10 Blueberry

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10.1 Introduction

Blueberries are members of the *Ericaceae* or Heath family, genus *Vaccinium*, subgenus *Cyanococcus*. Genus *Vaccinium* consists of blueberries, cranberries, lingonberries and many related wild species. The genus is very diverse, containing about 400 species, mostly found in the tropics at high elevation, but also in temperate and boreal regions. *Vaccinium* is widely distributed plant genus and exhibits a high level of morphological diversity. Fruits from several *Vaccinium* species are collected from the wild for food. *Vaccinium* species in section *Cyanococcus* are used to develop blueberry cultivars which are grown for their edible fruit. Most are shrubs like the blueberries; however diverse range of growth forms from epiphytes to trees exists.

Blueberry is an important small fruit crop and the most recent major fruit crop to be cultivated, having being domesticated during the twentieth century. They are high value crop which can thrive on acidic imperfectly drained sandy soils, once considered worthless for agricultural crop production. North America is the major producer of blueberries. The total area devoted to growing commercial blueberries in North America is approximately 74,000 ha. Blueberries are one of the richest sources of antioxidants of all fresh fruits and vegetables (Prior et al. 1998). In addition, fresh blueberries are fair source of vitamin C (Matzner 1967). Blueberries are produced commercially in 16 countries worldwide. Worldwide average yields have increased almost by 50% in the last 10 years to just over 4,000 lbs/acre. Acreage has also increased by 37%, causing production to double in the last 10 years. Major countries of blueberry production are USA and Canada, together account for the major blueberry production. In addition to these, other countries including Poland, The Netherlands, France, Italy, Mexico, New Zealand and Lithuania also produce blueberries.

10.1.1 Cytology

According to Longley (1927), the genus *Vaccinium* has a basic chromosome number of 12. Blueberry exists at three ploidy levels: $2\times$ ($2n = 24$), $4\times$ ($2n = 48$) and $6\times$ ($2n = 72$). Diploid population ($2n = 24$) includes species: *V. myrtilloides* Michx, *V. corymbosum* L., *V. pallidum* Ait., *V. darrowi* Camp, *V. elliottii* Chapm, *V. tenellum* Ait. *V. boreale*. Important tetraploids are *V. angustifolium* Ait, *V. corymbosum*, *V. hirsutum* Buckley, *V. simulatum* Small, *V. myrsinites* Lam. These tetraploids may have resulted from crosses between members of the same species, resulting in autotetraploids, or between members of different species as allotetraploids. Species *V. corymbosum* L. and *V. australe* Small, are natural tetraploids. The third important group of species is represented by the hexaploid ($2n = 72$) population of which *V. ashei* Reade, and *V. constablaei* Gray, are members. There are seven diploid, six tetraploid and two hexaploid species (Rowland and Hammerschlag 2005). Since no fundamental sterility barrier exists between homoploid *Vaccinium* species, many polyploids have arisen naturally (Coville 1927; Newcomber 1941). These polyploids particularly tetraploids ($2n = 48$), are thought to be responsible for the wide range of adaptation of the genus (Newcomber 1941). Ahokas (1972) concluded that the diploid Vaccinum genome ($x = 12$) is actually "homoeologous tetraploidy" (secondary polyploidy), based on Hall and Galletta's findings (1971).

Three species are of major economic importance: (1) The highbush blueberry, *Vaccinium corymbosum* (2) The lowbush blueberry *Vaccinium angustifolium* and (3) the rabbiteye blueberry, *V. ashei*. Most of the worldwide blueberry production comes from the highbush blueberry.

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10.1.2 Commercial Blueberries

Commercially grown blueberries can be divided into five major groups.

