SNiPs, Chips, BACs, and YACs: Are Small Fruits Part of the Party Mix?

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Considerable interest has been expressed in utilizing molecular markers to map trait loci, tag traits of interest, and to pursue marker-assisted selection (MAS) for various small fruit crops. Arising from this body of work are a number of recent efforts directed toward cloning and characterizing cDNA clones from differentially expressed libraries derived from various plant parts, particularly fruit. Similarly, a number of efforts are under way to genetically engineer DNA coding for desired traits into small fruit genotypes of proven quality. The volume of recent literature pertaining to small fruit molecular biology necessitated limiting this review in several respects. First, several crops that are legitimate small fruits, including *Vitis* and *Ribes*, for which a substantial body of literature exists, were excluded. I will focus instead on blueberry (*Vaccinium* sp.), strawberry (*Fragaria* sp.), cranberry (*Vaccinium macrocarpon* Aiton), and brambles (*Rubus* sp.). Second, only papers published in the last 10 years are reviewed in detail, although earlier papers are cited. A number of fairly recent reviews of various molecular approaches to fruit research effectively cover previous work [Antonius-Klemola, 1999; Janick and Moore, 1996 (and references therein); McNicol and Graham, 1992; Scorza, 1991; Weeden et al., 1994]. Several of these are from workshops and colloquia held in conjunction with recent annual meetings of The American Society for Horticultural Science (Kays, 1991; Mehlenbacher, 1995; Scorza, 1996). In this paper, genetic fingerprinting, gene tagging, and mapping (marker technology) and gene cloning, and genetic transformation will be reviewed for each of the four previously mentioned crops.

BLUEBERRY (*Vaccinium* sp.)

While blueberries have been gathered by humans from native stands for thousands of years (Barker et al., 1964; Darrow and Camp, 1945; Hall et al., 1979), blueberry is also the most recently domesticated major fruit crop (Galletta and Ballington, 1996). The first fruit resulting from hybrid plants were harvested in 1916 in New Jersey (Coville, 1921). Currently, five major groups of blueberries are commercially grown; 1) lowbush types, which include managed wild populations of *V. angustifolium* Aiton, *V. myrtilloides* Michaux, and *V. boreale* Hall and Aalders, and improved lowbush cultivars; 2) half-high blueberries, which are hybrid or backcross derivatives of highbush-lowbush hybridizations; 3) highbush types, including both wild *V. corymbosum* L. selections and hybrids; 4) southern highbush blueberries, which are basically the same as the more common highbush types, with the addition of genes from the low-chilling species *V. darrowii* Camp, as well as from *V. angustifolium* Ait., *V. ashei* Reade, and *V. tenellum* Ait. (Ballington et al., 1991a; Lyrene, 1990a); and 5) rabbiteye blueberries, which are all wild selections or hybrids derived from *V. ashei*.

In general, current breeding objectives for the numerous blueberry breeding programs include: 1) expansion of the range of production through broader soil adaptation (upland mineral soils); 2) adaptation to lower chill regions or colder production regions through increased bud tolerance or delayed flowering combined with earlier fruit maturation; and 3) increased disease resistance.

Genetic fingerprinting, gene tagging, and mapping

Rabbiteye blueberry cultivars constitute a very narrow germplasm base, which has been recognized as a problem (Lyrene, 1981b). To determine the magnitude of the problem and devise a method to widen the germplasm base, Aruna et al. (1993) utilized 20 randomly amplified, polymorphic DNA (RAPD) primers to determine genetic relatedness among four native clones and 15 improved cultivars of rabbiteye blueberry. In an extension of the previous study, a cultivar key for economically important rabbiteye blueberry was created using 11 DNA fragments amplified by four RAPD primers (Aruna et al., 1995). Clustering of genotypes generally made sense in light of known pedigree information. Overall, there was a trend toward lower genetic distances within advanced generations from the wild selections.

Levi and Rowland (1997) utilized 15 RAPD markers and three simple-sequence repeat (SSR)-anchored primers to differentiate 15 highbush blueberry and two rabbiteye cultivars and one southern lowbush selection from the wild. However, the molecular data showed clustering within *V. corymbosum* that did not agree with pedigree information. In a similar study, 26 wild lowbush blueberry clones (*V. angustifolium*), including six named cultivars and 12 selections, were screened with a total of 30 RAPD primers (Burgher et al., 1998). All 26 clones could be unambiguously identified with 11 of the primers, and clusters matched known relationships among the clones fairly well, showing trends relating to geographic origin.

