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Crop Improvement in Blueberry Utilizing its Genetic Diversity: A Path to Sustainable Berry Farming

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Abstract: Blueberries (*Vaccinium* spp.) are deciduous shrubs renowned for their high antioxidant-rich properties, support cardiovascular health, lower blood pressure, strengthen bones, and help prevent chronic illnesses like diabetes, cancer, and neurological disorders. With around 450 species in the *Vaccinium* genus, exploring their genetic diversity is critical for crop improvement. Advanced genomics can unravel their evolutionary pathways, aiding informed breeding strategies. Key areas of improvement include developing low-chill varieties, enhancing rootstocks, and improving traits like texture, flavour, shelf life, and nutritional quality. Genetic approaches such as crossing, hybridization, and genomic selection can help introduce economically important traits. However, challenges like their superficial root system, which limits water and nutrient absorption and increases vulnerability to drought stress, must be addressed. Harnessing the genetic variability of *Vaccinium* species through innovative breeding efforts is essential to overcome production challenges and support the global blueberry industry's growth.

Keywords: Genetic Diversity • Genetic Improvement • Hybridisation • Plant Selection • *Vaccinium*

Introduction

Blueberries (*Vaccinium* spp., Fam: *Ericaceae*) are economically important berry fruits prized for their trade and export value. They rank among the richest sources of antioxidants, primarily anthocyanins, which confer numerous health benefits including enhanced bone strength, reduced blood pressure, improved cardiovascular health, and neuroprotection (Prior et al 1998; Basu et al 2010; Norberto et al 2013). Blueberries have also been linked to the prevention and treatment of chronic diseases such as cancer, diabetes, cardiovascular, and neurodegenerative disorders (Rimando et al 2004; Martin et al 2006). Their antioxidant capacity correlates with combined phenolic, flavonoid, anthocyanin, and pro-anthocyanidin contents (Lobo et al 2010). Additionally, rutin has been identified as a major bioactive compound in blueberry leaves (Stefanescu et al 2020). Blueberries are processed into diverse products including jams, jellies, sauces, baked goods, frozen fruit, wines, and cordials due to their excellent culinary qualities and flavorful juice (Hancock et al 2008). Rising global demand, driven by their nutritional value, has accelerated breeding efforts aimed at sustainable production. Key breeding goals include larger fruit size, improved flavour and texture, ease of mechanical harvesting, resistance to biotic and abiotic stresses, and extended shelf life (Gallardo et al 2018).

The genus *Vaccinium* comprises approximately 450 species worldwide (Fang et al 2005) with varying ploidy levels: diploids (2n=2x=24), tetraploids (2n=4x=48), and hexaploids (2n=6x=72). The primary commercial gene pool includes lowbush blueberry (*V. angustifolium*), highbush blueberry (*V. corymbosum*), and rabbit-eye blueberry (*V. virgatum* syn. *V. ashei*) (Retamale et al., 2018). Wild lowbush blueberries exhibit strong abiotic stress tolerance. Cultivated highbush blueberries are classified into Northern highbush (*V. corymbosum*, 2n=4x=48) and Southern highbush (*V. corymbosum* × *V. darrowii* hybrids, 2n=4x=48), with Southern highbush showing drought tolerance and superior fruit quality in size, texture, and flavor (Hancock et al 2008). Breeding programs leveraging crosses between Southern and Northern germplasm have developed advanced Southern cultivars with chilling requirements between 0 and 750 hours, suitable for warmer climates (Gallardo et al 2018). Rabbit-eye blueberries display greater drought and heat tolerance than lowbush and highbush types. The current review underscores the extensive genetic diversity within *Vaccinium*, which underpins innovative breeding strategies to develop superior cultivars with enhanced fruit traits and stress resilience. It also highlights challenges such as complex genetics and environmental adaptability, proposing hybridization and genomic-assisted breeding as key solutions. These advances support sustainable blueberry cultivation and bolster the future growth of the global blueberry industry.

Crop Improvement utilizing Genetic Diversity

Accelerating genetic improvement in blueberries relies on genomic tools to integrate climatic adaptation with desirable horticultural traits. Most breeding efforts began in the U.S. after blueberries were introduced in the early 20th century, focusing on developing cultivars adapted to diverse climates and soils, disease and pest resistance, mechanical tolerance, and high fruit quality (Galletta and Ballington 1996). Woody perennials like blueberries benefit from marker-assisted selection due to their long generation times, high heterozygosity, self-incompatibility, inbreeding depression, and polyploidy. A genomic study of 195 accessions from five *Vaccinium* species (*V. corymbosum, V. boreale, V. darrowii, V. myrsinites,* and *V. tenellum*) utilized SNPs from genotyping-by-sequencing (GBS) to explore evolutionary relationships and genetic diversity (Manzanero et al 2023).

