Assessment of Genetic Diversity among Selected Raspberry Cultivars

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Growing raspberries in southern United States is a challenging task as they are high chill-loving plants. Cultivation of raspberry in Florida is significantly hampered due to prevailing hot and humid conditions. The characteristics of raspberry cultivars have been categorized based on morphological descriptions that are dependent on the environment. With this view our program is geared toward identifying low chill/high temperature raspberry genotypes. In this regard, genetic diversity among raspberry cultivars being evaluated in our breeding program for low-chill/high temperature tolerance was assessed employing molecular techniques. A high quality DNA was isolated from raspberry leaf tissue and subjected to molecular analysis. DNA typing was carried out using RAPD assay. Of the 100 primers screened, 10 primers yielded 142 usable DNA bands. Pairwise comparisons of unique and shared amplification products were used to construct a phylogenetic tree using Euclidean linkage distances that clearly separated low- and high-chill raspberry genotypes. These data showed the potentiality of RAPD markers for assessing genetic diversity among raspberry cultivars. Further studies coupled with field analysis would aid in identifying low-chill/high temperature fruit set raspberry genotypes useful for developing raspberry cultivars suitable for Florida.

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Raspberries (*Rubus* spp.), often termed “bramble,” belong to the genus *Rubus*, which is a member of the Rosaceae family, closely related to strawberry in the subfamily Rosoideae (Anonymous, 2010). *Rubus* is one of the most diverse genera of flowering plants, consisting of 12 subgenera, some with hundreds of species. Raspberries are diploid (2n = 2x = 14) in nature with a very small genome (275 Mbp) (Graham et al., 2007). The raspberry is a plant of the northern hemisphere. As a crop, it is mainly cultivated in Russia, eastern and western Europe, the United States, and Canada. There are two horticulturally important raspberry species: red raspberry (*R. idaeus* L.; Fig. 1A) and black raspberry (*R. occidentalis* L.; Fig. 1B). In addition, there are also erect and vine-type raspberry plants. Raspberries are used in the production of ice cream, yogurt, jams and jellies, and other dessert and frozen fruit products. A key production problem associated with these fruit is the need for superior quality fruit for processing, high yield, and disease and pest resistant cultivars. Fruit quality characteristics include size, firmness, color, flavor, texture, and overall appeal. Maintaining a desirable flavor with acceptable color and flesh texture, while increasing fruit size, yield, and postharvest storage capacity is essential. Although some of these traits can be incorporated into new cultivars using a conventional breeding approach, the process is time consuming and unstable. Biotechnology has now become a preferred tool for adding value to biodiversity by allowing more effective identification and utilization of genes.

Raspberry production in the southern U.S. requires tolerance to high temperature coupled with low chilling requirement (<500 h below 45 °F) for flowering and fruit set. The major problem associated with cultivation of raspberry in Florida is prevalence...
of hot and humid conditions. This has significantly hampered the successful cultivation of raspberry as they are “cold-loving plants.” Therefore a study was carried out to unveil the diversity among raspberry cultivars related to their heat tolerance ability, which will aid in a breeding program. As the efficiency of a selection scheme or genetic analysis based on phenotype is a function of the heritability of the trait, factors like environment, traits of multigenic and quantitative inheritance, or partial and complete dominance often confound the expression of genetic traits. Many of these complications of a phenotype-based assay can be overcome through direct identification of genotypes with DNA-based genetic diagnostic assay (Kumar et al., 2001). For this reason, DNA-based genetic markers are being integrated into several plant systems and are expected to play an important role in future plant improvement programs.

Random amplified polymorphic DNA (RAPD) assay detects nucleotide sequence of polymorphisms in DNA using only a single primer of arbitrary nucleotide sequence. The protocol is also relatively quick and easy to perform and uses fluorescence instead of radioactivity. Because the RAPD technique is an amplification-based assay, only nanogram quantities of DNA are required. One of the strengths of these new assays is that they are more amenable to automation than conventional techniques. It is simple to perform and is preferable to experimentation where the genotypes of a large number of individuals are to be determined at a few genetic loci.

Materials and Methods

**PLANT MATERIAL.** Leaf samples of the 11 raspberry cultivars (ORUS 2571-2, ORUS 3240-1, Jingu Juegal, Southland, Fall Gold, Haida, Dormanred, Scepter, Rossana, Heritage, and Indian Summer) used in this research were obtained from the field and greenhouse-grown plants maintained at the Center for Viticulture and Small Fruit Research, Tallahassee, FL. Genotypes were chosen based on their varying degree of chill requirements as recommended by National Germplasm Repository, Corvallis, OR. The leaf samples were collected, frozen in liquid nitrogen, and stored at −80 °C.

**EXTRACTION OF DNA.** DNA was extracted using the ZR Plant/Seed DNA kit (catalogue no. D6020) following the manufacturers instruction (Zymo Research Corp.).

