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Short communication

DETERMINATION OF F_1 HYBRID PURITY OF *CAPSICUM ANNUM* L. USING RAPD MARKERS

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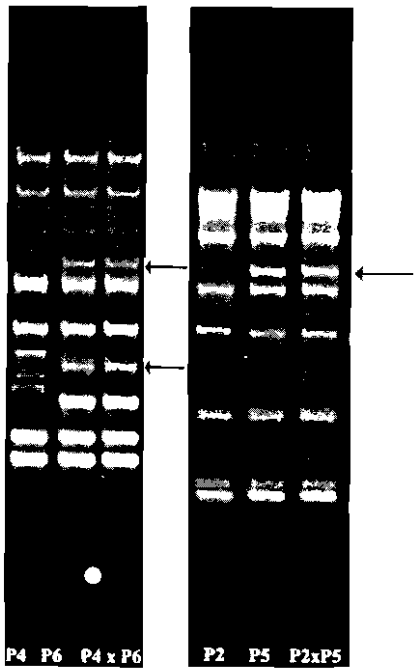
Determination of F_1 hybrid purity is a vital component of hybrid seed production for both commercial and breeding purposes. For years, the method used to check hybrid seed purity has been the grow-out test. This consists of growing a representative sample of the F_1 seed and later classifying it using morphological descriptors of differences as true hybrid or off types. This method is time consuming, space demanding and certain extent limited to varietal identification. Fast determination of true hybrid is also essential in breeding so that advanced generations can be produced without delay. The advent of Polymerase Chain Reaction (PCR) had a direct impact on the development of new markers more suited to plant breeding related practices. PCR-based on RAPD analysis has been shown to be effective in testing hybridity of Capsicum (Ballester and de Vicente, 1998; Ilbi, 2003; Mongkolporrn *et al.*, 2004).

The present work reports the results of RAPD analysis to assess the genetic purity of two chilli hybrids. Two F_1 hybrids, P2 x P6 and P4 x P5, and their correspondent parents (P2, P6, P4 and P5) were analyzed in this study during 2005. Total DNA of the hybrids and parents was extracted from young leaves using modified CTAB. The quantity of DNA present in each sample was determined by recording the absorbance at 260 nm in fluorometer (Model DNA Quant 200, Hoefer, California, USA). To access the quality, all the genomic DNA samples were run on 0.8% agarose gel. Thirty decameter oligonucleotide primers were screened by PCR. The PCR reactions were performed using a 20il mixture containing 0.20 il $MgCl_2$, 0.25 il dNTP mix, 1.00 il random decamer, 0.20 il *Taq* polymerase and 2.00 il genomic DNA for DNA amplification. The amplification program was the following, initial denaturation at 94°C for 1 minute followed by 35 cycles of incubation at 94°C of 1 minute, 36°C for 1 minute and 72°C for 3 minute. The final extension was done at 72°C for 10 minutes. Along with the PCR amplified products, Lambda DNA (*EcoR*I and *Hind*III double digest) as standard marker were subjected to electrophoresis in 1.5% agarose gel in 1XTBE buffer at 8V/centimeter for 4-5 hours. The electronic image of the ethidium bromide stained gel was visualized and documented in gel documentation system (Model Alpha imager 1200, Alpha Innotech Corp, USA).

Out of the 30 primers tested, 20 produced clear amplification products and the remaining primers either did not amplify or showed in consistent amplification. On an average, each primer amplified three scorable bands. To test the hybrid purity, it should be able to distinguish cross from a self pollination of a female parent. Further screening among 20 clear primers, 12 (60%) showed a polymorphism between the inbred lines. Data on those 12 primers were used to select the presence of bands specific to the male parent that might reveal a proper pattern of a true hybrid. Finally, three primers were found useful in determining the hybridity of two hybrids. The efficiency to find a RAPD marker useful for purity determination was 10%. This value is similar to that found for pepper hybrids, in which 8 primers showed

Table 1. List of RAPD markers useful for checking hybrid purity for the two hybrids

Hybrid	Primer	Marker size (bp)	Female	Male
P2 x P5	OPC 5	840	-	+
	OPF 7	950		
	OPF 2	1150		
P4 x P6	OPC 5	840		
	OPF 7	950		
	OPF 7	1700		
	OPF 2	1850		



Arrow indicates male parent specific markers

Fig. 1. Male specific markers amplified by OPF 7 and OPC 5 in hybrids (P4 X P6 and P2 X P5)

good polymorphism between parents out of 100 primers tested (Ballester and de Vincente, 1998). In our study, these primers (3) generated 4 RAPD markers. Two primers (OPF 2 and OPC 5) gave one marker and the primer OPF 7 presented two useful bands. On the other hand, all the three primers were good to solve the purity test of two hybrids. In total, the number of RAPD markers useful for checking hybridity of the two hybrids was 3 (P2 x P5) and 4 (P4 x P6). Our results demonstrate that RAPD markers amplify reproducible polymorphic bands that can be used for hybrid purity determination.

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