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.

The first reports of linkage mapping came from Dr. Tom Davis's group at the Univ. of New Hampshire and involved the use of isozymes. Williamson et al. (1995) reported the inheritance of shikimate dehydrogenase (*SDH*) allozymes and their linkage relationships with phosphoglucoisomerase (*PGI-2*) and a locus governing red vs. yellow fruit color in the diploid strawberry *F. vesca*. The *SDH* and fruit color loci were found to be linked with a recombination frequency of 1.1%. Yu and Davis (1995) reported the linkage between runnering and *PGI-2* allozymes in four F_2 and two F_3 populations. Contingency chi-square tests revealed a significant association of the two loci in all six populations, with a recombination frequency of 18.1% for the pooled data.

Davis and Yu (1997) reported the first genetic linkage map for strawberry, which was created in a diploid F_2 mapping population. They used 28 RAPD primers to detect 73 dominant and 11 codominant markers in the mapping population of 80 individuals. In addition, they combined these 84 RAPD markers with segregation data for an alcohol dehydrogenase sequence tagged site (STS) marker and *SDH* and *PGI-2* isozyme loci. The map they presented contained 80 markers distributed over seven linkage groups, which corresponds with the basic chromosome set for the diploid *F. vesca*. The authors reported significant skewing for entire linkage groups toward an excess of maternal alleles. They speculate that the maternal cytoplasm may have provided a cellular environment more favorable for transmission of maternal alleles. Yu and Davis (1995) reported similar, maternally biased, transmission of alleles in several crosses between 'Alpine' and non-'Alpine' parents. The diploid map could serve as a first step toward map-based cloning of economically important genes controlling traits such as fruit color, disease resistance, and vegetative and floral characteristics.

Red stele root rot, incited by the fungus *Phytophthora fragariae* Hickman var. *fragariae*, interacts with resistance genes in cultivated strawberry in a gene-for-gene fashion (Van de Weg, 1989, 1997). Bulked segregant analysis (BSA) was used to identify seven RAPD markers, developed from four RAPD primers, that were linked to the red stele resistance gene *Rpf* 1 (Haymes et al., 1997). A linkage map of the *Rpf* 1 gene region represents the first genetic linkage map for cultivated octoploid strawberry. The work demonstrates that portions of the strawberry genome segregate in a diploid fashion, which was noted previously with isozyme loci in cultivated strawberry (Arulsekar et al., 1981; Kong and Sjulin, 1993).

The RAPD markers described above could have utility for a marker-assisted selection (MAS) scheme in a breeding program. For MAS to be completely successful, it must be rapid and highly reproducible. Although quick and easy, the RAPD technique has not proven to be highly reproducible (C.J. Jones et al., 1997, and references therein). Haymes et al. (2000) developed two dominant, sequence-characterized, amplified region (SCAR) markers from one of the repulsion-linked RAPD markers described in the preceding study. The markers were linked to the *Rpf* 1 gene and mapped to the same location as the original RAPD marker from which they were developed. When these SCAR markers and three RAPD markers were tested with a diverse cultivar collection, their linkage was highly conserved to the gene.

At least five specific genes for red stele resistance are known to exist; these confer resistance to some of the 30 known races of red stele that have been identified. Haymes et al. (pers. comm.) utilized the BSA technique to identify AFLP markers linked to three such genes in cultivated strawberry. Eleven AFLP markers were linked to the *Rpf* 1 gene and the previously reported seven RAPD markers. Linkage maps were created for the *Rpf* 3 and *Rpf* 6 gene regions with five and six AFLP markers placed on the respective linkage maps. The three genes appear to be clustered and are loosely linked.

At the Univ. of Reading, the research group of Drs. Nick Battey and Mike Wilkinson are investigating the antagonistic relationship between vegetative and floral development in the diploid wild strawberry *F. vesca* (Battey et al., 1998). Their ultimate objective is to clone the alleles controlling seasonal flowering and runnering by using positional cloning. They are developing saturated maps around the loci controlling the two traits in a BC_1 population with inter-simple sequence repeat–polymerase chain reaction (ISSR–PCR) and AFLP analysis. Flanking markers tightly linked to the seasonal flowering locus have been identified and will be used to probe genomic and cDNA libraries to select the gene-containing clones.

Gene cloning and genetic transformation

Numerous studies are now being reported that detail the cloning and/or characterization of genes in cultivated strawberry (Table 1). The majority of the reports involve work being done with cDNA or genomic DNA isolated from fruit at different ripening stages. However, several studies have involved expression of genes under differential environmental stresses or at different developmental stages. The emergence of DNA chip technology, which has already been utilized for strawberry studies (Aharoni, 2000; Frans Krens, pers. comm.; Lemieux et al., 1998) will greatly accelerate progress in understanding gene expression for complex traits as well as the potential for cloning various genes.

The *A. tumefaciens*–mediated transformation of strawberry leaf disks was first reported by James et al. (1990) and Nehra et al. (1990b). In the James study, one plant from 'Rapella' leaf explants was transformed with a binary vector carrying the neomycin phosphotransferase gene (*NPT*II), which confers resistance to the antibiotic kanamycin.

An *A. tumefaciens*–mediated transformation of leaf disks with a callus intermediary stage (Nehra et al., 1990a) and a similar leaf disk transformation with a binary vector containing the *NPT*II and *GUS* genes, which generated shoots directly from leaf disks (Nehra et al., 1990b), were developed for the cultivar Redcoat. Initially, 17 clones were selected that expressed NPTII activity and showed GUS activity in histochemical staining assays. Southern hybridizations demonstrated that the various transgenic plants contained one to two copies of the genes.

Electroporation was used to mediate direct gene transfer into protoplasts from a strawberry selection (Nyman and Wallin, 1992). The protoplasts were transformed with a plasmid containing a hygromycin phosphotransferase and β-glucuronidase gene. Graham (1990) and Graham et al. (1995a) reported *A. tumefaciens*–mediated transformation of 'Melody' with a binary vector containing the cowpea (*Vigna unguiculata* L.) protease trypsin inhibitor (*CpTi*) gene (Hilder et al., 1987). Transgenic plants containing the *CpTi* gene were less susceptible to vine weevil (*Otiorhynchus sulcatus* F., Coleoptera: Curculionidae) (Graham et al., 1997c). Preliminary results from a 3-year field test with 'Melody' and 'Symphony' transformed with the *CpTi* constructs suggested that highly expressing lines survived significantly better and had more root mass than did either nontransgenic plants or the *GUS* transgenic line.

