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Abstract. **Randomly amplified polymorphic DNA (RAPD) techniques were used to compare the DNA from leaf tissues of nine commercial poinsettia (*Euphorbia pulcherrima* Wild ex Klotzsch) cultivars. Amplification occurred in 57 out of 60 (95%) tested primers. Nine primers that revealed polymorphisms among cultivars were selected for further evaluation. Forty-eight RAPD bands were scored from these primers, and 33 (69%) were polymorphic. All tested cultivars could be discriminated with seven bands generated from primers OPB7 and OPC13. Results of a UPGMA cluster analysis and principal components analysis placed the nine cultivars into two groups: one group consisted of 'Jingle Bells', 'Supjibi', and 'V-17 Angelika', the other of 'V-14 Glory', 'Red Sails', 'Jolly Red', and 'Freedom'. 'Lilo Red' and 'Pink Peppermint' belonged to the latter group, but were relatively distant from other cultivars in that group. These results indicate that RAPDs are efficient for identification of poinsettia cultivars and for determination of the genetic relationships among cultivars.**

Poinsettia is the most important ornamental plant grown for the Christmas season in the United States. Because it is a major contributor to the ornamental plant industry, poinsettia has become an important target for breeding new varieties with novel or improved characteristics. Thus, new cultivars are continuously being released (Ecke, 1990a, 1990b). Traditional cultivar identification in poinsettia is based on an evaluation of morphological characteristics that requires a lengthy survey of plant growth. Morphology-based identification is difficult because morphological characteristics can vary considerably with environmental conditions. Some cultivars are morphologically similar, making their identification more difficult. Furthermore, the same poinsettia cultivars may be registered in different countries under differing names (Ecke, 1990b). This cultivar registration system, coupled with the close morphological similarity among some cultivars, and the environmental influences on morphological characteristics, can cause confusion to growers, breeders, and retailers. As an alternative, biochemical markers, such as flavonoids (Asen, 1979; Stewart et al., 1979b) and anthocyanins (Stewart et al., 1979a), have been used for cultivar identification and genetic analysis of sports. The main drawback of such biochemical markers is that their expression is influenced by the developmental stage of the plant and may also be influenced by environmental conditions. In recent years, randomly amplified polymorphic DNAs (RAPDs) (Welsh and McClelland, 1990; Williams et al., 1990) have been used for cultivar identification in many plants (Newbury and Ford-Lloyd, 1993). In contrast to biochemical markers, RAPD markers are simple, consistent, and can be analyzed at any stage of plant growth. The use of RAPD markers to differentiate poinsettia cultivars would provide for a rapid and accurate technique for cultivar identification.

The cultivated poinsettia has been domesticated from a single species of *Euphorbia.* Poinsettia are generally considered to have a narrow range of genetic variation (Stewart et al., 1979a). Additionally, current commercial poinsettia cultivars are hybrids or sports of hybrids (Ecke, 1990b) and their genetic relationships are not well known. This study was conducted to investigate the feasibility of using RAPD markers for the identification of poinsettia cultivars and to estimate genetic relationships among these cultivars.

Materials and Methods

Nine commercial poinsettia cultivars ('Jingle Bells', 'Supjibi Red', 'V-17 Angelika', 'V-14 Glory', 'Pink Peppermint', 'Red Sails', 'Lilo Red', 'Freedom Red', and 'Jolly Red') were tested. Fully expanded leaves were collected from greenhouse-grown plants. DNA was extracted from ≈1 g of fresh or frozen (–80 °C) leaves. DNA extraction was performed following the CTAB procedure (Saghai-Maroof et al., 1984) with modifications of Gawel and Jarret (1991). Precipitated DNA was hooked out using a bent glass rod, rinsed several times in ethanol (70%) and air-dried. The dried DNA was then dissolved in 500 µL TE (10 mM Tris·Cl, 1 mM EDTA, pH 8.0) and extracted once with an equal volume of 1 phenol : 1 chloroform (v/v). Finally, the DNA was ethanol precipitated and redissolved in 300 µL TE. DNA concentrations were determined with a DNA minifluorometer (TKO 100; Hoeffer Scientific, San Francisco). To confirm concentrations and to check for fragmentation of the DNA, the samples were electrophoresed in a 1.4% agarose gel in 1 × TAE (40 mM Tris·acetate, 1 mM EDTA, pH 8.0) and compared with a known concentration of λ DNA.