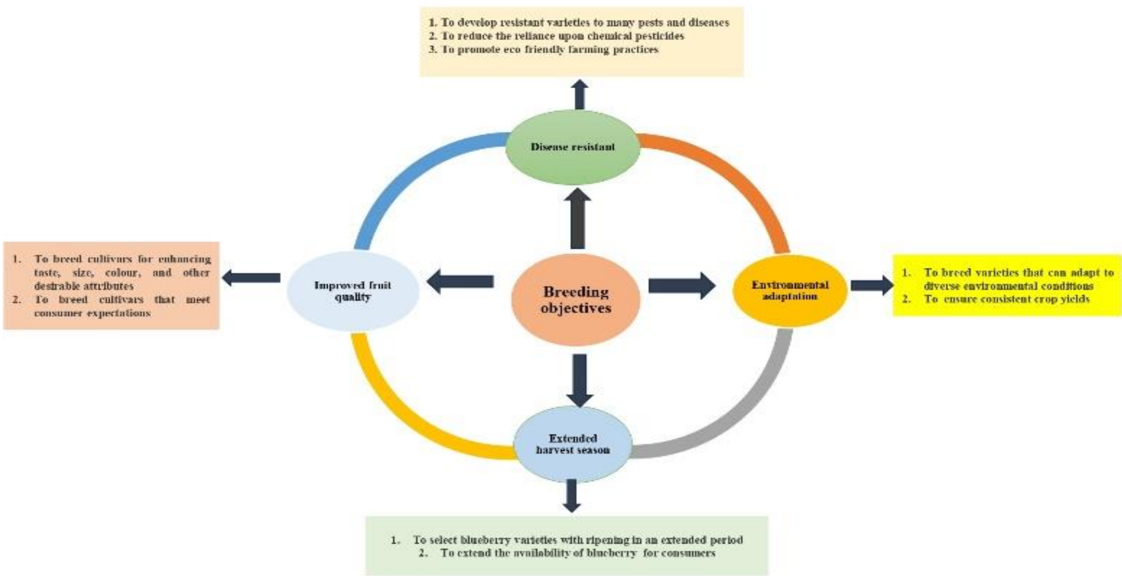

Fig 1. Breeding objectives of Blueberry

Four distinct species under the genus *Vaccinium* viz. *V. angustifolium* (Highbush), *V. ashei* (Rabbiteye), *V. corymbosum* (Southern Highbush) and *V. darrowi* have contributed most of the germplasm to the current blueberry cultivars (Ballington 1990). More recently, half-high blueberry genotypes (*V. corymbosum* x *V. angustifolium*) have been developed that are similar in growth and fruiting characteristics to highbush blueberry, yet exhibit greater cold hardiness (Wildung and Sargent 1989). An increasing trend of interest was observed in blueberry production in warm temperate, sub-tropical, and even tropical areas of the world. Current low chilling cultivars viz. Sharp blue, Gulf Coast, Wannabe, Misty, Becky blue, Bonita and Climax are grown in traditional production systems, are not generally amenable to production in areas with extremely low or no chilling temperatures availability during the dormant season (Luby *et al* 1991).

Achievements in breeding and crop Improvement

United States being the leader in blueberry breeding programs, the major goals were to improve the crop in these types viz. Northen highbush blueberry (NHB), Southern highbush blueberry (SHB), Rabbiteye (RY) cultivars (Table 1) and their interspecific hybrids (Hancock et al., 2008). Fruit breeding using marker assisted selection became more popular in the last decade due to its cost effectiveness with enhanced selection efficiency and reduced time period towards the development and release of new cultivars. Let's discuss in brief about the different breeding strategies used in blueberry crop improvement at global level.