Mathews et al. (1995a) reported the *A. tumefaciens*–mediated transformation of cultivars Tristar and Totem. 'Tristar' was transformed with two strains of *A. tumefaciens* containing a binary vector with marker genes *UIDA* and *NPTII*, and 'Totem' with *A. tumefaciens* strains engineered with binary vectors containing the selectable marker genes *NPTII* or *HPT* and the S-adenosylmethionine hydrolase gene (*SAMase*). In transgenic tomatoes (*Lycopersicon esculentum* L.), expression of *SAMase* impedes ethylene production and significantly extends the shelf life of the fruit (Good et al., 1994). The same strategy is being employed to extend the postharvest shelf life of strawberry. Insertion of the gene in plants from 250 independent transgenic events was confirmed using Southern hybridization. Their protocol is referred to as iterative culture, wherein primary regenerants were subjected to increasingly stringent levels of antibiotic to recover "pure" transgenic types and eliminate chimeras (Mathews et al., 1998).

Random leaf samples of soil-grown transgenic plants analyzed for the presence of *GUS* sequence by Southern hybridization and *GUS* expression through X-Gluc histological assays showed that the transgenics produced through iterative culturing were routinely transgenic, while those produced through traditional noniterative culturing were chimeras containing both transgenic and nontransgenic tissues.

Leaf disks from 'Selektra' were transformed with *A. tumefaciens* containing the *GUS* gene or the phosphinothricin acetyl transferase gene (*PAT*), which confers resistance to the herbicide glufosinate-

Table 1. Gene cloning and characterization studies reported for strawberry.

Gene, trait and reference	cDNA library source	Conclusion
StAG1- An AGAMOUS-like MADS-box[z] gene (Rosin and Hannapel, 1999)	'Calypso'	High nucleotide sequence homology to AGAMOUS family genes, amino acid homology 70% to 75% overall and 90% to 95% within MADS-box. *StAG1* RNA expression specific to stamens, carpels and all stages of fruit and seed development.
Fifty fruit-ripening related mRNAs (Manning, 1994)	'Brighton' receptacle tissue	Most changes in abundance of RNAs occurred with onset of anthocyanin accumulation. Expression found to be coordinated by auxin(s) produced by achenes.
Twenty-six ripening-related cDNA clones (Manning, 1998)	'Brighton' ripe fruit	Up-regulated from 16 d after anthesis to orange fruit stage. Several of the clones were fruit specific. Based on sequence homology, putative functions involved pathways related to color development, texture, respiration, carbohydrate composition, and flavor.
Five ripening-enhanced mRNAs (Wilkinson et al., 1995)	'Pajaro' ripe fruit w/differential display	All five clones most abundant in ripe fruit with little or no expression at earlier stages of fruit development. Three of the five appeared to be fruit-specific and showed sequence homology to a 40S ribosomal protein gene and various members of the annexin protein superfamily.
Eight ripening-related cDNAs (Medina-Escobar et al., 1997	'Chandler' subtractive fruit	Comparison of nucleotide sequence to database information suggested all eight clones were involved in different pathways that could all be involved in fruit maturation.
Low molecular weight heat shock proteins (LMW-HSP) (Medina-Escobar et al., 1998)	'Chandler' subtractive fruit	Showed strongest expression at initial ripening events with declining expression during "turning ripe" and "full-ripe" stages. Differential expression pattern was detected in the achenes and receptacle. Southern analysis suggested a family of LMW-HSP genes may exist in strawberry.
Pectate lyase-like genes (Medina-Escobar et al., 1997b)	'Chandler' subtractive fruit	Predominantly expressed in ripe fruit with increasing expression during ripening process and low levels in other tissues. Removal of achenes from green fruit induced expression, which could be inhibited by naphthalene acetic acid (NAA). Gene product could be partially responsible for pectin degradation resulting in fruit softening.
Dihydroflavonol 4-reductase (DFR) ripening-related gene (Moyano et al., 1998)	'Chandler' subtractive fruit	Predominantly expressed in fruit, first detected at the green stage, reaching highest levels when achenes begin to color through full-ripe stage. Induced by removal of achenes and inhibited by NAA. May function in the biosynthesis of anthocyanin during fruit coloring.
*Cel*1 endo-1,4-ß glucanase (Egase) (Harpster et al., 1998)	'Chandler' ripe fruit	*Cel*1 appears to exist as a single gene per diploid genome. May be part of a larger multi-gene family of Egases. Expression found to be fruit specific, continuing from white fruit to deterioration. *Cel*1 appeared to be ripening-specific and auxin repressed.
*FaEG*10 and *FaEG*30 endo-1,4-ß glucanase (Egases) (Trainotti et al., 1999a, 1999b)	'Chandler' fruit	Both Egases produced increasing amounts of mRNA through the ripening process. *FaEG*10 expression patterns were similar to those for other higher plant Egases, beginning at the onset of ripening and increasing many-fold through full-ripe. The pattern for *FaEG*30 was similar, although it was also observed in large green fruits and in low amounts in some vegetative green tissue. Expression of the two genes was down-regulated by NAA treatments.
*FaExp*2 ripening-related expansin gene (Civello et al., 1999)	'Chandler' ripe fruit	As strawberry fruit ripen, *FaExp*2 expression increases. Unlike other ripening-related genes, expression of *FaExp*2 is largely unaffected by achene removal or auxin treatments. Although *FaExp*2 had an expression pattern similar to that seen for a tomato expansion gene, the amino acid sequence was more closely related to an expansin gene expressed in early fruit development in tomato and to expansins expressed in apricot (*Prunus armeniaca* L.) fruit.
APX-c cytosolic form of ascorbate peroxidase (Kim and Chung, 1998)	'Yoho' half-ripe fruit	Strongly expressed in ripening fruit, weakly expressed in root, leaf, and petiole tissue and not in seed. Fruit expression was lowest in the small green stage and highest at the half-ripe stage. Authors speculate the gene may be induced by increased hydrogen peroxide levels in plant tissues.
*SGR*101 and *SGR*701 fruit-development related genes (Hamano et al., 1998)	'Noyoho' fruit	The two clones were differentially expressed 7 to 10 d after pollination. Nucleotide sequence homology searches revealed *SGR*101 to be similar to an *Arabadopsis thaliana* ribosomal-protein S6 knase gene. *SGR*701 shared high similarity with a *Phaseolus vulgaris* hydroxyproline-rich glycoprotein (HRGP) gene.
*Fcor*1, *Fcor*2 and *Fcor*3 cold acclimation-related genes (NDong et al., 1997)	'Chambly' cold acclimated	Differential screening of the library revealed the three clones showed differential expression at low temperature. Expression of *Fcor*1 peaked after 2 d of low-temperature (LT) exposure, while *Fcor*2 peaked after 2 weeks exposure. Expression of *Fcor*3 decreased within 24 h of LT exposure and remained low during the 8-week acclimation period. *Fcor*1 and 2 were expressed in all tissues, while *Fcor*3 was specific to leaves. The expression of *Fcor*1 correlated with freezing tolerance in the cultivars used in the study.