Several efforts are under way to create genetic linkage maps for cultivated blueberry in order to tag quantitative trait loci (QTLs) related to chilling requirement, cold hardiness, and soil adaptation. Rowland and Levi (1994) constructed a RAPD-based genetic linkage map of diploid blueberry based on a cross between an F_1 hybrid created between *V. darrowi* x *V. elliottii* Chapm. and another *V. darrowi* genotype. The map was constructed from over 70 markers and consisted of 12 linkage groups, which represented the basic chromosome number for blueberry. More recently, Rowland et al. (1999) developed RAPD-based genetic linkage maps based on crosses between F_1 hybrids of *V. darrowi* and *V. caesanence* Mackenz., in which ≈30 RAPD markers in the *V. darrowi* testcross and 40 RAPD markers in the *V. caesanence* testcross have been mapped.

Based on a tetraploid mapping population resulting from a cross of US 75, a tetraploid hybrid of Fla 4B (a selection of diploid *V. darrowi* x tetraploid *V. corymbosum* 'Bluecrop') x the tetraploid 'Bluetta', 140 markers unique for Fla 4B were mapped to 29 linkage groups (Qu and Hancock, 1997). This map is of interest because US 75 transmits reduced chilling requirements to hybrid progeny, along with high fruit quality, adaptation to mineral soils, and adaptive photosynthetic capacity in hot and dry conditions.

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Gene cloning and genetic transformation

Currently, the only effort to characterize and clone genes from blueberry has been with dehydrins, which become the most abundant proteins in flower buds during cold acclimation. The genes controlling their production may be useful in improving cold hardiness. Levi et al. (1999) cloned and sequenced a cDNA clone screened from a library prepared from RNA extracted from cold-acclimated blueberry floral buds. The RNA hybridized to a probe prepared using degenerate primers based on peptide sequence information from 65 and 60 kDA dehydrins that accumulate in blueberry in response to chilling (Muthalif and Rowland, 1994). The cDNA was a full-length clone encoding a 60-kDA dehydrin.

Some interest has been expressed in improving blueberry cultivars through direct gene transfer. Progress has been limited by lack of a reliable, highly efficient tissue culture regeneration system. Shoot regeneration of a few commercial cultivars has been documented (Billings et al., 1988; Callow et al., 1989; Dweikat and Lyrene, 1988; Hruskoci and Read, 1993; Rowland and Ogden, 1992, 1993). The regeneration efficiency of some commercial cultivars appears to increase with the use of zeatin riboside. However, Cao and Hammerschlag (unpublished data) found that, although thidiazuron was not as effective as zeatin riboside in inducing shoot proliferation, it is significantly less expensive than either 6-(γ,γ -dimethylallylamino)-purine (2iP) or zeatin riboside.

The successful transformation of blueberry was reported by Graham et al. (1996a). Blueberry shoots of 'Northcountry', a *V. corymbosum* x *V. angustifolium* half-high hybrid, were inoculated with *Agrobacterium tumefaciens* isolate LBA4404 with a vector containing the β-glucuronidase (*GUS*) gene. Polymerase chain reaction (PCR) analysis confirmed the presence of the *GUS* and *NPTII* marker genes in five transgenic, in vitro plantlets 2 years after transformation. More recently, Cao et al. (1998) investigated several factors influencing the *Agrobacterium*-mediated transfer of the *GUS* gene into blueberry leaf explants. The *A. tumefaciens* strain EHA 105 was significantly more effective for transformation than was strain LBA 4404. Extended co-cultivation period, explant age, and length of time following transfer of explants to fresh media generally influenced the frequency of *GUS*-expression.

STRAWBERRY (*Fragaria ×ananassa* Duch.)

The high levels of heterozygosity within the genome of the domesticated strawberry (octoploid, 2n = 8x = 56), combined with a wealth of highly compatible *Fragaria* species, has allowed modern strawberry breeders to make good progress in developing improved cultivars. To some degree, the high level of success in conventional breeding, based on these factors, has reduced the need for utilizing molecular techniques. However, despite the level of heterozygosity, several investigations of genetic relatedness in cultivated strawberry, utilizing both pedigree information (Sjulin and Dale, 1987) and molecular marker data (Graham et al., 1996b; Harrison et al., 1997) have revealed a restricted genetic base upon which strawberry breeders depend. Moreover, the complexities of manipulating quantitative traits, such as flavor, and of inheritance in the polyploid genome, and the ever-increasing need for resistance to biotic and abiotic stresses has prompted some gene mapping investigations and initial attempts at gene transfer.