(1) Highbush Blueberry: In Highbush types (4×) *Vaccinium corymbosum* makes up much of the genetic material of the northern highbush. It is a very variable deciduous shrub that is typically 4 to 6 ft. tall. It is most widely planted blueberries, popular with home gardeners throughout the northern US and southern Canada. There are over 100 named varieties of northern highbush blueberries.
(2) The wild or lowbush blueberries of North America: The lowbush types (2× and 4×) include managed wild populations of *V. angustifolium*, *V. myrtilloides, V. boreale* and improved lowbush cultivars. In North America most are *Vaccinium angustifolium* and are known as sweet lowbush blueberry. The lowbush is not commercially planted, but thousands of acres of natural stands are pruned, sprayed and harvested. It is a dwarf, woody, usually deciduous shrub that is found growing in wide range of areas. The term lowbush applies to those species that are less than 3 ft.
(3) Rabbiteye: The Rabbiteyes (6×) are all wild selections and hybrid cultivars of *V. ashei*.
(4) Southern Highbush: Southern highbush blueberries (4×) cover hybrids that may contain genetic material from two, three and sometimes four *Vaccinium* species. These are predominantly highbush *V. corymbosum* germplasm but which have the low-chilling species *V. darrowi* in their parentage, as well as *V. angustifolium* and in some cases *V. ashei* and *V. tenellum* (Lyrene 1990; Ballington et al. 1991). Southern highbush were specifically hybridized for superior fruit, soil adaptability, heat tolerance, and low winter chilling.
(5) Halfhigh Blueberries: Halfhigh blueberry (4×) is a term given to a group of blueberries that do not exceed about 3 ft. at maturity, but most have the bushy, woody habit of highbush cultivars. These are species hybrid or backcross derivatives of lowbush-highbush hybrids, usually involving *Vaccinium angustifolium and V. corymbosum* parentage. The half high category refers to bushes intermediate in height between high bush and low bush (Galletta and Ballington 1996; Hokanson 2001; Rowland and Hammerschlag 2005).

10.1.3 Breeding Objectives

10.1.3.1 Horticultural Attributes

The early objectives in blueberry breeding included large berry size, light blue color, small scar, firmness of fruit, good dessert quality and productivity. Each of these objectives has been realized, but all are not yet combined in a single variety. The trait that receive emphasis in selection vary with the location and type of blueberry, but generally include plant vigor, disease resistance, desirable plant architecture, easy of clonal propagation, large fruit size, good flavor, light blue fruit color, long storage life, season of ripening, and consistent high yields. Other objectives of breeding work involve the development of cultivars with greater winter hardiness, drought resistance and adaptation to mechanical harvesting.

10.1.3.2 Biotic Stress Resistance

Insects The control of blueberry insects is one of the most important phases of blueberry culture. Some of these insects seriously reduce the productivity of the bush, while others impair the quality of berries lowering their value. Blueberries are subject to attack from many different insects. Blueberry maggot is the major fruit pest of blueberry. The adult is a small fly which lays an egg under the skin of the developing fruit. The tiny larva feeds within the fruit. Cranberry fruitworm, plum curculio, blueberry bud mite are other important insects. Mites are tiny pests (<1/100th inch) inhabit the leaf and flower buds, feeding on them before they emerge. The other problematic insects to blueberries are: blueberry blossom weevil, blueberry leafminer, blueberry stem borer, cherry fruitworm, cranberry fruitworm, cranberry rootworms and grubs, scale insects, sharp nosed leafhopper, blueberry crown girdler, black vine borer, and cranberry rootworm.

Diseases Blueberries can be attacked by a host of fungi, bacteria, and viruses. Most of the diseases vary in severity and economic importance from one blueberry growing region to another. Lowbush blueberries, highbush blueberries, and rabbiteye blueberries have similar types of diseases, but the disease that is most important in one type may be minor in the other. Mummy berry is probably the most

Table 1. Important diseases and pests of blueberry

	Causal Agents
Diseases	
Fusicoccum canker	*Fusicoccum putrefaciens*
Anthracnose	*Colletotrichum gloeosporioides*
Botrytis blight	*Botrytis cinerea*
Mummy berry	*Monilinia vaccinii-corymbosi*
Red leaf rose bloom	*Exobasidium vaccinii*
Blueberry stunt	Mycoplasma-like organism
Blueberry Shoestring	Blueberry Shoestring Virus
Stem blight	*Botryosphaeria dothidea*
Stem canker	*Botryosphaeria corticis*
Phomopsis twig blight and canker	*Phomopsis vaccinii*
Alternaria fruit rot	*Alternaria* spp.
Insects	
Blueberry maggot	*Rhagoletis mendax* Curran
Sharp-nosed leafhopper	*Scaphytopius magdalensis* Provancher
Blueberry aphid	*Illinoia pepperi* MacGillivray
Cranberry fruitworm	*Acrobasis vaccinii* Riley
Cherry fruitworm	*Grapholita packardi* Zeller
Plum curculio	*Conotrachelus nenuphar* Herbst
Blueberry bud mite	*Acalitus Vaccinii* Keifer