[z]AGAMOUS-like MADS-box gene refers to a C-class gene (AGAMOUS), of the A, B, C classes of genes known to direct development of four floral organs, that is expressed in the third and fourth floral whorls of angiosperm flowers and is associated with stamen and carpel development. Most ABC genes contain a highly conserved, 56 amino acid domain called the MADS-box domain.

ammonium [(butanoic acid, 2-amino-4-hydroxymethylphosphinyl)-monoammonium salt] (du Plessis et al., 1997). All but one of the 23 regenerated plants selected on kanamycin revealed the presence of 1 to 34 inserts when a probe containing the *PAT* sequence was used for Southern hybridizations. Four transgenic lines appeared to be fairly similar to the nontransgenic controls, although they yielded significantly less. All transgenics were resistant to field treatments of glufosinate-ammonium that killed all weeds and nontransgenic strawberry plants.

Barceló et al. (1998) reported *A. tumefaciens*–mediated transformation and regeneration of nine putatively transgenic 'Chandler' shoots. The presence of the *NPT*II gene was confirmed in six plants by PCR. *Agrobacterium*-induced false positives were ruled out because no *VirD1* gene product was PCR amplified from the same samples.

The antifreeze protein gene (*AFP*) from winter flounder (*Pseudopleuronectes americanus* Walbaum) was transformed with *A. tumefaciens* into the cultivar Firework (Firsov and Dolgov, 1999). This gene delays the formation of ice crystals by binding antifreeze proteins with water molecules (Davies and Hew, 1990). Eight transgenic lines of strawberry were confirmed with PCR amplification of inserted *NPT* II and *AFP* sequences. The morphology of the transgenic plants was the same as that of the original cultivar.

At the USDA Horticultural Crops Research Laboratory, Dr. Robert Martin's group is developing genetically engineered, virus-resistant strawberry cultivars. They are transforming 'Hood' and 'Totem' with mutated movement protein, mutated polymerase, and mutated helicase of strawberry mild yellow edge virus. The lines producing coat protein have been planted in the field, but have not shown acceptable resistance (R. Martin, pers. comm.).

Interest in *F. vesca* has primarily been in its utility as a model system or a bridge for transferring traits of interest to octoploid cultivated strawberry. As a model system, several groups have pursued in vitro studies with the species to optimize transformation protocols. El Mansouri et al. (1996) reported the *A. tumefaciens*–mediated transformation of *F. vesca* leaf disks with a vector containing the *NPT*II and *GUS* genes. Among the 7.7% (25/144) of the explants that were kanamycin-resistant and the 6.9% (23/144) showing *GUS* activity, the presence of the *NPT*II gene was documented in one plant by PCR.

Haymes and Davis (1998) reported the stable *A. tumefaciens*–mediated transformation of β-glucuronidase (*GUS*) and neomycin phosphotransferase (*NPT*II) marker genes into diploid *F. vesca* 'Alpine' plants. Southern analysis suggested two sites of insertion for *NPT*II in first generation transformants. When these plants were selfed, the resulting progeny segregated in a 15:1 fashion for *GUS* expression, which is consistent with the proposed two-gene insertion model.

Dr. Frans Krens of the Dept. of Cell Biology, Plant Research International in Wageningen, reports that their group has prepared a cDNA library from red-ripe fruit of the cultivar Elsanta. They have sequenced 1800 expressed sequence tagged (EST) clones and are currently transforming some of the interesting genes into strawberry to confirm their putative functions. The group is particularly interested in genes controlling or regulating flavor, color, ripening, and postharvest quality. They have also developed heterologous and homologous, fruit-specific promoter (fbp7) *GUS* constructs and have transformed them into strawberry to test expression at various fruit development stages. One of the gene constructs being tested is designed for resistance to diseases caused by species of *Botrytis*, *Verticillium*, and *Phytophthora*.

DNA Plant Technologies, Inc. (DNAP) acquired Monsanto's strawberry development program in 1998 with exclusive rights to their existing technology and nonexclusive rights to future Monsanto enabling and trait technology. They have produced a large number of primary RoundUp Ready® transformants of 'Camarosa', 'Selva', and several promising lines that are currently under testing for glyphosate tolerance as well as field and quality performance. They are also investigating fungal resistance, principally to *Botrytis*, *Verticillium*, and *Phytophthora* species, using broad fungal resistance strategies based on the assumption that other pathogens will be affected by the gene combinations developed for these primary targets. A long-term project is directed toward "fumigation independence.". The ultimate product would contain several genes directed at weed, pathogen, and nematode control. Much of this work is oriented toward the impending loss of methyl bromide as a soil fumigant. In addition, the company will continue its work to engineer increased sweetness, color, and postharvest quality, and changes in flowering or response to daylength.

CRANBERRY [*Vaccinium macrocarpon* (Ait.)] Pursh

Current cranberry breeding objectives for plant characters generally include increased resistance to biotic and abiotic stress, increased vine and bud hardiness, rapid root initiation in cuttings, minimum runnering after the field is established, high density of shorter, stout uprights that bear fruit uniformly above the flood level, and precocious, highly productive plants. Increased emphasis is being placed on resistance to diseases and insects. For fruit characters, sweeter and better colored fruit for raw consumption, increased aroma, firmness, size, flavor, organic acids, anthocyanin content, glossiness, postharvest qualities, pectin content, and early and uniform ripening would encompass most breeding program objectives.

Genetic fingerprinting, gene tagging, and mapping

Cranberry bogs originally composed of one cultivar can become "contaminated" with volunteer seedlings and native genotypes over the long periods the bogs are in production. The fact that new bogs have often been established with stolons raked from existing plantings compounds the "contamination" problem. Few qualitative morphological descriptors exist for cultivated cranberry, leading to a great need for molecular-based genetic fingerprints. In a series of investigations, the research group led by Dr. Nicoli Vorsa at the Rutgers Univ. Cranberry and Blueberry Research Center have used RAPD primers, in combination with silver staining techniques, to address these issues. In an initial report, Novy et al. (1994) attempted to differentiate 22 cranberry cultivars. Fourteen cultivars showed unique single-stranded RAPD banding profiles, while the remaining eight were represented by three profiles. Identical banding profiles among the cultivars were attributed to cultivar misclassification, with one genotype represented by several cultivar names.