Genomic DNA (20 ng) was mixed in 50 µL of a reaction mixture that contained 1X PCR buffer (10 mM KCl, 100 mM Tri-HCl, pH 8.3), 1.5 mM $MgCl_2$, 200 mM dNTPs (Promega Biotech, Madison, Wis.), 0.2 µM primer, and 1.5 unit *Taq* polymerase (Promega Biotech). Concentrations of genomic DNA, $MgCl_2$, and *Taq* polymerase were based on the optimization of RAPD-PCR using genomic DNA (5, 10, 15, 20, 25, 30 ng), $MgCl_2$ (0.75, 1.0, 1.25, 1.5, 2.0, 2.5, 3.0 mM), and *Taq* polymerase (0.75, 1.0, 1.2, 1.5, 1.75, 2.0 units/reaction). The primers used were 10-bp RAPD primers from Random Primer Kit A, B, and C (Operon Technologies, Alameda, Calif.). Only one primer was used in each reaction. Amplification was carried out with a PTC-100 Programmable Thermal Controller (MJ Research, Watertown, Mass.) using the following temperature profile: 1) 80 °C, 15 min; 2) 94 °C, 2 min; 3) 92 °C, 1 min; 4) 35 °C, 1 min; 5) slope 1 °C every 8 s to 72 °C; 6) 72 °C, 2 min; 7) cycle to step 3, 45 times; 8) 72 °C, 10 min; 9) 4 °C for storage.

Following amplification, the RAPD products (20 µL) were loaded in 2.0% agarose gels in TAE buffer and separated by electrophoresis. A pGEM marker DNA (Promega Biotech) was included as a size marker. Gels were stained with ethidium bromide and photographed on a UV transilluminator.

Bands were scored as present (1) or absent (0) using RFLPScan (CSPI, Bilerica, Mass.). Only bands that were between 250 and 1700 bp and in the upper 65 percentile of brightness were included in the analysis. Bands that differed in size by more than 1.2% were considered different. Band-sharing analysis was conducted using NTSYS-pc (Rohlf, 1992).

Results and Discussion

The typical yields of DNA were 200 to 300 $\mu g \cdot g^{-1}$ of leaf tissue. All extracted DNA was of high molecular weight with very little fragmentation, as indicated by gel electrophoresis. In preliminary RAPD-PCR experiments, opti-

Table 1. RAPD markers produced by nine selected primers among poinsettia cultivars. Percent polymorphism reflects the number of total bands from each primer that distinguish at least one cultivar.

	Bands		
Primer	No. obtained	No. polymorphic	Percent polymorphic
OPA 2	6	2	33
OPA 4	5	3	60
OPA11	4	3	75
OPA12	9	3	33
OPB 6	3	1	33
OPB 7	5	2	40
OPB11	4	3	75
OPC 6	4	1	25
OPC13	8	5	63
Total	48	33	69

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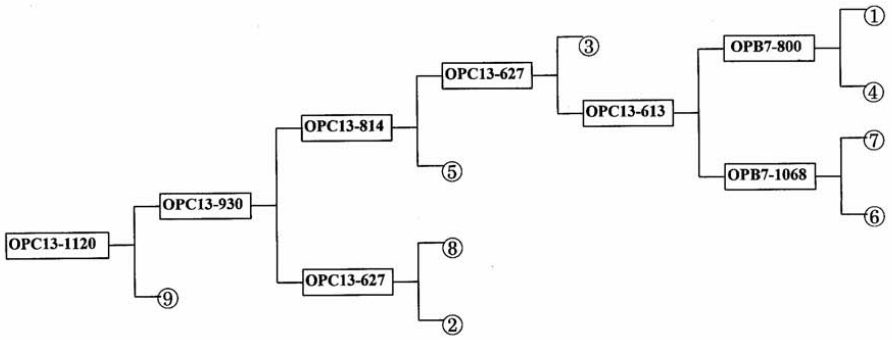


Fig. 1. Identification of poinsettia cultivars using RAPD markers. (**1**) 'Jingle Bells', (**2**) 'Supjibi Red', (**3**) 'V-17 Angelika', (**4**) 'V-14 Glory', (**5**) 'Pink Peppermint', (**6**) 'Red Sails', (**7**) 'Lilo Red', (**8**) 'Freedom Red', (**9**) 'Jolly Red'.