Plant selections

The pivotal and foremost stage in blueberry breeding is the selection of cultivars. The inaugural highbush berry cultivar, 'Brooks,' was chosen by 'Coville' in New Hampshire, USA in the year 1908. Following this selection, the introduction of a novel lowbush blueberry cultivar named 'Russel' took place in the in the year 1909 (Mainland 2012). The 'Ochlocknee' cultivar, developed through planned crossing and hybridization work in 1963 at Tifton, USA, is distinguished by its excellent storage quality, high-quality berries, and suitability for mechanical harvesting (Ne Smith et al 2006). In 1981, the 'Snowflake' cultivar was meticulously selected through the hybridization of Florida-K (female) and NC1830 (male), noted for its large berry size and high wax surface (Lyrene 1993). In 1984, the low-chill blueberry cultivar 'Windsor' was developed through a planned hybridization program, resulting from a cross between FL-83132 (female) and Sharp Blue (male). This cultivar is recognized for its larger berries, deep blue colour, and resistance to stem blight and Phytophthora root rot (Lyrene 2002). The Northern highbush blueberry cultivar 'Hort Blue Poppins' was selected in 1988, known for its early season harvest, cold hardiness, good texture, flavor, along with its desirable architecture (Patel 2011). Another novel cultivar, 'Vernon,' was selected in 1990 at the Coastal Plain Experiment Station in Tifton, characterized by its high yield and large berry size (Ne Smith et al 2007).

In 1992, the University of Georgia selected the variety 'Alapaha,' which demonstrated good productivity, cold hardiness, and excellent storage quality (Ne Smith et al 2006). The same year, the University of Florida introduced the cultivar 'FLX-2,' notable for its self-fruitfulness and resistance to mites and insects. The cultivar 'Pearl River' was selected in 1995, notable for its dark blue berries with excellent texture (Ehlenfeldt et al 2006). Another cv., 'Snow Chaser,' was selected in the same year, characterized by a very low chilling requirement and exhibits resistance to various diseases, insects, and pests. In 1998, cv. 'Sweet Crisp,' was crossbred with distinct low chilling requirement of 200-300 hours, producing sweet, firm berries and demonstrated a vigorous growth habit (Lyrene

2009). The novel cultivar 'Talisman' was selected in 2000 from a cross between Magnolia (female) and Elizabeth (male) with a mid-late to late-season bearing, characterized by its mild flavour, higher yield, and large-sized berries (Ehlenfeldt 2021). Another cultivar, 'Dris Blue Fourteen,' was selected in 2004, distinguished by its higher productivity, large firm berries, and low acidity (Caster et al 2017). A novel cultivar, 'Scintilla,' was cross bed from lines FL 96-43 (F) x Fl 96-26 (M) in the University of Florida and features powdery blue-coloured berries in loose clusters, with excellent texture and firmness. It also exhibited early ripening in spring and highly resistant to stem canker disease (Lyrene 2008). The U.S. Department of Agriculture selected a Rabbit eye blueberry cultivar ‘T-959’ during the year 2005, developed from the cross T-460 (female) × FL 80-11 (male) with chilling requirement between 500 to 550 hours. This blueberry cultivar is characterized by its large berries and vigorous plant growth (Ne Smith 2014).

Table 1. Details of different types of blueberry cultivars

Type	**Cultivar**	**Parent**	**Characters**	**Origin**	**Inventor**	**References**
Northern Highbush Blueberry (NHB)	Dris Blue Fourteen	Ms122(F) ×G455(M)	Large Size, Firm, Low Acidity	Skagit County, August 2004	Brian K. Caster Et Al	Caster et al., 2017
Northern Highbush Blueberry (NHB)	Hort blue Poppins	Nui unnamed Seedling selection called 1386	Small fruit, medium to light	Ruakura, Hamilton, New Zealand, in 1988	Narandra Patel	Patel, 2011
Northern Highbush Blueberry (NHB)	Talisman	Magnolia(F)× Elizabeth (M)	Late-midseason ripening, uniform size, light-blue berries	Usda-Ars, 2000	Mark K. Ehlenfeldt	Ehlenfeldt, 2020
Northern Highbush Blueberry (NHB)	Windsor	FL83132(F)×Sharp blue(M)	Very large berry, dark-blue colour, resistance to cane canker	Gaines ville, 1984	Paul M. Lyrene	Lyrene, 2002
Southern Highbush Blueberry (SHB)	Sweet crisp	Southern Belle (F)x FL 95-3 (M)	low chilling, extremely firm	University of Florida, Spring of 1998	Paul M. Lyrene	Lyrene, 2009
Southern Highbush Blueberry (SHB)	Snow chaser	FL 95-57 (F) x FL 89-119 (M)	Very low chilling, firm berries, light-blue colour	University of Florida, March, 1995	Paul M. Lyrene	Lyrene, 2008
Southern Highbush Blueberry (SHB)	Scintilla	FL 96-43 (F)x FL 96-26 (M)	Very low chilling, Early ripening, large size berries, powdery blue	University of Florida, Gainesville, United States 1997	Paul M. Lyrene	Lyrene, 2008
Southern Highbush Blueberry (SHB)	Flx-2	Fl 92-9 (F) x Sunshine blue (M)	Very low chilling, medium sized berries, light-blue	University of Florida, March, 1992	Paul M. Lyrene	Lyrene, 2008
Rabbit Eye Blueberry	Snowflake	Florida-K (F)×NC1830 (M)	Late flowering with early ripening, high resistance to cane canker, moderate resistance to Phytophthora root rot and stem blight.	FAES in Gainesville in 1981	Paul M. Lyrene	Lyrene, 1993
Rabbit Eye Blueberry	T-959	T-460 (F)× FL 80-11(M)	Excellent fruit firmness, very large berry.	U.S. Department of Agriculture in 2002	Dr. Scott NeSmith	NeSmith, 2014
Rabbit Eye Blueberry	Alapaha	T-65 (F) x ‘Brightwell (M)	Late bloom-early ripening, small berry scar, moderate twig die-back, good tolerance to Stem canker.	University of Georgia in 1992	NeSmith 2014	NeSmith et al., 2006

