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Short Communication

Identification of a RAPD marker linked to sex determination in *Momordica dioica* Roxb.

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Sexual dimorphism is the rule in most animals. In plant kingdom however, dioecy is found only in 4% of the angiosperms. Dioecism has originated independently in different families and genera [1] and several distinct genetic mechanisms regulating dioecy have been found in different plant species [2, 3]. Sex is the queen problem in evolutionary biology and tracing of molecular factor(s) 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 development [4]. The presence of sex chromosomes has been documented in some plants [1]. More often, the sex ratio in dioecious plant species is controlled by the expression of alleles at one to several loci [2]. 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 pistachio [5], hemp [6] and basket willow [7]. There are three major sex strategies in angiosperms such as bisexual, monoecious and dioecious forms. Cucurbitaceae family exhibits similar sex spectrum. *Momordica dioica,* commonly known as spine gourd, a perennial cucurbitaceous rhizomatous distinctly dioecious climber found in the forests of southern India and Bengal. Tender fruits and tuberous roots are used as vegetable and ayurvedic medicine. Medicinal properties of this plant are sex specific and each sex has its own medicinal value [8]. Karyomorphological studies [9] reveal that male and female *M. dioica* exhibit no morphological markers and possess homomorphic chromosomes which enable sex screening. The sex ratio in natural distribution is 3:1 (Male:Female). In the present study, *M. dioica* plant was investigated for the molecular basis of genotypic differentiation between male and female plants using randomly amplified polymorphic DNA (RAPD) technique. Identification of sex through RAPD markers have been reported in *Salix viminalis* [10], *Carica papaya* and *Cycas circinalis* [11]. A method to determine the gender 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.

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 protocol [12]. Quantification and purity of isolated DNA was checked through uv-spectrophotometer. Purified genomic DNA was subjected to PCR amplification for RAPD analysis, using twenty decamer random primers. Each 20µl of the PCR reaction mixture consisted of 50ng genomic DNA, 1.5mM $MgCl_2$, 200µM each dNTP, 15pM primer (Operon Tech., Alameda, USA) and 0.5 units of *Taq* DNA polymerase (Banglore Genei, Bangalore, India). Amplifications were carried out in a Gradient Palm

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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, 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.

Among the 20 random primers used in the present investigation, OPA-15 yielded a unique amplicon of 1625 bp only in male lines in all 20 populations studied (results of only four populations shown in Fig. 1. A RAPD marker OPA-15_{1625} band consistently appeared exclusively in male genotypes, suggesting thereby male associated nature of this DNA marker in *M. dioica*. The OPA-15_{1625} apparently constitutes a marker closely linked to a male determining chromosome segment of *M. dioica.* As a consequence, this marker can be fruitfully and adequately used for determination of sex in *M. dioica*, well before the plant reaches reproductive maturity. OPA-15_{1625} 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 markers from sequenced RAPD markers. A SCAR can indeed further simplify the massive screening of dioecious *M. dioica* cultivars and breeding lines.

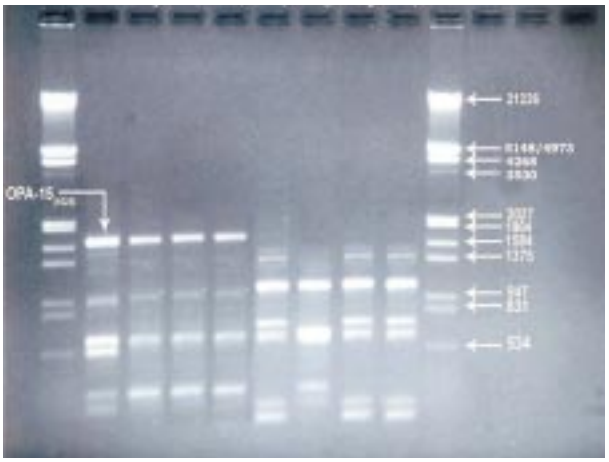

Fig. 1. Fractionation of RAPD products developed by OPA-15 primer in male and female *M. dioica* on 1.2% agarose gel. Lane 1 and 10-molecular weight marker: Lane 2 through 5-RAPD products from male plants; Lane 6 through 9 RAPD products from female plants. Arrow head indicates male-related RAPD band OPA-15_{1625}.

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