widespread threat to blueberry in almost all countries. It is characterized by the formation of dried-out mummified fruit at harvest. The disease kills leaves, shoots, and flowers and then produces the spores on these dead tissues that infect the fruit later. It may reduce yields by up to 10% in severe infestations of some main commercial areas. Other common diseases of blueberry are blueberry stunt, blueberry shoestring, leaf mottle, scorch and red ringspot viruses, stem blight, stem canker, botrytis, anthracnose, phomopsis twig blight, canker, alternaria and fruit rot, fusicoccum canker and red leaf rose bloom (Galletta and Ballington 1996; Rowland and Hammerschlag 2005).

Viruses cause several diseases in blueberry. Two viruses of importance in North America are Blueberry scorch and red ringspot viruses. Virus diseases are the most difficult to control since infection may occur several months, or possibly years, before symptoms are seen, and the only effective control usually involves removing infected bushes. Fruit and foliar diseases are controlled with a combination of proper cultivar selection, cultural practices, and fungicides. Stem and root diseases are more difficult to control. Disease-free planting stock, promotion of good plant growth, removal and destruction of infected plant parts, and the selection of well drained ground all help reduce the incidence and severity of root and stem diseases. Table 1 lists important diseases and pests of blueberry (Galletta 1975; Luby et al 1991; Galletta and Ballington 1996).

10.1.4 Blueberry Breeding

There has been a great breeding effort in the highbush blueberry than any other *Vaccinium* species (Draper and Scott 1971). The breeding of highbush blueberries began in about 1900 while rabbiteye breeding began in about 1940. Of the three types of blueberries, lowbush blueberries have benefited the least from cultivar development, and most lowbush blueberries still come from native plants. Breeders have released several blueberry cultivars comprised of diverse species and from widely different geographical areas (Ballington 1990; Lyrene 1990).

Three themes have been evident in the breeding of both highbush and rabbiteye cultivars: recurrent selection (Lyrene 1988), the proven-cross method,

and interspecific hybridization (Lyrene and Ballington 1986; Lyrene and Perry 1988). Interspecific hybridization continues to be the keystone to the success of the cultivated blueberry improvement program. The genus *Vaccinium* has many species. Within section *Cyanococcus*, interspecific crosses are easy to make and interspecific hybrids are usually vigorous and fertile if the two species involved have the same chromosome number. Coville (1937) undertook interspecific hybridization for blueberry breeding. Most of the native species of blueberry could be hybridized with the cultivated types and provide unique genes. (Draper 1977; Draper et al. 1982). Crosses of diploid × tetraploid, diploid × hexaploid, and tetraploid × hexaploid species give varying result, depending on the species involved. Many breeders utilized various species for blueberry improvement. (Moore 1965; Sharp and Sherman 1971; Ballington 1990; Lyrene 1990). Wild *V. corymbosums* selections have been used for many years for developing blueberry cultivars.

The first varieties to be introduced were hybrids of *V. corymbosum* and *V. australe*. Modern cultivars were derived from the hybrids of *Vaccinium angustifolium* and *Vaccinium corymbosum* and from the hexaploid hybrids of *V. ashei* and *V. constablaei*. (Ballington 1980). There has been an emphasis in several breeding programs in the past to develop highbush blueberry varieties (*V. corymbosum*, 4×) with low chilling requirements, suitable for growing in the southern United States because they are early ripening. Crosses between high-chilling northern highbush cultivars and the low-chilling southern evergreen diploid, *V. darrowi* Camp, have been important in the development of low-chilling highbush cultivars (Sharpe and Darrow 1959; Sharp and Sherman 1971). Crosses between *V. ashei* and *V. constablaei* have resulted in the release of two rabbiteye hybrid cultivars (Ballington 2001). However traditional breeding approaches for blueberry are labor-intensive due to heterozygosity, polyploidy, and length of evaluation trails. Hybridization between certain species has been difficult to achieve due to chromosome number difference and the inability to easily induce polyploidy. Attempts to transfer genes between tetraploid highbush cultivars and hexaploid rabbiteye cultivars have not been highly successful.

