Random Amplified Polymorphic DNA Markers Are Useful for Purity Determination of Tomato Hybrids

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Identifying breeding lines and determining hybrid purity are major quality requirements in plant breeding and seed production. Cultivar contaminations may affect yield, uniformity, and quality of the product. In addition, identifying cultivars is essential for proprietary reasons.

Until recently, morphological markers have been used to identify cultivars and to determine hybrid purity. However, such tests are time consuming and expensive, and often insufficient characteristic morphological traits exist to allow for cultivar discrimination. Molecular markers such as isozymes and restriction fragment length polymorphisms (RFLP) also have been used for cultivar identification (Bunce et al., 1986; Tanksley and Jones, 1981). More recently, random amplified polymorphic DNA (RAPD) markers have been used for fingerprinting plant species (Hu and Quiros, 1991). Using RAPDs has gained popularity because 1) a small amount of DNA is required per reaction, 2) numerous oligonucleotide primers allow for polymorphism screening, 3) each primer detects multiple bands, and 4) the technique is fast and simple, requiring no sophisticated equipment and no radioactive isotopes.

In tomato (*Lycopersicon esculentum* L.), several molecular marker techniques have been used for cultivar identification. Alcohol dehydrogenase allozymes were used for testing hybrid purity by Tanksley and Jones (1981). Highly polymorphic telomeric sequences allowed the tomato cultivar distinction using pulsed field gel electrophoresis (Broun et al., 1992). An oligonucleotide repeat of GATA

was used in RFLP analysis to distinguish 15 cultivars (Vosman et al., 1992). In this report, we demonstrate that RAPD markers can distinguish inbred lines used as parents for hybrids and, therefore, are suitable for testing the purity of these hybrids.

Pioneer Vegetable Genetics (Kfar Ruth, Israel) provided four tomato hybrids with their male and female parents. Hybrids PH236 and PH254 have a determinate plant habit; hybrids PH198 and PH176 are indeterminate types. DNA was extracted from 10-plant bulks per line as described by Saghai-Marouf et al. (1984). Primers that amplified polymorphic bands between the parents were repeated four times using DNA that was extracted twice from each line. Conditions for polymerase chain reaction amplification and agarose gel electrophoresis were as described by Paran and Michelmore (1993). We purchased RAPD primers from Operon Technologies, Alameda, Calif. Amplification was performed in a thermal cycler (MJ Research, Watertown, Mass).

A total of 160 arbitrary 10-mer oligonucleotide primers were used to screen for polymorphisms between the four pairs of parents. On average, each primer amplified eight bands. Thirteen primers amplified polymorphic bands between the pairs of parents, of which three (OPH13, OPI09, and OPL11) detected polymorphisms for more than one hybrid (Table 1). Only the primers that detected bands specific to the male parent were useful for distinguishing a true hybrid from a self-pollination of the female parent. The male-specific polymorphic bands segregated as expected (3:1) in a sample of 20 selfed progeny of each hybrid (data not shown). The four hybrids could be distinguished from each other using the three RAPDs (G02₄₂₀, F08₈₄₀, and K12₆₅₀).

We have demonstrated that RAPD markers can be used for purity determination and identification of tomato hybrids that originated from diverse pedigrees. Purity testing of these hybrids previously has been accomplished using a morphological marker (jointless, hybrid PH176) or by isozymes (hybrids PH236 and PH254, ADH and GOT,

respectively). For hybrid PH198, no characteristic morphological or molecular markers were available for purity tests. Therefore, the RAPD identification for determining the purity of hybrid PH198 is particularly important.

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Table 1. Random amplified polymorphic DNA primers used for distinguishing the parents of four tomato hybrids.

Hybrid	Primer ^a	Sequence	M ^y	F ^x
PH236	E03 ₉₆₀	CCAGATGCAC	+	–
	E03 ₉₀₀	CCAGATGCAC	–	+
	F10 ₁₆₀₀	GGAAGCTTGG	+	–
	F10 ₇₂₀	GGAAGCTTGG	+	–
	F10 ₆₆₀	GGAAGCTTGG	–	+
	F08 ₈₄₀	GGGATATCGG	+	–
	G16 ₆₅₀	AGCGTCCTCC	–	+
	H09 ₁₁₈₀	TGTAGCTGGG	–	+
	I02 ₁₄₀₀	GGAGGAGAGG	+	–
	I09 ₅₀₀	TGGAGAGCAG	+	–
	I09 ₄₇₀	TGGAGAGCAG	–	+
	J17 ₁₀₀₀	ACGCCAGTTC	–	+
	L11 ₆₀₀	ACGATGAGCC	–	+
PH254	G02 ₄₂₀	GGCACTGAGG	+	–
	H13 ₁₀₀₀	GACGCCACAC	+	–
PH198	I09 ₅₀₀	TGGAGAGCAG	+	–
	I09 ₄₇₀	TGGAGAGCAG	–	+
PH176	H13 ₁₀₀₀	GACGCCACAC	+	–
	K12 ₆₅₀	TGGCCCTCAC	+	–
	L11 ₇₀₀	ACGATGAGCC	+	–

^aPrimer number refers to Operon Technologies kits. The number in subscript refers to the band size in base pairs.

^yM = male.

^xF = female.

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