Genetic fingerprinting, gene tagging, and mapping

Genetic fingerprinting of strawberry cultivars and genotypes utilizing RAPD primers, first described by Williams et al. (1990), has been accomplished. Several of these studies also investigated the genetic relatedness of the germplasm based on the marker data. Hancock et al. (1994) used 10 RAPD primers to uniquely identify eight strawberry cultivars and advanced selections from the UC–Davis strawberry breeding program, and detected a fairly high product–moment correlation between the number of shared banding profiles and pairwise coefficients of co-ancestry. Levi et al. (1994) used eight RAPD primers to differentiate eight strawberry cultivars and one *F. chiloensis* (L.) Duch. genotype. Dissimilarity values based on the number of bands shared by genotypes revealed that the *F. chiloensis* genotype 'Del Norte' was most dissimilar. Gidoni et al. (1994) used four RAPD primers, which unambiguously differentiated eight cultivars, including the closely related Israeli cultivars Ofra, Dorit, and Nurit. The DNA fragments used to differentiate the cultivars were consistently reproduced in amplifications with independently isolated DNA samples from several independent accessions of each cultivar.

Degani et al. (1998) differentiated 41 North American cultivars with 15 reproducible fragments produced from 10 RAPD primers. Similarity coefficients based on the RAPD data produced mixed results. In some instances the coefficients made sense when compared with known pedigree information; i.e., some cultivars showed high levels of affinity to a parent, others showed little or none. In a follow-up study, amplified fragment-length polymorphisms (AFLPs) were compared with RAPDs for their ability to produce fingerprints and predict relationships (Degani et al., 2000) among 19 cultivars. Each of the four AFLP primer sets used in the study uniquely differentiated all 19 cultivars. Dendrograms produced with the AFLP and RAPD data sets differed considerably. Surprisingly, a better correlation was found between pedigree-based and RAPD data than between pedigree and AFLP data, despite the fact that many more AFLP markers than RAPDs were utilized in the study and that RAPD markers are generally considered less reproducible than AFLPs. The authors speculate that the AFLP markers utilized in this study were not evenly distributed across the strawberry genome, a phenomenon noted in other AFLP studies (Ellis et al., 1997; Schut et al., 1997).

Other RAPD markers were used to identify strawberry cultivars in a legal case to determine whether the patented cultivar Marmolada had been illegally propagated and used for commercial purposes (Congiu et al., 2000). In this double-blind test, six RAPD markers unequivocally identified all 'Marmolada' plants in the test array and the results were accepted as court evidence.

Graham et al. (1996b) reported on the use of 10 RAPD primers to uniquely identify and examine genetic relatedness among eight strawberry cultivars released from four breeding programs around the world. All eight cultivars displayed unique banding profiles. The authors used the RAPD data to generate similarity matrices; the degree of similarity calculated with these matrices was higher than that calculated from pedigree information. The authors attribute this finding to the possibility that some founding clones were not distinct genotypes as had been commonly assumed. The overall similarity matrix based on the molecular data indicated a 70% similarity between the eight cultivars, suggesting a restricted genetic base for cultivated strawberry, despite the fact that the cultivars originated from widely different breeding programs.

In order to collect and utilize wild relatives of crop plants successfully, such germplasm resources need to be well characterized. Several recent studies have utilized molecular markers to evaluate *Fragaria* germplasm. Chloroplast DNA restriction fragment variation was examined among 26 *Fragaria* taxa and two closely related species, *Potentilla fruticosa* L. and *Duchesnea indica* (Andrews) Focke (Harrison et al., 1997). Low levels of variation were detected among the *Fragaria* taxa, with lower levels of variation being detected as ploidy increased. The lack of variation among the octoploid group suggested the possibility that they are of relatively recent origin. The most divergent groups were the diploids, *F. iinumae* Makino, *F. nilgerrensis* Schlect., and *F. vesca* L., with *F. iinumae* appearing to be the most ancestral. Contrary to general opinion (Bringhurst and Khan, 1963; Senanayake and Bringhurst, 1967), the diploid species *F. vesca* did not show levels of affinity with the cultivated octoploid strawberry that would necessarily suggest it to be ancestral.

Porebski and Catling (1998) used 12 RAPD primers to investigate intraspecific relationships between five North American *F. chiloensis* ssp. *lucida* accessions, 15 North American ssp. *pacifica* accessions and 15 ssp. *chiloensis* accessions from South America. A phenogram based on unweighted pair-group method averaging (UPGMA) clustering showed a distinct difference between North American and South American *F. chiloensis* accessions. Interestingly, more variation was found within the subspecies originating from the Canadian Pacific coast than within those originating from the South American Pacific coast.