10.2 Application of Marker Technologies

10.2.1 Protein Markers

Isozymes have been used in genome analysis of higher plants both to determine phylogenetic and evolutionary relationships and in genetic linkage analysis. Hill and Vander Kloet (1983) used isozyme markers for genetic studies in blueberry and reported limited variation in four enzyme systems. Among four *Vaccinium* sections Vorsa et al. (1988) studied diploid, tetraploid and hexaploid *Cyanococcus* species for isozyme polymorphisms using 12 enzyme systems. Further Kreb and Hancock (1989) used isozyme markers to investigate the mode of inheritance in tetraploid *V. corymbosum* and reported that it has tetrasomic inheritance in the four enzyme systems analyzed. Bruederle et al. (1991) extended isozyme analysis of 20 loci to the investigation of population genetic structure among diploid blueberry species *V. elliotii*, *V. myrtilloids*, and *V. tenellum*. They found that the diploid species exhibit high level of variation within populations as expected for highly self-sterile, outcrossing crops in taxa. Hokanson and Hancock (1998) examined levels of allozymic diversity in native Michigan populations of diploid *V. myrtilloids* and the tetraploid *V. angustifolium* and *V. corymbosum*. The number of polymorphic loci is very limited within a gene pool, and polymorphism is low. DNA markers were favored for most purposes.

10.2.2 Molecular Markers

Several types of DNA markers are now available for use in genetic mapping. PCR-based markers such as random amplified polymorphic DNA (RAPD), arbitrary primer-PCR (AP-PCR), inter-simple sequence repeat (ISSR), expressed sequence tag (EST), cleaved amplified polymorphic sequences (CAPS) and microsatellites have also been used mainly for the analysis of plant genomes. With respect to molecular genetic research in blueberry molecular markers have been developed for DNA fingerprinting, analysis of genetic relationship and mapping. Several cDNA and genomics clones have been isolated and an EST database has been made publicly available. The type of markers currently available includes isozyme, RFLP, RAPD, ISSR, EST-PCR, CAPS and microsatellite markers.

Molecular markers have been identified that are useful for DNA fingerprinting of representative selections and cultivars of three major commercial grown types of blueberries: the highbush, lowbush and rabbiteye types. Haghighi and Hancock (1992), performed RFLP analysis on various genotypes representing the blueberry species *V. corymbosum*, *V. angustifolium*, *V. darrowi* and *V. ashei*, using chloroplast specific and mitochondria specific probes. In this study, high polymorphism was observed in mitochondrial genome while no polymorphism was detected in chloroplast genome. Aruna et al. (1993) and Levi et al. (1993) reported successful amplification of RAPD markers from blueberry DNA. Aruna et al. (1993, 1995) reported good results from DNA of native selections and improved cultivars of rabbiteye blueberry, *V. ashei*. In this study the extent of genetic relatedness among 19 cultivars of rabbiteye blueberry, 15 improved cultivars and the four original selections from the wild were used. Their analysis was consistent with phylogenetic data provided for rabbiteye blueberries. Levi et al. (1993) described an RAPD protocol from several different woody plants including blueberry, cherry, peach, pear and apple.

