Testing for Genetic Purity in Petunia and Cyclamen Seed Using Random Amplified Polymorphic DNA Markers

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Abstract. This study examined the use of random amplified polymorphic DNA (RAPD) markers as a means to identify cultivars of petunia (*Petunia hybrida* Vilm) seedlings and cyclamen (*Cyclamen persicum* Mill.) seeds and to determine the genetic purity within cyclamen seeds. Bulk samples of six petunia and five cyclamen hybrid cultivars, respectively, produced consistent RAPD marker profiles. Evaluation of individual seeds from a single cyclamen hybrid produced polymorphic banding patterns that were attributed to genetic variability present in the female and male inbred parents. These results show that RAPD markers can be used to quickly assess the genetic purity of selected cultivars of these two flower seed crops.

Seed purity depends on the proportion of pure seed, other crop seed, weed seed, and inert matter in a seed lot. Of these four components, the pure seed portion is critical because it represents the fraction of the seed lot purported to be the cultivar on the seed label. Genetic purity testing has two objectives: 1) confirmation that the stated cultivar is present in the seed lot and 2) determination of the percentage of seeds in the seed lot that is the cultivar. In self-pollinated crops, where pure line cultivars are marketed, the first objective is the most important because a high proportion of seed is expected to be identical. In cross-pollinated crops for the production of hybrid or synthetic cultivars, the second objective is of equal or greater interest, as it reflects the level of outcrossing vs. selfing and the expected degree of uniformity for a particular seed lot. Most genetic purity tests have traditionally used morphological features for discrimination. The increasing number of new cultivars developed by breeders challenges this approach, and makes the need for new genetic purity tests more important (McDonald, 1995).

Polymerase chain reaction protocols are rapidly becoming established as useful molecular biology techniques in taxonomic stud-

ies. Among the most powerful of these is random amplified polymorphic DNA (RAPD), which is a more sensitive measure of genetic purity than morphological traits. For RAPD technology to be used routinely in genetic purity programs, the procedure must be standardized. Staub et al. (1996) concluded that RAPD assays in cucumber (*Cucumis sativus* L.) were reliable and reproducible providing that efforts were made to minimize error including such factors as the use of high-quality DNA, consistent and optimized PCR conditions, and conservative band scoring. Similarly, Shatters et al. (1995) showed that reproducible results were obtained from multiple soybean [*Glycine max* (L.) Merr.] DNA isolations using three separate isolation procedures involving either multiple seed or a single seed as the template source. Jianhua et al. (1996) reported that, in most cases, seed deterioration, pathogen infestation, and production environment were not factors that influenced RAPD marker results for soybean. Collectively, these findings indicate that RAPD markers can be helpful in routinely discriminating the heritage of cultivars of numerous crops.

Floriculture is a rapidly expanding industry with increasing focus on superior flower traits such as size, color, and shape. This focus has led to a greater emphasis on cultivar development, most of which is hybrid production. For hybrids to perform optimally and with the highest vigor, each parental inbred line should be homozygous and homogenous. However, due to inbreeding depression, a high degree of homozygosity is not always achievable. In some floriculture crops, the degree of homogeneity in parental lines and resulting hybrids is determined primarily on the basis of plant morphological features. These can be greatly compromised by environmental interactions during plant development; thus, interpretation depends on the expertise of the evaluator (McDonald, 1995). In view of the value of RAPD markers in genetic purity determina-

tions for other crops, the objectives of this study were to determine 1) whether RAPD markers could be of value in genetic purity evaluations of two important floral crops—petunia and cyclamen—and 2) the level of genetic purity found in commercial inbred and hybrid lines of cyclamen based on RAPD markers.

Materials and Methods

Seeds. Six petunia hybrid cultivars were obtained from Goldsmith Seeds, Gilroy, Calif. Ball Seed Co., West Chicago, Ill., provided five cyclamen hybrids and their respective inbred parents.

RAPD analyses. Petunia seeds were germinated at 20 °C for 7 days in 9-cm petri plates containing one blue blotter filter paper disk (Anchor Paper Co., St. Paul, Minn.) moistened with distilled water. Twenty germinated petunia seedlings and 10 cyclamen seeds were bulked for each cultivar and used for the cultivar identification. The DNA extraction, amplification, and electrophoresis procedures were the same as described by McDonald et al. (1994) except that 10 µL of proteinase K (Life Technologies, Gaithersburg, Md.) at 10 mg·L⁻¹ and 10 µL of 1.0 mM CaCl₂ were added following DNA extraction and incubated for 1.5 h at 37 °C to digest any remaining protein from the DNA. The 10-mer oligonucleotide primer 262 (Ransom Hill Bioscience, Ramona, Calif.) with a base sequence of CGGCCACTGT was used in all studies. Genetic purity studies were conducted on 10 seeds each of the hybrid progeny and 10 seeds each of the inbred parents of the F₁ 97222 cyclamen cultivar. Each lane represented a RAPD marker profile for an individual seed. All studies were repeated three times.