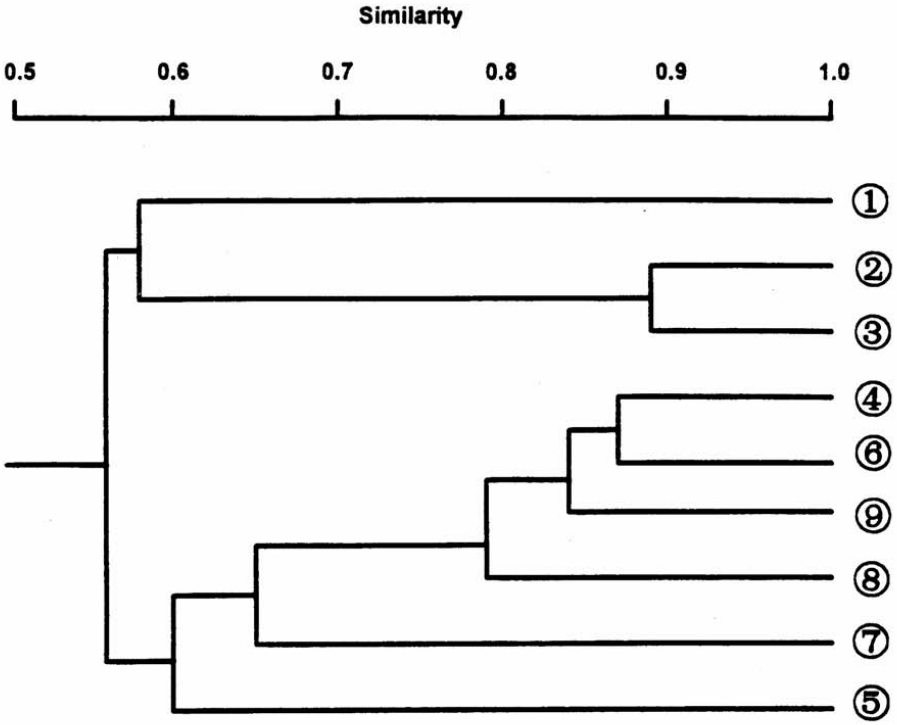
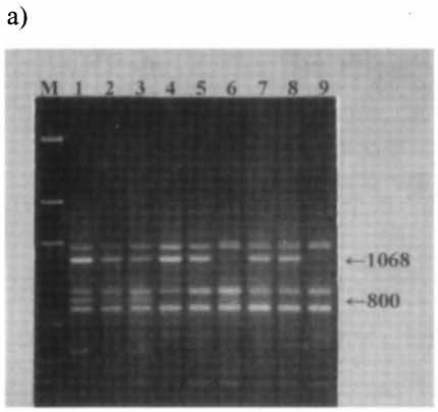


Fig. 2. Relationship of poinsettia cultivars as determined by UPGMA using simple matching coefficient computed from RAPD data. (**1**) 'Jingle Bells', (**2**) 'Supjibi Red', (**3**) 'V-17 Angelika', (**4**) 'V-14 Glory', (**5**) 'Pink Peppermint', (**6**) 'Red Sails', (**7**) 'Lilo Red', (**8**) 'Freedom Red', (**9**) 'Jolly Red'.



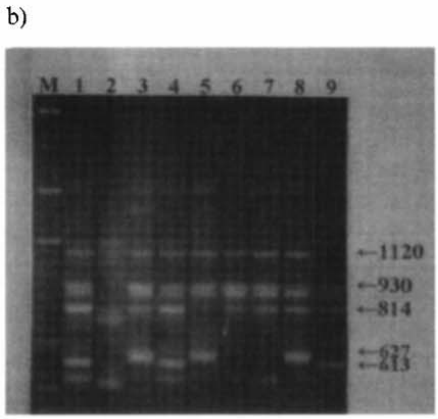


Fig. 3. RAPD analysis of poinsettia cultivars. (**a**) Primer OPB07, (**b**) Primer OPC13. (**1**) 'Jingle Bells', (**2**) 'Supjibi Red', (**3**) 'V-17 Angelika', (**4**) 'V-14 Glory', (**5**) 'Pink Peppermint', (**6**) 'Red Sails', (**7**) 'Lilo Red', (**8**) 'Freedom Red', (**9**) 'Jolly Red'. M = pGEM markers. Fragments used in the key are marked by arrows.

mization was carried out to determine the optimal concentrations of template DNA, $MgCl_2$, and *Taq* polymerase. In general, either no product or products with inconsistent bands were obtained when the concentration of template DNA was <10 ng or $MgCl_2$ was <1.0 mM. If >25 ng of template DNA or >2.5 mM $MgCl_2$ were used, smear DNA banding patterns were produced. The optimal *Taq* polymerase concentrations were with 1.2 and 1.5 units/reaction. There was no significant difference in the concentrations of *Taq* polymerase between 1.2 and 1.5 units/reaction. The optimal RAPD-PCR conditions were 20 ng template DNA, 1.5 mM $MgCl_2$ and 1.5 units of polymerase. Data presented here were produced using these RAPD-PCR conditions.

The quality of DNA amplification and resolution of DNA bands in agarose gels varied considerably with primers. Of the 60 oligonucleotides primers tested, three primers (OPA5, OPA6, and OPB16) produced either no amplification or unreadable amplification patterns. The remaining 57 primers (95%) produced 1 to 13 bands with an average of 5.4 per primer. Fifteen primers revealed polymorphisms among the nine poinsettia cultivars; the other 42 were monomorphic. Based on band resolution, nine primers were selected for further evaluation and repeated in at least two replicate RAPD reactions. Only bands that consistently amplified in each replication were scored. A total of 48 bands were scored from these primers and 33 of them were polymorphic (Table 1). Primers OPA11 and OPB11 produced the highest level of polymorphism.