Novy and Vorsa (1995) examined intracultivar variability and genetic relatedness among accessions from the four major cranberry cultivars currently planted and found that each cultivar was represented by multiple genotypes, which in many cases were not closely related to each other. Partitioning of the genetic variation revealed that fully 90% of the variation occurred within the groups. The authors demonstrate that consensus fingerprints can be developed among regional representatives of a cultivar that could allow for "true types" to be identified. Applying these techniques and a phenotypic analysis, Novy et al. (1996) examined uprights from 12 'McFarlin' Washington State cranberry bogs that had been reported to be highly variable in terms of yield, fruit set, shape, and coloration. All seven yield, fruit, and flower characters varied significantly among the bogs in both years. Variable RAPD banding profiles were obtained, indicating that more than one genotype was present in the 'McFarlin' samples. More than one RAPD profile was present in nine of the 12 bogs. One RAPD profile that predominated in the higher producing bogs matched RAPD profiles of 19 'McFarlin' samples from other states, suggesting that this profile represents the true 'McFarlin'. The high levels of variation between the 'McFarlin' profile and several of the other profiles found suggest that the latter were not selfed or hybrid progeny of 'McFarlin', but rather instances where the bogs were established with diverse genotypes.

Although RAPDs have generally been considered dominant markers, scored on the basis of their presence or absence, Novy and Vorsa (1996) reported evidence for the existence of RAPD heteroduplex molecules in cranberry. The heteroduplex molecules result from reannealing of two single-stranded RAPD products that are amplified from alternative sites, but share enough homology to form a double-stranded molecule. These RAPD markers, always found to be jointly present or absent (non-independent) in the RAPD profiles of 22 cranberry cultivars, had the potential to bias estimates of genetic

similarity and relatedness. Segregation analysis, and the ability to create the associated RAPD markers through mixing of DNA amplification products from individuals lacking the associated markers, provided the evidence for the existence of heteroduplex formation. While such markers have the potential to confound various genetic analyses, the heteroduplexes also provide a source of co-dominant RAPD markers, the lack of which has been an oft-cited weakness of the RAPD technique.

Gene cloning and genetic transformation

Currently, the only cranberry transformations that have been reported are from Dr. Brent McCown's laboratory at the Univ. of Wisconsin, using the "gene-gun." The cranberry cultivar Stevens was transformed with plasmid DNA containing the genes *GUS* (β-glucuronidase), *NPT*II (neomycin phosphotransferase II), and *BT* (*Bacillus thuringiensis* ssp. *kurstaki* crystal protein) (Serres et al., 1992). Stem sections derived from in vitro plant cultures that had been induced to form adventitious buds were transformed. About 90% of the transformed shoots were shown to contain all three genes via PCR and Southern analysis. The group has also reported the successful "gene-gun"–mediated transformation of the cranberry cultivar Stevens with a gene conferring tolerance to the herbicide glufosinate (B. McCown, pers. comm.).

Effectiveness of the *BT* gene against economically important cranberry lepidopteran pests in bioassays was inconsistent. Serres and McCown (pers. comm.) found that crude cranberry leaf extracts combined with B.t.-endotoxin solution reduced the insecticidal activity of the endotoxin in feeding studies with lepidopteran pests. Further studies revealed that leaf extracts enhanced for flavonolic compounds and devoid of proteins inhibited insecticidal activity of the endotoxin to the same degree as did the crude extracts. Serres et al. (1997) reported similar findings relating to the inactivation of β-glucuronidase (*GUS*) in either purified bacterial *GUS* preparations or preparations from transgenic tobacco (*Nicotiana tabacum* L.) or cranberry.

Qu et al. (1999) reported a highly efficient in vitro system for regeneration of cranberry from leaf explants. The basal medium consisted of Anderson's salts and Murashige and Skoog's organics supplemented with thiadiazol (TDZ) and 2iP. Qu et al. (1999) examined the effects of varying levels of TDZ, 2iP, NAA, explant orientation, and leaf explant age (leaf position relative to distal end of the shoot) on the regeneration efficiency for the genotypes Early Black, Pilgrim, Stevens, Ben Lear, and US35, which differed significantly in regeneration capacity. Elongated shoot cuttings were easily rooted in vitro in basal medium, as well as ex vitro in shredded sphagnum moss.

RASPBERRIES AND BLACKBERRIES (*Rubus* sp. L.)

Modern breeding objectives for raspberry include increased fruit size and yield, ease of harvest, upright cane habit with reduced to nonexistent spines (thornless), and heavier yielding, fall fruiting or primocane types. Resistance to biotic and abiotic stresses is becoming of increased importance. Cultivars are needed that display increased cold, heat, and drought tolerance, as well as adaptation to other climatic conditions and a wide range of soil types. Additionally, increased genetic-based resistance to various biotic stresses will become critically important. Many of the same objectives will hold for blackberry cultivar improvement, with the addition of free-suckering roots to facilitate propagation, reduction in acid content and seed size, and an increase in fruit soluble solids content. There is a growing interest in primocane fruit production in blackberry, which might be a way to alleviate the low cold hardiness problems generally associated with the thornless character.

Gene fingerprinting, gene tagging, and mapping

Recent molecular marker–based studies in *Rubus* have mainly involved use of restriction fragment length polymorphisms (RFLPs) based on minisatellite DNA (series of tandem repeats of a core consensus sequence with 10–60 base pairs) and RAPDs. These markers have been used to fingerprint various cultivars and genotypes, and to investigate both genetic relationships among species and cultivars at several taxonomic levels and population genetics among naturally occurring populations. Along similar lines, they facilitate maintenance of trueness-to-type during micropropagation of raspberry and detect somaclonal raspberry variants regenerated through tissue culture (Table 2).

I have found no work describing mapping efforts in *Rubus* in the literature. At Sweetbriar Development, Inc., linkage maps are being developed using SCAR and arbitrarily primed PCR (APPCR) markers (Gavin Sills, pers. comm.). The work began in a population that was segregating mainly for some easily measured phenotypic traits, i.e., fruit size, drupelet number and size, seed weight, and floricane vs. primocane flowering. Putative QTLs were identified for all traits; these accounted for 45% of the variation for some traits. The map covered ≈600 cM of the genome and consisted of six linkage groups. Currently efforts are being focused on four high-priority mapping populations that are segregating for traits such as resistance to fruit rot.

In an effort to begin the process of developing physical maps for *Rubus*, which will provide a means for distinguishing divergent genomes and tracing introgression in hybrids, Lim et al. (1998) reported genomic in situ hybridization (GISH) and fluorescent in situ hybridization (FISH) data for chromosomes of raspberry, blackberry and their allopolyploid hybrids. With GISH, they were able to differentiate chromosomes from raspberry vs. those from blackberry in allopolyploid hybrid plants. Furthermore, the techniques permitted the detection of blackberry chromosomes carrying raspberry translocations.