Levi and Rowland (1997) used RAPD and SSR-anchored primers for the usefulness for amplifying blueberry DNA. These markers were used to differentiate and evaluate genetic relationship among 15 highbush (*V. corymbosum*) or highbush hybrid cultivars, two rabbiteye (*V. ashei*) cultivars and one southern lowbush (*V. darrowi*) selection from the wild. The *V. ashei* cultivars and *V. darrowi* selection grouped out separately from the *V. corymbosum* cultivars. The study indicated that RAPD and SSR-anchored primers are useful for identifying blueberry cultivars or selection. Burgher et al. (1998) screened 26 wild lowbush (*V. angustifolium*) clones, including six named cultivars and 12 selections. Clustering of genotypes correlated fairly well with geographic origin of clones. Further Bugher et al. (2002) used RAPD analysis with low bush blueberry selections and native accessions that has been collected from various geographic regions in Atlantic Canada and Maine. This analysis successfully distinguished all the clones. Arce-Johnson et al. (2002) reported using two RAPD primers to distinguish five highbush Chilean cultivars. Rowland (2003a) reported development of EST-PCR markers for fingerprinting and genetic relationship studies in blueberry. The polymorphic EST-PCR and CAPS marker developed in this study distinguished all the genotypes indicating that these markers should have general utility for DNA fingerprinting and examination of genetic diversity in blueberry. Further, EST-PCR primers were tested for their ability to amplify fragments in related *Ericaceae*, cranberry and rhododendron (Rowland et al. 2003b).

Microsatellite or SSRs have recently become important genetic markers in plant genome research. The development of SSR in plants is accelerating, and SSR loci are now being incorporated into established genetic maps of the major plant species. SSRs are particularly attractive for distinguishing between cultivars because the level of polymorphism detected at SSRs loci is higher than that detected with any other molecular marker assay. Boches (2005) reported microsatellite markers for *Vaccinium* from EST and genomic libraries. SSR markers were derived from two Expressed Sequence Tags (EST) libraries and from microsatellite enriched genomic library constructed from *V. corymbosum* cultivar Blue crop DNA. Recently, Boches et al. (2006) used 20 EST-SSR and eight genomic microsatellite loci to determine genetic diversity in 69 *Vaccinium corymbosum* L. accessions consisting of 13 wild accessions and 56 cultivars (one half-high, 18 southern highbush and 37 northern highbush).

10.3 Genetic Linkage Mapping

Different types of markers have been used for generating the linkage maps. PCR-based markers are being used extensively for the construction of linkage maps. Within the *Ericaceae* molecular linkage maps have been developed only for blueberry (Rowland and Levi 1994; Qu and Hancock 1997). Initial RAPD based genetic linkage maps have been developed for three diploid and one tetraploid blueberry populations. Rowland and Levi (1994) reported the construction of an initial genetic linkage map for diploid blueberry using a population resulting from a test cross between the F_1 interspecific hybrid US 388 (*V. darrowi* × *V. elliottii*) and another *V. darrowi* clone US 799. The map comprises 70 RAPD markers mapped to 12 linkage groups in agreement with the basic blueberry chromosome number and cover a total genetic distance of over 950 cM, with a range of 3–30 cM between adjacent markers. Qu and Hancock (1995) have used RAPD markers to establish a tetrasomic mode of inheritance in interspecific hybrids of diploid *V. darrowi* and tetraploid *V. corymbosum*.

Vorsa and Rowland (1997) reported RAPD for the estimation of 2*n* megagametophyte heterozygosity in a diploid blueberry *Vaccinium darrowi* Camp. Qu and Hancock (1997) reported construction of an RAPD based genetic linkage map of tetraploid blueberry population that should be segregating for high fruit quality, heat tolerance and cold tolerance. The population resulted from a cross of US75 (a tetraploid hybrid of a diploid *V. darrowi* selection Fla 4B and tetraploid *V. corymbosum* 'Bluecrop') and another *V. corymbosum* 'Bluetta'. A total of 140 RAPD markers unique to Fla 4B that segregated 1:1 in the tetraploid population were mapped into 29 linkage groups. Rowland et al. (1999) constructed RAPD-based genetic linkage map using diploid blueberry populations shown to be segregating for both chilling requirements and cold hardiness. The population resulted from test crosses between F_1 interspecific hybrids, *V. darrowi* × diploid *V. corymbosum*, and another *V. darrowi* clone and another diploid *V. corymbosum* clone. Recently a few EST-PCR markers have been added to these maps; the map of the *V. corymbosum* test cross currently comprises approximately 90 RAPD and EST-PCR markers and the map of the *V. darrowi* test cross comprises approximately 70 RAPD and EST-PCR markers (Rowland et al. 2003c; Rowland and Hammerschlag 2005).