**DNA AMPLIFICATION.** The basic protocol reported by Williams et al. (1990) for PCR was followed with slight modifications to suit raspberry. A single decamer of arbitrary sequence was used in each PCR reaction. Amplification reaction were carried out in 25-µL reaction mixture containing template DNA (25 ng), 5 pmols of primer (Operon USA, Inc.), 2 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl, 0.1% Triton X-100, 1 unit of Taq DNA polymerase (Promega), and 200 mM of each dNTP (Promega). Amplification was performed in a thermal cycler (Eppendorf) for 45 cycles after an initial denaturation at 94 °C for 2 min. In each cycle, denaturation for one min at 94 °C, annealing for 1 min at 35 °C and extension for 2 min at 72 °C was programmed with a final extension step at 72 °C for 5 min after 45th cycle. Negative control was used initially to check the fidelity of the PCR reaction.

**DNA ELECTROPHORESIS.** The amplified DNA fragments were separated on a 2% agarose gel (polysaccharide derivative of agar) and stained with ethidium bromide. Running buffer containing Tris-base, boric acid, and EDTA (pH 8.0) was used for electrophoresis and for preparing the gels. Wells were loaded with 25 µL of reaction volume and 5 µL of loading buffer (Sucrose and Bromo-Cresol green dye) together. Electrophoresis was conducted at 60–75 V for 2–4 h and the gel was photographed under ultraviolet light by using a gel doc system (BioRad).

**DENDROGRAM CONSTRUCTION.** Each sample was amplified at least twice to verify reproducibility. Only those RAPD markers that reproduced consistently across successful PCR reaction and across DNA extractions were included in the analysis. Amplification products were scored as “present” or “absent” and transferred to a binary code with 1 or 2, respectively. Euclidean distances were estimated using a program of Statistica version 6.0 and a dendrogram was constructed using unweighted pair-group mean analysis (UPGMA) method.

**Results and Discussion**

Leaf samples were collected from 11 raspberry cultivars and DNA was extracted. The yield of resulting DNA was about 50–200 µg·mL⁻¹ per gram of leaf tissue. The quality of DNA was very high (260 nm/280 nm = 1.8 to 1.9), and its purity, as confirmed by gel electrophoresis, was excellent (data not shown).

In this study, 100 Operon random 10-base long, single-stranded primers (OPA to OPE with 20 primers in each group) were used to screen the raspberry cultivars. At least one product was amplified in all the primers used. A representative of the PCR amplification product of 11 raspberry cultivars is shown in Fig. 2, which yielded sufficient polymorphisms to distinguish between cultivars. Furthermore, the 10 primers (OPA1, OPA5, OPA7, OPA13, OPB15, OPC3, OPD13, OPE3, OPE15, OPE19) yielded an average of 12 bands per primer with sizes ranging from 200 bp to 3000 bp. All of the resulting 142 bands were either monomorphic or polymorphic and were considered for the precise calculation of genetic diversity.

Cluster analysis using pairwise comparisons of unique and shared amplification products using Euclidean linkage distances grouped the 11 raspberry cultivars into two separate clusters (Fig. 3). The first group represents the low-chill loving plants (ORUS, Jingu Juegal, Southland, Fall Gold, Haida, Heritage, and Dormanred) and the second group represents the high-chill loving plants (Scepter, Rossana, and Indian Summer). The analysis of

![Fig. 2. A representative gel profile depicting the polymorphic bands obtained using arbitrary Operon primer OPE15. Lane M: Molecular marker 100bp. Lane 1 to 11 raspberry cultivars: Haida, Fall Gold, Rossana, Scepter, Southland, Heritage, Dormanred, ORUS 3240-1, ORUS 2571-2, Indian Summer, and Jingu Juegal. *Arrow indicates the polymorphic markers.](image-url)
these raspberry cultivars varying in their chilling requirement suggested that the diversity is moderate and has shown differences in their similarity, which indicated that the reasons for absence of bands are not the same and hence they are more diverse. These results are consistent with the report of Patamsyte et al. (2009) who also found high level of genetic variation among raspberry accessions using Euclidean and molecular genetic distance matrices constructed based on 48 polymorphic RAPD markers. In addition, Stafne et al. (2003) also discriminated raspberry cultivars using RAPD markers. These studies indicate the ability of RAPD makers to differentiate the low- and high-chill requiring raspberry genotypes. This information will be useful for germplasm characterization and cultivar identification including legal issues like assessing infringements on plant breeders’ rights (Fernández et al., 2008).

Overall, this study indicated that RAPD analysis reveals differences among the raspberry cultivars. RAPD technique is less restricting than other molecular techniques and hence, is readily applicable in germplasm screening. Further, RAPD markers in the cluster are also closely linked with important traits like cold or heat tolerance. Such an association could contribute for efficient selection and hybridization work in raspberry breeding program.

Conclusions

A rapid DNA fingerprinting technique for identifying raspberry cultivars was developed. Out of the 100 Operon primers screened, 10 produced 142 usable bands. Cluster analysis of the data based on Euclidean distances using UPGMA revealed moderate degree of diversity. The study indicated the efficiency of RAPD markers for the identification of cold- or heat-tolerant raspberry cultivars.

Literature Cited