Gene cloning and manipulation

As part of an ongoing effort to utilize biotechnology in improving postharvest qualities of raspberry, Jones et al. (1997) reported on a new method to obtain high-quality RNA from this species. Conventional methods have proven quite inefficient, due in large part to high levels of RNAses, polysaccharides, and acidity. Key features of the protocol include a high buffering capacity lysis buffer and the use of a high concentration of insoluble polyvinylpolpyrrolidone to prevent oxidation of polyphenols and their subsequent binding to nucleic acids and proteins. The protocol worked well for extracting RNA from fruit at all developmental stages, as well as from leaf, stem, and root tissues.

Three techniques were used to isolate genes that are differentially expressed at various stages of the fruit ripening process (Jones et al., 1999). Using differential screening with cDNA probes from early and late fruit ripening stages, they isolated three genes that were up-regulated during the ripening process, including: a) a latex-like protein with sequence similarity to the wound-responsive major latex protein gene from opium poppy; b) a metallothionein-like protein that binds heavy metals, is involved in metal ion detoxification and has ability to protect against oxidative stress; and c) a polygalacturonase, which is a cell-wall hydrolase involved in softening of ripening fruit. With the differential display technique they made a comparison of mRNA profiles of green, white, pink, and red fruit, and isolated 16 cDNA clones that were up-regulated during fruit ripening. Sequence similarity between these clones and several genes were found, including: a) a dioxygenase-related protein, which is part of the ethylene forming process; b) a metallothionein-like protein with no similarity to the one found with differential screening, but still involved in binding of metal ions; and c) a protease inhibitor gene that might code for insecticidal properties. With a cDNA-AFLP analysis, 26 sequence tags specific to ripe fruit were made. One showed similarity to pectin methyl esterase, which is another cell-wall hydrolase.

As part of an effort to control gray mold (caused by *Botrytis cinerea* Pers.:Fr.), two cDNAs that may control production of polygalacturonase-inhibiting proteins (PGIPs) were cloned from raspberry fruit (Ramanathan et al., 1997). Plant PGIPs inhibit fungal endopolygalacturonases, which are released by invasive fungi to begin the degradation of cell walls. The raspberry cDNAs show a high degree of amino acid sequence identity with PGIPs isolated from other plant species. Although PGIP activity decreases during the fruit ripening process, coincident with the onset of fruit fungal infections, PGIP

Table 2. Genetic marker studies reported in *Rubus* and a summary of findings.

Marker (reference)	Application	Summary
RFLP, M13 minisatellite (Nybom et al., 1989; Nybom and Hall, 1991)	Genetic fingerprinting	Identified unique fingerprints for several closely related blackberry, raspberry, and hybrid cultivars. Many polymorphisms were detected, resulting in a low probability that any two cultivars in the study would share a banding profile.
RFLP, M13 and (AC)/(TG) minisatellite (Antonius et al., 1997)	Genetic fingerprinting and diversity assessment	Established fingerprints for 39 blackberry accessions in the Helsinki clone collection. Thirty-seven had identical banding patterns to 'Majestät'. The other two accessions had identical patterns that differed from the rest by one additional band, suggesting they might be somatic mutants.
RFLP, M13 minisatellite (Kraft et al., 1996)	Genetic fingerprinting and taxonomic differentiation	Seven facultatively apomictic blackberry species were fingerprinted to determine the relationship between morphological differentiation and genetic variation as determined by molecular markers. Levels of intraspecific variation were quite low in the *R. nessensis* group, and although they were not completely homogeneous, few widespread genotypes existed for each species. Even less variation was found for the more apomictic group related to *R. gracilis*. The slight variation in fingerprint patterns coupled with limited morphological variations leads the authors to conclude that species designations for some of the groups is unwarranted. The authors also conclude that DNA fingerprinting should be used in conjunction with morphological characters when making higher taxonomic comparisons.
RFLP, M13 minisatellite (Nybom, 1995)	Genetic fingerprinting, ascertainment of hybrid status, determination of inheritance, and regulation of apomixis	Several interspecific hybrids and apomictically derived *Rubus* plants were used in a set of hybridization experiments. Pollen was more highly stainable for hybrid offspring than for apomictically derived seedlings. Hybrid seedlings had more variable fingerprints. Determination of inheritance and regulation of apomixis could not be made with the data set generated in the experiment.
RFLP, M13 and (AC)/(TG) minisatellite (Antonius and Nybom, 1995)	Genetic fingerprinting and ascertainment of hybrid status	Nineteen interspecific hybrids between raspberry and blackberry were fingerprinted, along with the parental genotypes. All of the parental plants had unique fingerprints. All of the progeny were unique; however, two were identical to their maternal parents. Results suggest that hybridization had taken place in most cases. In some plants with reduced vigor and fruiting ability, DNA fingerprints showed no paternal contribution and a reduction in maternal bands, suggesting meiosis had taken place before parthenogenetic egg development (automixis).
RFLP, M13 minisatellite (Kraft et al., 1995)	Genetic fingerprinting and ascertainment of hybrid status	The genetic fingerprint of *R. vestervicensis*, a purported hybrid between *R. grabowskii* and *R. pedemontanus*, was compared to the genetic fingerprints of seven and six clones of each of the purported progenitors respectively. All of the bands found in *R. grabowskii* and some bands from *R. pedemontanus* comprised the fingerprint for *R. vestervicensis* suggesting it was the result of the fertilization of an unreduced *R. grabowskii* egg cell with *R. pedemontanus* pollen.
RFLP, M13, 33.15 and (AC)/(TG) minisatellite (Hoepfner et al., 1993; 1996)	Genetic fingerprinting, maintaining trueness-to-type, and detection of somaclonal variation	All raspberry genotypes were readily differentiated. No intracultivar variation was detected among vegetatively propagated samples from different sites, from within micropropagated clones of raspberry selections, or within callus-derived cell lines. When 26 regenerates classified as "off-types," based on phenotype, were compared to controls, no differences in banding pattern were detected. The authors speculate the mutations occurring in tissue culture may be of too fine a scale to be detected with microsatellites.
RFLP, M13 minisatellite (Nybom et al., 1990; Nybom and Schaal, 1990)	Genetic fingerprinting and influence of reproductive mode on distribution of genetic variation in naturally occurring populations	*R. allegheniensis*, *R. flagellaris*, *R. occidentalis* and *R. pensilvanicus* plants separated by only a few meters had identical banding patterns. Subsequently, 20 plants each of *R. occidentalis* and *R. pensilvanicus* sampled along a 600-m transect were found to be comprised of 15 and 5 genotypes respectively. The difference was attributed to the largely apomictic reproductive modes of the polyploid *R. pensilvanicus*.
RFLP, minisatellite DNA (Parent and Pagé, 1992)	Genetic fingerprinting	Thirteen red and two purple raspberry cultivars were fingerprinted by alkaline phosphatase-labeled probes. All cultivars were uniquely fingerprinted and the fingerprints were reproducible when samples were collected from plants of different ages, at different developmental stages, or from different locations on the plant.
RFLP, M13 and (AC)/(TG) minisatellite (Heiberg et al., 1999)	Genetic fingerprinting	'Winklers Sämling' and 'Asker' two *Phytophthora* root rot resistant cultivars were found to share almost identical banding patterns. One additional band in 'Asker' appears to be due to a point mutation subsequent to vegetative propagation from 'Winklers Sämling'.
RFLP, M13 and (AC)/(TA) minisatellite (Antonius and Nybom, 1994; Nybom, 1998)	Genetic fingerprinting and distribution of genetic variation in natural populations	Twenty-four *R. idaeus* plants sampled in a 50 × 150-m study site exhibited unique genetic fingerprints, suggesting vegetative reproduction for the species may be more limited than thought. In contrast, nine samples of *R. nessensis* collected on the same site had identical fingerprints. Additional studies of *R. nessensis* populations that varied for several biometrical characters also had identical fingerprints, suggesting high levels of vegetative or apomictic reproduction for the species.
RFLP, M13 and (AC)/(TG) minisatellite, RAPDs, and $(GACA)_4$ (Korpelainen et al., 1999)	Genetic diversity and clonal structure in naturally occurring populations of *Rubus chamaemorus*	Although the three populations showed morphological variation, the amount of genetic variation detected with markers was fairly low. The three populations investigated N = 38, 31, and 40 contained 2.4, 1.5, and 2.5 effective genotypes, respectively, showing some genotypes were quite widespread in the populations.
RFLP, GACA and GATA minisatellite (Busemeyer et al., 1997)	Genetic diversity	Sampled 22 individuals from each of 4 isolated Philippine populations of *Rubus moluccanus*. No two individuals shared banding profiles, suggesting no apomictic reproduction. Results suggested intra-island gene flow was relatively high. However, significant differentiation of the species occurred over short distances on individual islands.