Results and Discussion

Preliminary studies showed RAPD markers obtained from petunia seeds were not reproducible. This difficulty was attributed to the small seed size and the difficulty in extracting DNA for RAPD analyses. As a result, reproducible RAPD marker profiles were obtained when DNA was extracted from bulked samples of either 20 seedlings of six petunia cultivars or 10 seeds of five cyclamen cultivars (Fig. 1). Bulking leaf tissue from several individuals has been reported as necessary in achieving reproducible DNA amplification fingerprinting results for petunia cultivars and species (Starman, 1995). However, seeds are the preferred DNA template in seed testing laboratories since results are more quickly obtained, thereby increasing efficiency. McDonald et al. (1994) reported a DNA extraction protocol for agronomic seeds that was applied to flower seeds in this study. This approach resulted in each cultivar of both floral crops displaying a unique polymorphic pattern when bulked seed/seedling samples were assayed.

Heretofore, the only traits used to discriminate cultivars for floriculture crops have been primarily morphological features such as

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flower color, size, and shape. These often require long periods of vegetative growth before such reproductive features are expressed. For example, genetic purity determinations of cyclamen are not possible until 8 months after planting (Widmer and Lyons, 1985). The RAPD technique described here affords the seed industry a useful genetic purity analysis test that is both more sensitive and faster (completed within 24 h) than current methods.

Since this technique provided reproducible results for the bulked samples, the genetic identity of individual seeds in the cyclamen hybrid seed lot was determined to confirm hybridization success. The incidence of heterogeneity in the seed lot was high (Fig. 2). While several factors may be responsible for this observation, the most likely is that the inbred parental lines were not genetically pure (e.g., inbreds had not reached homozygosity and/or lots were not homogenous). To test this, RAPD analyses of individual seeds from the female and male parents of the hybrid seed lot were conducted. The results confirmed that both inbred parental lines used in making the hybrid cross were not homogenous (Fig. 3).

The determination of genetic purity for hybrid crops is essential to verify that hybridization of agronomic crops such as maize (*Zea mays* L.) has occurred so that increased grain yields through heterosis results. To assure this success, the maize seed industry routinely screens inbred and hybrid lines for genetic purity using starch gel electrophoresis (McDonald, 1990). Floriculture breeders, in contrast, emphasize morphological traits such as flower color intensity, plant habit, size and shape of flowers and leaves, and earliness of the crop. While heterosis would be beneficial in many floral crops, it is often difficult to achieve for others. For example, in cyclamen, inbreeding depression is so severe that the inbred is unable to produce seed after only a few selfing cycles (Wellensiek, 1961). This depression culminates in inbred parents being seldom genetically pure, a conclusion confirmed by our findings (Fig. 3), which may explain the observation that batches of seedlings from individual parents often vary widely in appearance (Grey-Wilson, 1988). Consequently, selection of F_1 hybrids based solely on morphological traits produces plants that vary in physical and developmental attributes: characteristics not desired by the producer or consumer. The use of RAPD markers in floriculture breeding may facilitate greater genetic purity in the parents and progeny producing superior floral crops.

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Fig. 1. RAPD markers for 20 bulked seedling and 10 seed samples for petunia and cyclamen cultivars, respectively. Lanes: M = molecular weight DNA marker, lanes 1-6 petunia cultivars (Fantasy pink morn, Primetime blue, Ultra crimson star, Ultra blue, Primetime red vein, Fantasy salmon) and lanes 7-11 cyclamen cultivars (F_1 80360, F_1 97222, F_1 97294, F_1 97358, 72229721) and C = control lane.

Fig. 2. RAPD markers for individual cyclamen seeds of hybrid F_1 97222 cultivar. M = molecular weight DNA marker, lanes 1-10 = individual hybrid seeds, C = control lane.

Fig. 3. RAPD markers for individual cyclamen seeds of the female and male inbred parents of F_1 97222. M = molecular weight DNA marker, female and male lanes 1-10 = individual inbred seeds, C = control lane.