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Short communication

Molecular marker to identify gynoecious lines in bitter gourd

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ABSTRACT

We report on the identification of an inter-simple sequence repeat marker (ISSR) associated with the gynoecious trait in bitter gourd. Twenty-four gynoecious plants belonging to the F_5 generation derived from a previously identified gynoecious line DBGy-201were screened using 200 RAPD and 28 ISSR markers. A 1000 base pair fragment specific to the gynoecious plants was amplified by the primer $(AC)_8T$. Since bitter gourd flowers 35-40 days after sowing, identification of this marker associated with gynoecy is of great significance to ascertain purity of gynoecious lines at an early stage of development for a cost effective hybrid seed production.

Key words: Bitter gourd, gynoecious-specific, ISSR marker.

Bitter gourd or bitter melon (*Momordica charantia* L.) is a monoecious crop with small flowers and a high male to female ratio that varies from 9:1 to 48:1. Hand pollination for hybrid seed production is thus a labour intensive process. Experienced hands can pollinate 11 to 12 flowers per hour to each produce 15 seeds per fruit in an average commercial operation (Devdas and Ramadas, 3). Although, in cucumber F_1 hybrids are commonly produced by open pollination of inbred gynoecious plants, limited availability of gynoecious lines in bitter gourd hamper the utilization of such a strategy. Zhou *et al*. (7) have reported gynoecious plants from among the Chinese germplasm and Ram *et al.* (6), and Behera *et al.* (1) have identified gynoecious plants from the Indian germplasm. Behera *et al*. (1) developed gynoecious bitter gourd lines derived from two plants DBGy-201 and DBGy-202. These lines have been characterized for inheritance of gynoecy and development of hybrids (Behera *et al*., 2). They hold immense potential in future breeding programmes for improvement of yield and earliness in bitter gourd. The present investigation has been carried out with the purpose of identifying molecular markers associated with the gynoecious trait in these lines to enable their identification at an early stage of development for cost effective hybrid seed production.

The plant material used in this study comprised of 24 plants belonging to the lines G1, G2 and G3 that are the F_5 families of DBGy-201. These lines were developed from segregating population (F_4) of gynoecious line (DBGy-201) derived through sib-mating followed by selfing after modifying into hermaphrodite sex form. Twenty six genotypes analyzed in this study include nine plants each from the gynoecious lines G1 and G2 and six from the line G3 along with the varieties Pusa Do Mausami and Pusa Vishesh that represent the monoecious genotype. DNA was extracted from young leaves using DNeasy plant mini kit (Qiagen, Germany) as per the manufacturer's protocol. For primer screening, DNA of plants from each of the gynoecious line was pooled separately and analysed against the profile of Pusa Do Mausami and Pusa Vishesh to screen for amplicon size polymorphism. Genomic DNA (20 ng) was amplified in a reaction mixture containing 2.0 mM $MgCl_2$, 1X reaction buffer, 0.2 mM dNTPs, 1.0 µM primer and 1 unit *Taq* DNA polymerase (MBI Fermentas, Germany) in a thermal cycler (Biometra, Germany) programmed at 94°C for 4 min; 35 cycles of 94°C for 1 min, 35°C for 1 min. and 72°C for 2 min. and a final extension for 10 min. at 72°C. For ISSRs the annealing temperature of each primer was optimized based on its melting temperature. The amplified products were electrophoresed through 1.4% agarose gel in TBE buffer at 1 V/cm^2 and photographed on the gel documentation system.

A total of 200 RAPD and 28 ISSR primers were employed for screening bulked samples from each of the three gynoecious lines and the two monoecious varieties Pusa Do Mausami and Pusa Vishesh. Based on the screening, 17 RAPD primers and 5 ISSR primers were short listed for generating molecular profiles of all the 26 samples, *i.e.* 24 gynoecious plants and 2 monoecious varieties. The results showed that none of the 17 RAPD primers differentiated the gynoecious lines from the monoecious types. One primer OPD03, however differentiated the individuals of the line G3 from those of G1 and G2 (Fig.1). Out of the five ISSR primers tested on all the 26 samples, four yielded monomorphic bands. One primer containing

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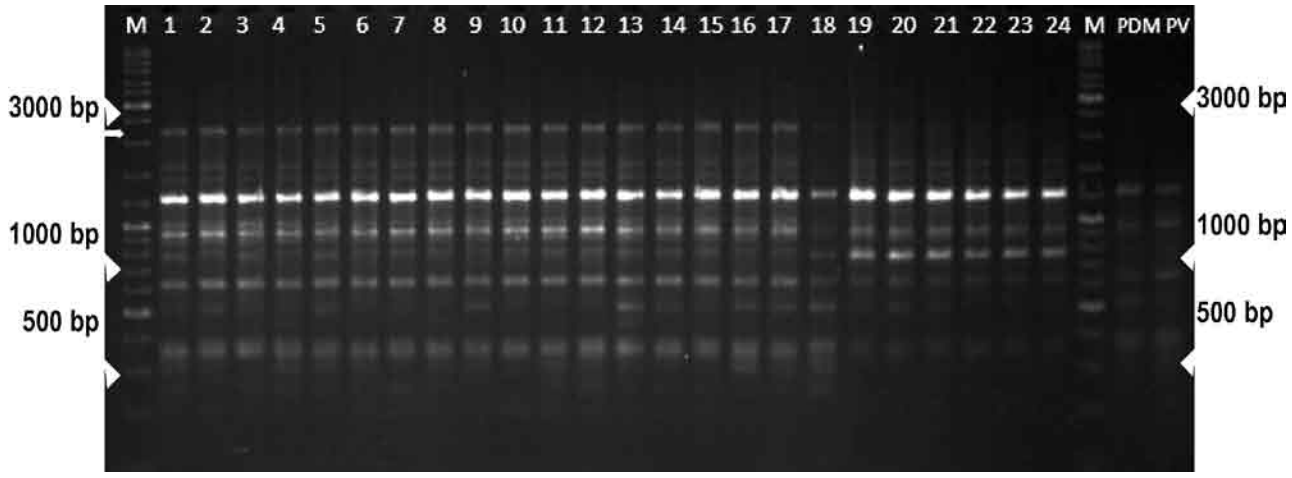