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Table 2. Continued.

Marker (reference)	Application	Summary
RFLP, GACA and GATA minisatellite (Keane et al., 1998)	Genetic variation	The genetic variation for *R. idaeus* plants collected at sites contaminated by several hazardous organic compounds and high iron concentrations were compared to plants collected at adjacent, noncontaminated sites. Mean genetic similarity and heterozygosity did not differ significantly between sites. However, plants from contaminated sites possessed significantly fewer alleles per individual and private alleles, suggesting a loss of genetic variation among *R. idaeus* growing on contaminated sites.
RFLP, chloroplast DNA (Waugh et al., 1990)	Genetic relatedness in the genus	Phylogenetic tree based on chloroplast data generally agreed with that based on morphology with the notable exception of *R. illecebrosus*, which was shown to have diverged considerably from other members of the subgenera *Idaeobatus.*
RFLP, chloroplast DNA (Moore, 1993)	Chloroplast genome diversity	In most cases, origins of the chloroplasts could be determined. Two misidentified genotypes were revealed and several long-standing pedigree questions were resolved. Overall, low levels of chloroplast DNA variation were detected. However, variation among cultivars with different species origins, between subspecies within the species *R. idaeus*, and within the subspecies *R. vulgatus* were revealed.
RAPD, *ndhF* chloroplast DNA sequencing (Howarth et al., 1997)	Phylogenetic analysis of endemic Hawaiian *Rubus*	Results from a DNA sequence analysis of the chloroplast *ndhF* gene contradict the previous hypothesis regarding the origin of Hawaiian *Rubus*. *Rubus hawaiensis* was found to be closely related to *R. spectabilis*. However, *R. macraei* was found to be distantly related to these species. All evidence suggested *R. macraei* was distantly related to New World and Asian species of the *Idaeobatus* and that the two Hawaiian endemics may have arisen from two separate colonization events.
RAPD (Parent et al., 1993)	Genetic fingerprinting, trueness to type	Fifteen raspberry cultivars were differentiated with three RAPD primers. DNA samples collected from plants at different times of the growing season and different stages of the certification program produced identical fingerprints.
RAPD (Eriksson and Bremer, 1993)	Genetic fingerprinting and population genetics	Genetic diversity estimated with RAPDs for 20 and 25 ramets of *R. saxatilis* collected from two distinct localities showed considerable genetic diversity. This result was in direct contrast to pollination studies that suggested the distinct patches consisted of single genets or incompatibility types. High genet density was linked with increased fruit production and increased genetic diversity in this species.
RAPD (Graham et al., 1994)	Genetic fingerprinting and relatedness	Nine RAPD primers successfully differentiated 10 red raspberry cultivars. Five PCR reactions were carried out per primer/genotype to determine reliability of the primers. Genetic relatedness estimates based on pedigree and molecular information were in general agreement. The degree of relationship between the cultivars based on pedigree was generally lower than when based on marker data. The authors speculate the marker data more truly reflects the degree of similarity based on the premise that some founding clones, although given different names, were very similar in genetic constitution and in some cases may have been identical.
RAPD (Pirinen et al., 1998)	Genetic fingerprinting and relatedness	Three RAPD primers differentiated five arctic bramble cultivars and variants and revealed a close relationship between 'Pima' and 'Mespi'. However, the authors do not recommend RAPDs as an absolute or routine criterion for cultivar identification due to problems with reproducibility.
RAPD (Pamfil et al., 2000)	Genetic relatedness	Estimated the relatedness of 40 *Rubus* species using molecular markers to help predict breeding success in wide hybridizations. Dendrograms produced with the molecular data were in general agreement with traditional taxonomies, although several discrepant groupings were detected that did not predict crossability.
RAPD (Graham and McNicol, 1995)	Genetic relatedness	Estimated genetic relatedness between 24 accessions arising from 13 *Rubus* species. A dendrogram based on the marker data resulted in four groupings that corresponded with the subgenera and hybrid groups studied. The data were in good agreement with existing pedigree information with two notable exceptions. *Rubus macraeii* was found to be as similar to the *Eubatus* as to the *Idaeobatus.* 'Black River', considered to be a *R. occidentalis* wild selection, was found to be as similar to cultivated red raspberry as to *R. occidentalis*.
RAPD (Graham et al., 1997b)	Genetic diversity and relatedness	Six RAPD primers were used to investigate genetic diversity of wild European red raspberry (*Rubus idaeus*), and the degree of relatedness to cultivated 'Glen Clova'. Clonal reproduction was found to occur at a distance of 20 m. The majority of populations were comprised of genetically distinct individuals, with plants more similar within a site than between sites. None of the populations were closely related to 'Glen Clova'.
RAPD (Trople and Moore, 1999)	Genetic similarity	Six RAPD primers were used to calculate genetic similarities among 42 *Rubus* accessions representing 37 species. The study revealed two mislabeled genotypes. A dendrogram was created using the RAPD data that resulted in two clusters. One contained the genotypes in the *Malachobatus, Anaplobatus,* and *Dalibardastrum* and some *Idaeobatus.* The other cluster contained the rest of the *Idaeobatus* the *Rubus* and *Cylactis.* Interestingly, *R. idaeus* was more closely related to species in other subgenera than to species in the *Idaeobatus.* Some species with low similarities hybridize easily with *R. idaeus.*
RAPD (Graham et al., 1997c)	Genetic fingerprinting, trueness to type, genetic similarity, and correlation to regeneration capacity	Five RAPD primers were used to establish genetic fingerprints for eight *Rubus* genotypes regenerated on various tissue culture media. In some cases the genotype groupings based on marker data could be correlated with regenerative capacity on certain medium combinations.