No single primer produced polymorphic bands in all cultivars. However, all cultivars were distinguishable with the combinations of polymorphic bands generated by various primers. We developed a key for the cultivars using seven polymorphic bands (Fig. 1). These polymorphic bands included OPC13-1120, OPC13-930, OPC13-814, OPC13-627, OPC13-613 (Fig. 2b), OPB7-1068 and OPB7-800 (Fig. 2a). The nine cultivars used represent a group of commercially cultivated poinsettias. The ability to differentiate all tested cultivars by RAPD bands suggests that this technique may be practically applied for poinsettia cultivar identification.

With UPGMA cluster analysis based on the RAPD markers, the nine poinsettia cultivars fell into two clusters: 'Jingle Bells', 'Supjibi', and 'V-17 Angelika' comprised one cluster; 'V-14 Glory', 'Red Sails', 'Jolly Red', 'Freedom', 'Lilo Red', and 'Pink Peppermint' comprised the other cluster (Fig. 3). Interestingly, the tetraploid 'Supjibi' and the diploid 'V-17 Angelika' are closely related. Results of principal component analysis (PCA) (Fig. 4) were similar to those obtained from UPGMA cluster analysis. With PCA, however, 'Pink Peppermint' and 'Lilo Red' were distant from 'V-14 Glory', 'Red Sails', 'Jolly Red', and 'Freedom'. Correlations between specific morphological traits and groupings depicted by the UPGMA and PCA have not been determined.

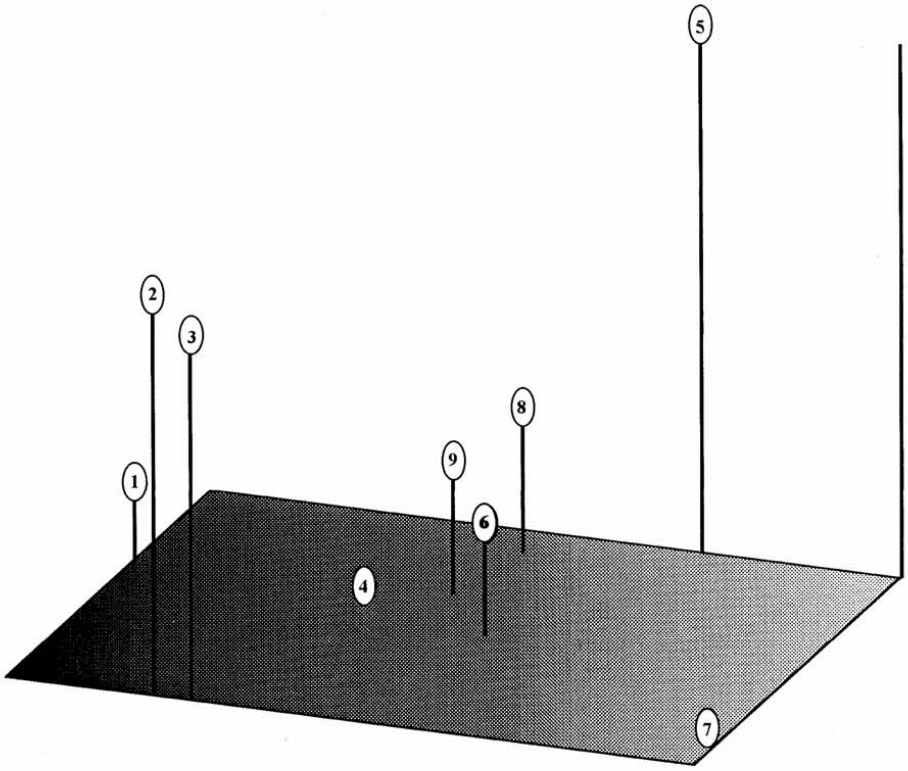


Fig. 4. Principal components analysis of poinsettia RAPD data. (**1**)' Jingle Bells', (**2**) 'Supjibi Red', (**3**) 'V-17 Angelika', (**4**) 'V-14 Glory', (**5**) 'Pink Peppermint', (**6**) 'Red Sails', (**7**) 'Lilo Red', (**8**) 'Freedom Red', (**9**) 'Jolly Red'.

We have shown that RAPD markers can be used for the identification of poinsettia cultivars. The application of this technique may alleviate some of the confusion of cultivar identity associated with morphological characteristics and multiple cultivar registrations. These results also indicate that RAPDs can be used to determine the genetic relationships among cultivars and to estimate the genetic diversity between cultivars.

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