One of the unique contributions of genetic mapping is the possibility of detection of genomic regions controlling quantitative traits. Most of the agronomically and economically important traits are controlled by a relatively large number of loci. Such loci are called as quantitative trait loci (QTL). A preliminary QTL analysis using current genetic linkage map and cold hardiness data for the *V. corymbosum* test cross population have identified one putative QTL associated with cold hardiness that explains ~20% of the genotypic variance (Rowland et al. 2003c). With further saturation, these maps and segregating populations should allow researchers to map genes and QTLs controlling the important traits.

Muthalif and Rowland (1994a, b) studied changes in protein levels associated with low temperature exposure in floral buds of blueberry cultivars with different levels of cold hardiness. Characterization of cold-responsive proteins revealed them to be members of a family of proteins known as dehydrins. Levi et al. (1999) reported isolation of 2.0 kb dehydrin cDNA which encodes the 60 kDa dehydrin. The sequence of 2 kb cDNA was further used to design primers to amplify alleles of two dehydrin-related genes from the cold sensitive and cold tolerant parent plants. Panta et al. (2004) reported mapping of dehydrin related gene to linkage group 12 of the current genetic linkage map of blueberry. Dhanraj et al. (2004, 2005) reported that family of dehydrins of 65, 60 and 14 kDa accumulates in floral buds during winter, and the levels of these proteins correlate with cold tolerance. A cDNA clone from blueberry floral bud RNA that encodes the 14 kDa dehydrin was identified and sequenced.

Genetic mapping provides a direct means of investigating the number of genes influencing a trait, the location of these genes along the chromosomes, and the affects of the variation in doses of these genes. The most successful applications will be in those species with well developed molecular marker maps. A large number of monogenic and polygenic loci for various traits have been identified in a number of plants, which are currently being exploited by breeders and molecular biologists together for marker-assisted selection. Tagging of useful genes like the ones responsible for conferring resistance to plant pathogen, insect, drought tolerance and a variety of other important developmental pathway genes, is a major target. Identification of the marker loci that are linked to the trait of interest is followed by the utilization of linkage association in genetic improvement program. Once linkage between a trait and a marker locus is established, it is possible to use the information in the selection of the breeding lines. The availability of more molecular marker based maps in blueberry would facilitate the localization of genes controlling traits such as fruit quality, fruit size, plant vigor, disease resistance and various environmental tolerances.

10.4 In Vitro Culture and Genetic Engineering

Most blueberry genotypes can be cloned in large numbers with great rapidity from small amount of starting by means of in vitro shoot culture (Nickerson 1978; Cohen and Elliott 1979). Shoot tip propagation of blueberry was initiated by Layrene (1978), who reported successful in vitro propagation of rabbiteye blueberry seedling. Since then, several reviews (Smagula and Lyrene 1984; George et al. 1987; Zimmerman 1980, 1991; Galletta and Ballington 1996) have summarized the in vitro technology for blueberry propagation. The first studies on shoot tip propagation of lowbush blueberry (*V. angustifolium*) were conducted by Frett and Smagula (1983). Further Smagula

and Litten (1989) and Litten et al. (1992) studied mycorrhizial inoculation of lowbush blueberry as an aid to micro-propagation. The earliest studies on shoot tip propagation of highbush blueberry (*V. corymbosum*) date back to 1979-1980 (Cohen and Elliott 1979; Cohen 1980; Zimmerman and Broome 1980).

Wolfe et al. (1983) conducted studies to compare various media and to determine the optimum medium for micropropagating highbush blueberry. Young and Cameron (1985a, b) studied influence of growth regulators, nitrogen form and effect of light on micropropagation of rabitteye blueberries. Grout and read (1986) studies the influence of the stock plant propagation method on propagation and rooting of halfhigh blueberry 'Northblue'. Rooting and establishment of in vitro blueberry plantlets in the presence of mycorrhizal fungi was studied by Lareau (1985). Chandler and Draper (1986) studied the effect of zeatin and 2iP on shoot proliferation of highbush blueberry clones. Grout et al (1986) conducted studies on the influence of stock plant propagation method, tissue culture and leaf-bud propagation of 'Northblue' blueberry. Long-term effects of in vitro propagation of 'Northblue' halfhigh blueberry under greenhouse and field conditions have been reported (El-Shiekh et al. 1996). Noè and Echher (1994) and Noè (1998) studied the influence of irradiance on the in vitro growth of highbush blueberry. Growth vigour and yielding of highbush blueberry from semi-woody cuttings and in vitro was studied by Smolarz and Chiebowska (1997). Isutsa et al. (1994) conducted investigations to identify environmental conditions that would accelerate rooting and acclimatization and improve survival of ex vitro blueberry microcuttings. Gonzalez et al. (2000) initiated studies to develop a uniform method of micropropagation using nodal segment from mature field-grown highbush blueberry plants. Further Cao et al. (2003) investigated the effect of sucrose concentration in the propagation medium on shoot proliferation and on gene delivery into highbush blueberry shoots.