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Table 2. Continued.

Marker (reference)	Application	Summary
RAPD (Chen et al., 1996)	Genotype differentiation	Work related to an attempt to produce thornless periclinal chimeras in *Rubus*. Authors tried to differentiate specific genotypes in mixtures of DNA from two genotypes. DNA from one genotype was detected in concentrations as low as one part in 30 parts of another DNA.
Sequence characterized amplified region (SCAR) – (Parent and Page, 1998)	Genetic fingerprinting	Sequence information from five previously characterized RAPD markers was used to produce 24-mer SCAR primers. The SCARs produced more repeatable results than the RAPD primers and segregated in a co-dominant fashion. Four SCAR markers were capable of distinguishing 15 raspberry cultivars.
Internal transcribed spacer region (ITS) Nuclear ribosomal DNA (Alice and Campbell, 1996)	Phylogenetic relationships among the *Rubus* subgenera	Sequenced the ITS 1 and 2 for 14 representatives of subgenus *Rubus* and one to three from 10 of the 11 remaining subgenera. Of the subgenera with more than one representative, only *Malochobatus* and *Anoplobatus* are monophyletic. *Rubus alpinus* from the subgenera *Lampobatus* groups within the subgenus *Rubus*, the blackberries. *Rubus ursinus*, generally grouped in the subgenus *Rubus*, appeared more closely related to subgenus *Idaeobatus*. Sequence divergence among six North American and eight European species of subgenus *Rubus* is low.
Internal transcribed spacer region (ITS) Nuclear ribosomal DNA (Alice et al., 1997)	Molecular documentation of hybridization between *R. idaeus* and *R. caesius*	Nuclear ribosomal DNA of the ITS region was sequenced for one *R. caesius* two *R. idaeus* and five putative hybrid individuals. The putative hybrids had several morphological characters that appeared intermediate to the two progenitors. The putative progenitors differed at 20 nucleotide positions and one indel. For one hybrid, both parental ITS sequences were recovered intact.

transcription levels appear to be maintained at high levels throughout fruit development.

Lack of a reliable system for regenerating shoots from explants has hampered progress in genetically engineering bramble crops. Generally, cultivars with blackberry in their pedigree regenerate fairly efficiently (Hall et al., 1986; Hassan et al., 1993; Fiola et al., 1990; Swartz et al., 1990). Hassan et al. (1993) also tested several *A. tumefaciens* strains and found strain 516, originally isolated from *Rubus*, to be significantly more virulent on all plant parts than were the other strains tested.

The regeneration of red raspberry has proven more difficult. McNicol and Graham (1990) found that the most critical factors were hormone type and concentration, concentration of sucrose, absence of activated charcoal, necessity for light, and the need for orienting leaf disks with the abaxial surface in contact with the medium.

Graham et al. (1997a) attempted to correlate genetic relatedness among four raspberry cultivars, as determined through RAPD banding profiles, with regenerative capacity. The cultivar groupings based on the RAPD data could in some cases be correlated with regenerative capacity on certain media. Among the Glen raspberries, 'Glen Clova' was the most dissimilar, while it demonstrated a higher regenerative capacity.

The synthetic cytokinin-like compound *N*-(2-chloro-4-pyridyl)-*N*-phenylurea (CPPU) improved the micropropagation of whole shoots and induction of organogenesis in raspberry, a blackberry, and a raspberry/blackberry hybrid, with no significant genotype effect noted among the four red raspberry cultivars evaluated (Millan-Mendoza and Graham, 1999). Similarly, the efficiency of organogenesis from internodal segments of three red raspberry cultivars with CPPU was significantly higher than with any other growth factor tested, but a significant genotype effect was noted in response. An initial transformation experiment revealed that *A. tumefaciens* infection and antibiotic selection media were not overly inhibitory to the ability of CPPU to induce organogenesis in the red raspberry cultivar Glen Magna.

The first report of genetic transformation in *Rubus* was from the Scottish Crop Research Institute (Graham et al., 1990; McNicol and Graham, 1989). They reported the successful *A. tumefaciens*–mediated transformation of a red raspberry selection, the blackberry by red raspberry hybrids 'Tayberry' and 'Sunberry', and 'Loch Ness' blackberry. Leaf disks and internodal stem segments were transformed with a binary vector containing either the *GUS* gene or the *NPT*II gene. Positive *GUS* assays were detected in four 'Tayberry', one 'Sunberry', two raspberry, and seven 'Loch Ness' blackberry regenerates. Using kanamycin-containing media to select plants transformed with the *NPT*II gene almost totally inhibited shoot regeneration. The authors suggested early selection for transformants with *GUS* assays and subsequent *NPT*II assays when the plantlets were more resistant.

Hassan et al. (1993) reported the successful *A. tumefaciens*–mediated transformation of a 'Black Satin' blackberry/'Tayberry' hybrid (MD-ETCE-1). The plants were transformed with a construct containing the *GUS*, *NPT*II, and *CSR* (chlorsulfuron herbicide resistance) genes. Transformation was verified with Southern hybridizations to a labeled *GUS* probe.

Mathews et al. (1995) reported the successful transformation of 'Meeker', 'Chilliwack', and 'Canby' red raspberry. Leaf and petiole explants were inoculated with the *A. tumefaciens* strain EHA 105, carrying a vector encoding gene sequences for SAMase, under the control of the wound- and fruit-specific tomato E4 promoter. As in their work with strawberry, they used iterative culture of node, petiole, and leaf explants to eliminate chimeral tissue. Petiole explants produced higher rates of transformation in terms of callus and shoot regeneration, regardless of the selective agent. Overall, the recovery of transformants was higher with the selection marker gene hygromycin phosphotransferase (*HPT*) than with the neomycin phosphotransferase (*NPT*II) gene. Stable integration of the transgenes was confirmed by Southern analysis. Following a total of 218 independent transformation events, transgenic plants were established in soil in the greenhouse and are currently undergoing field testing for evaluation of the transgenic trait.