Fig. 1. Amplification profile of the gynoecious lines G1 (lanes 1 to 9), G2 (lanes 10 to 18), G3 (lanes 19 to 24), Pusa Do Mausmi (PDM) and Pusa Vishesh (PV) with the RAPD primer OPD03. M is the DNA ladder mix. Arrow on the left below 3 kb marker denotes the 2300 bp band.

$(AC)_8T$ repeat however amplified an approximately 1 kb fragment in all the gynoecious plants. This fragment was conspicuously absent in the monoecious Pusa Do Mausami and Pusa Vishesh (Fig. 2).The results obtained with the two discriminating primers are summarized in Table 1.

Since reproducibility of the amplification profile is one of the constraints while using ISSR markers (Fang and Roose, 4), the robustness and repeatability of this 1 kb amplicon was confirmed in order to identify it as a marker associated with the gynoecious trait. For this amplifications were carried out in two different thermocylclers (Biometra and Perkin Elmer) and by using different *Taq* DNA polymerase brands (New England Biolabs,UK and MBI Fermentas, Germany). The 1 Kb fragment was amplified in the gynoecious lines in all the combinations (Thermocyler and *Taq* DNA polymerase) with minor modifications in the reaction conditions, thus confirming the repeatability of the assay.

Bitter gourd, a member of the cucurbitaceous family, is typically a monoecious plant. Amongst the monoecious plant systems, cucumber, another member of the same family is a model system for floral biology and sex determination studies (Malepszy and Niemirowicz-Szczytt, 5). Reports on molecular mechanisms underlying sex-related differences indicate that the mechanism of sex determination is not the same in all species.The presence of naturally occurring gynoecious sex forms in bitter gourd

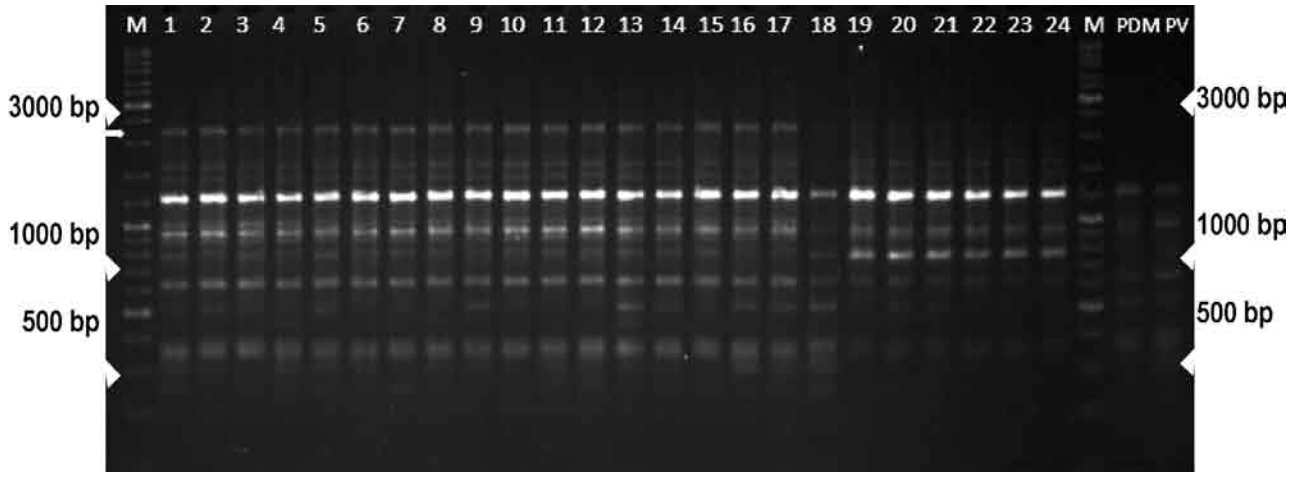

Fig. 2. Amplification profile of the gynoecious lines G1 (lanes 1 to 9), G2 (lanes 10 to 18), G3 (lanes 19 to 24), Pusa Do Mausmi (PDM) and Pusa Vishesh (PV) with the ISSR primer $(AC)_8T$. M is the DNA ladder mix. Arrow on the left denotes the 1000 bp band.

Table 1. Molecular markers differentiating the genotypes under study (-) indicates absence and (+) indicates presence of the band.

Marker	Primer sequence	Size (bp)	Gynoecious genotype			Monoecious genotype	
			G1	G2	G3	Pusa Do Mausami	Pusa Vishesh
OPD03	GTCGCCGTCA	2300	-	-	+	-	-
$(AC)_8T$	ACACACACACACACACT	1000	+	+	+	-	-

provides a tool for utilization not only in hybrid seed production but also for studying the molecular basis of sex expression in this crop. Given that the 1 kb band is present in only the gynoecious lines, it has high potential as a diagnostic marker for gynoecy. This is expected to assist the breeding programme of bitter gourd significantly. Further analysis of this 1 kb fragment is underway to explore its conversion to a SCAR (Sequence Characterized Amplified Region) marker and to evaluate its role in the gynoecious sex expression.

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