The first success with organogenesis from highbush blueberry (Billings et al. 1988) occurred from leaf explants of in vitro propagated shoots. Rowland and Ogden (1992, 1993) investigated zeatin riboside (ZR) for highbush blueberry regeneration from leaf explant. Hruskoci and Read (1993) studied the in vitro shoot shoot regeneration from internode segment and internode-derived callus in blueberry. Cao and Hammerschlag (2000) reported improved shoot organogenesis from leaf explant of highbush blueberry. Cao et al. (2002) also reported that growth regulator pre-treatments enhance shoot organogenesis from leaf explants of 'bluecrop'.

As a tool in cultivar breeding, in vitro chromosome doubling with colchicines is feasible with blueberry (Lyrene and Perry 1982). Lyrene and Perry (1982) reported that a combination of colchicine facilitates chromosome doubling in blueberry. Different methods have been used to induce tetraploids in *V. darrowi*, *V. elliottii* and *V. darrowi* × *V. elliottii* hybrids (Perry and Lyrene, 1984), 8× plants from 4× *V. corymbosum* clones (Goldy and Lyrene 1984) and 6× plants from triploid (*V. coryombosum* (4×) × *V. elliotii* (2×)) hybrid (Dweikat and Lyrene 1989). The use of 2*n* gametes to obtain elevated polyploids is also possible with blueberry, and is more efficient than colchicine doubling in many situations.

Transformation is a powerful approach to introduce genes of interest and accelerate the breeding process for many fruit crops. Transformation is particularly suited to blueberry since it has a polyploidy genome and is asexually propagated. To date, regeneration has been reported for only a few commercial blueberry cultivars (Billings et al. 1988; Callow et al. 1989; Rowland and Ogden 1992, 1993; Hruskosi and Read 1993). Cao et al. (1998) studied several factors that influenced the efficiency of *Agrobacterium*-mediated transfer of an intron containing β-glucuronidase (GUS) gene into leaf cells of several commercially important blueberry cultivars and into callus derived from these cells during the early stages of transformation. There is one report of transformation but has not been confirmed by Southern analysis (Graham et al. 1996).

Hancock et al. (1990) conducted transformation studies with the highbush, sierra with *Agrobacterium tumefaciens*. They investigated the effect of concentration of *A. tumifaciens*, length of co-cultivation and antibiotic treatments on transformation. Rowland and Ogden (1993) initiated transformation studies with *A. tumifaciens* strain C58C1/pGA482. Graham et al. (1996) reported transformation of half high North country using disarmed *A. tumifaciens* strain LBA4404 containing a binary vector with an intron containing GUS marker gene (Vancanneyt et al. 1990). Cao et al. (1998) conducted an in-depth study on factors that influence the early stages of transformation. They used 10 highbush blueberry cultivars and disarmed *Agrobacterium* strain LBA4404. Recently, Song and Sink (2004) described an efficient shoot regen-

eration method and results of transient transformation studies that led to *A. tumefaciens*-mediated stable transformation of four selected highbush blueberry cultivars.

10.5 Future Scope of Works

The increasing use of biotechnology in blueberry research, in fields such as diverse as linkage mapping, gene cloning, functional genomics, tissue culture and genetic transformation has increased ability to manipulate species for the advantage in breeding programs. There is still much work needed to provide a better understanding of gene regulation and phenotypic expression, generation of high-density genetic maps, and transformation system.

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