DeFaria et al. (1997) reported the optimization of regeneration and transformation protocols for 'Comet' red raspberry. Adventitious shoot regeneration was significantly increased when 10-mm-diameter leaf disks were used and explants were subcultured after 4 weeks. Regeneration was not affected by adding cefotaxime sodium to the medium at levels up to 500 $mg \cdot L^{-1}$. However, regeneration was moderately reduced when explants were inoculated with *Agrobacterium*, and inhibited when kanamycin levels reached 40 $mg \cdot L^{-1}$ or more. *Agrobacterium tumefaciens* strain C58 was the most virulent in terms of producing tumors, and a disarmed strain of C58, LBA4404, was used to transform 'Comet' leaf disks. From a total of 500 shoots, one was confirmed to have been transformed.

Mazzetti et al. (1999) attempted to fuse protoplasts from the primocane fruiting raspberry 'Autumn Bliss' and the thornless blackberry 'Hull Thornless'. Several factors influencing isolation, culturing, and fusion of protoplasts were investigated. Fusion products were identified using RAPD markers, and GISH and FISH techniques. Several factors were investigated for regenerating plants from the fusion calli, and, although roots and pre-embryonic structures were differentiated, no whole plants were recovered.

At the USDA Horticultural Crops Research Laboratory in Corvallis, Ore., Dr. Robert Martin's group is developing genetically engineered, virus-resistant raspberries. A number of different constructs are being used, including coat protein genes, mutated polymerase, and mutated movement protein, for raspberry bushy dwarf (RBDV), and nontranslatable RNA for RBDV and tomato ringspot virus (ToRSV). Currently, raspberry plants have been transformed with *A. tumefaciens*

for resistance to ToRSV and RBDV. Recently the group began to experiment with particle bombardment and are comparing this to vectoring with *A. tumefaciens*. They have induced expression of *GUS* and green fluorescent protein (*GFP*) in leaves that were bombarded, but transgenic plants have not been produced with the gene gun to date.

CONCLUSION

As we enter the 21st century, great anticipation surrounds the potential for molecular biology to address the myriad of problems associated with producing agricultural crops. Commensurate with that interest, considerable progress has been made in plant molecular biology. The wealth of literature encountered in the course of this review demonstrates that considerable progress has been achieved in small fruit molecular biology; upon closer examination; however, such progress has clearly not kept pace with that in some of the major commodities. Since the mid-1980s, the USDA Animal Plant Health Inspection Service (APHIS) has issued over 5000 U.S. permits for field tests of genetically engineered crops, but only 20 have been issued for strawberry, seven for raspberry and one for cranberry (K. Hokanson, USDA–APHIS, personal comm.). About 51 transgenic crop/gene combinations have now been deregulated by the USDA and given full commercial approval. In 1998, ≈27.4 million ha of genetically engineered crops were planted in the United States, including 10.9 million ha of soybean [*Glycine max* (L.) Merr.], 7.9 million ha of corn (*Zea mays* L.), 2.3 million ha of cotton (*Gossypium hirsutum* L.), and 24,280 ha of potato (*Solanum tuberosum* L.). To date, no transgenic small fruit has been deregulated and no genetically engineered small fruits have been grown commercially (K. Hokanson, pers. comm.).

Similarly, the small fruits lag far behind the major commodity crops in genetic mapping. The current integrated genetic map for soybean contains 1423 markers, including 689 RFLPs, 606 SSRs, 11 AFLPs, 79 RAPDs, 10 isozymes, and 26 classical markers (Cregan et al., 1999), and that for corn contains 5650 markers, including 4533 AFLP and 1117 RFLP and SSR markers (Peleman et al., 2000). Few maps exist for small fruit crops. Moreover, the linkage maps that do exist rely almost solely on RAPD markers, which have not been considered ideal for developing the robust, collaborative mapping programs that have been the basis for the great progress witnessed in soybean and corn.

Several questions need be asked with regard to the seeming lack of progress in small fruit molecular biology. One might ask, "Is there really a need?" For all the crops considered in this review, vast amounts of untapped genetic potential are readily available in highly compatible germplasm. Moreover, much of this wild germplasm can literally be found growing at the edges of our cultivated fields. This is in sharp contrast to many of our commodity crops, where much of the germplasm is difficult to access and that which is available has been heavily utilized.

"Are these crops too problematic?" Many of our small fruit crops are complex polyploids, which are proving to be recalcitrant for standard mapping approaches. Similarly, many have proven to be very difficult to manipulate in vitro. Significant amounts of time and money need to be invested to address these problems. This scenario highlights a potential, if not real, conundrum. Given the lack of progress, the daunting nature of the tasks, and the absence of a strong synergistic effort, the small fruit community has encountered great difficulty in attracting significant and reliable funding to address the problems.

"Is molecular biology too costly?" Without question, molecular research is expensive. The relatively small research community dedicated to small fruits does not command large amounts of research dollars. A realistic assessment of our professional and financial assets and the potential outcomes for various research thrusts would be a prudent step. In an accompanying manuscript, Luby and Shaw (2000) point out that only in certain specific instances will a marker-assisted selection (MAS) program really be cost-effective when compared with conventional breeding methods. Similar arguments could be made with regard to transgenic plant technologies.

"Is society ready for molecular biology?" Negative public perception regarding genetically engineered crops, especially in Europe, is another issue that requires consideration. Once a genetically modified crop is readied for market, will it be accepted by the consumer? Much has been said and could be said regarding the rightful place of genetic engineering in agriculture. However, the response to the release of a genetically modified small fruit should be considered and prepared for before large amounts of money are invested in its development.

Nearly 30 years of data on plant molecular biology have now accumulated, yet it is probably safe to say that we have only scratched the surface. The deserts have yet to bloom and hunger still exists in our world. Despite the seemingly intractable problems encountered in small fruit molecular biology, the high costs, and the negative public perceptions at this juncture, the potential for molecular biology still looms large on the horizon. In order to be positioned to take advantage of the future potential of the technology, continued advancements need to be made. New, robust markers capable of providing reliable, reproducible fingerprints and anchors for genetic maps need to be produced. When high-throughput techniques become cost-effective, the marker systems will be in place that will allow small fruit geneticists to produce saturated linkage maps useful for mapping quantitative traits. Reliable in vitro protocols need to be developed, which will allow breeders to quickly take advantage of new, significant genes that will be isolated with increasing frequency by molecular biologists. Insertion of these genes into small fruit cultivars will make a real difference for the producers and consumers of these crops. Prudent decisions need to be made regarding the appropriate amounts of human and capital resources the small fruit community should direct towards these long-term, high-potential research goals.

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