Lane 1,3,5,7,9,11-RAPD products from male plants; Lane 2,4,6,8,10,12 RAPD products from female plants Arrow indicates female-related RAPD band 900 bp.

RESULTS

Among the 50 random primers used in the present investigation, OPA-15 yielded a unique amplicon of 900 bp only in female lines in all 20 populations studied (results of only six male and six female populations shown in Figure 1).

DISCUSSION

Sex is the queen problem in evolutionary biology and tracing of molecular factors for sex expression has potential importance in basic and applied research. Sex determination mechanism in plants is not well understood. Neither the genetic nor the physiological basis of gender has been completely resolved in any plant species, in spite of the striking progress made over the floral development²². The presence of sex chromosomes has been documented in some plants²³. More often, the sex ratio in dioecious plant species is controlled by the expression of alleles at one to several loci²⁴. There are three major sex strategies in angiosperms such as bisexual, monoecious and dioecious forms. Cucurbitaceae family exhibits similar sex spectrum. As the *Momordica dioica* is a dioecious plant, use of molecular markers to discriminate sex is worthwhile in absence of morphological differences and genetic information. A RAPD marker OPA-15₉₀₀ band consistently appeared exclusively in female genotypes, suggesting thereby female associated nature of this DNA marker in *Momordica dioica*. The OPA-15₉₀₀ apparently constitutes a marker closely linked to a female determining chromosome segment of *Momordica dioica*. As a consequence, this marker can be fruitfully and adequately used for determination of sex in *Momordica dioica*, well before the plant reaches reproductive maturity. Michelmore *et al.*²⁵ described an application of the random amplified polymorphic DNA (RAPD) technique. A better approach to understand how sex determination operates in dioecious species is to study differences at the DNA level. The discovery of markers linked to sex determining genes could eventually allow us to clone the genes involved in this process. OPA-15₉₀₀ proved to be extremely reproducible under a wide variation of amplification conditions. Nevertheless, the reproducibility

of RAPD markers has often been questioned and currently the trend is to develop the reliable and simple to use SCAR (Sequence Characterized Amplified Region) markers from sequenced RAPD markers. A SCAR can indeed further simplify the massive screening of dioecious *Momordica dioica* cultivars and breeding lines.

CONCLUSION

As both male and female taxa of *Momordica dioica* exhibit homomorphic chromosome with 2n=28, sex of the plant cannot be identified based on karyomorphological data. Sexual dimorphism appears to be controlled by genes in nature and supports the development of molecular marker by which sex can be identified. In *Momordica dioica* OPA-15₉₀₀ amplicon was found to be female linked marker. In the present research work RAPD marker was developed. From this RAPD marker further OPA-15₉₀₀ amplicon can be sequenced to design SCAR marker, which further makes the sex identification of *Momordica dioica* at seedling stage fast and accurate.

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