Rabbit Eye Blueberry	Ochlockonee	Tifblue (F) x Menditoo(M)	Large berry size, Late ripening, Good tolerance to stem canker, Tolerant of various defoliating leaf diseases	Coastal Plain Experiment Station in Tifton 1963	D. Scott NeSmith Arlen D. Draper	NeSmith et al., 2006
Rabbit Eye Blueberry	Vernon	T-23(F)×T-260 (M).	Late bloom/early ripening, High and consistent yield Large Berry size.	Coastal Plain Experiment Station in Tifton 1990.	D. Scott NeSmith Arlen D. Draper	NeSmith et al., 2005
Rabbit Eye Blueberry	Pearl river	G 136 (SHB) (F) × Beckyblue (M)	Medium sized, Firm, dark blue berries	USDA–ARS, Poplarville, Miss,1995	James M. Siers	Ehlenfeldt et al., 2006
Rabbit Eye Blueberry	Snowflake	Florida- K (F)×NC1830 (M)	Late flowering with early ripening, high resistance to cane canker, moderate resistance to Phytophthora root rot and stem blight.	FAES in Gainesville in 1981	Paul M. Lyrene	Lyrene, 1993

Hybridization

Over a century of blueberry breeding has successfully integrated distinctive traits from wild germplasm into the primary gene pool through extensive homoploid and heteroploid interspecific hybridizations (Brevis et al 2008). Crossbreeding efforts initiated in the early 20th century involved combinations such as *V. stamineum* × *V. myrtilloides*, *V. melanocarpum* × *V. myrtilloides*, and *V. corymbosum* × *V. australe*. Notably, *V. corymbosum* is prized for its cold hardiness, superior fruit quality, and early ripening. Additionally, cultivars with reduced chilling requirements, adaptability to less acidic soils, and light blue fruit color have been developed using *V. darrowii* and *V. elliottii* (Wenslaff and Lyrene 2000). Northern high bush (NHB) cultivars are more tolerant to mid-winter temperature if compared to Southern high bush (SHB) (Table 1) cultivars even though there is a great variation both within and across Vaccinium species (Hanson et al 2007). Cold hardiness in blueberry cultivars such as cv. Blue-crop, Tifblue, and GulfCoast is closely associated with floral bud dehydrin concentration, making it a predictive marker in breeding programs (Dhanaraj et al 2005). The development of Southern highbush blueberry (SHB) cultivars at the University of Florida in 1948 marked a key advancement, incorporating low-chill (600 h below 7 °C) tolerance through interspecific hybrids with wild diploids (Ballington 2006). Crosses between *V. arboreum* and *V. darrowii* have produced robust hybrids, serving as a genetic bridge to tetraploid SHB types (Olmstead et al 2013). Late-blooming cultivars also show reduced frost damage compared to early bloomers, as frost risk correlates with floral bud development (Lin and Pliszka 2003). Despite the successful incorporation of wild diploids, outdated taxonomy and incomplete pedigrees hinder genetic traceability. Although wide hybridization enhances heterozygosity, its impact on DNA polymorphism remains limited (Lyrene 2002). This study aims to evaluate the genetic contribution of various *Vaccinium* species to cultivated highbush blueberries using microsatellite markers.

Genomic selection

Genomic research in blueberries, though nascent, has made notable advances recently. Approximately 5,000 expressed sequence tags (ESTs) from non-acclimated and cold-acclimated flower bud libraries have been generated and made publicly available, providing key genetic insights (Dhanaraj et al

2007). Plant & Food Research Ltd., New Zealand, is expanding EST collections from diverse tissues (fruit, flower buds, leaves, stems) to develop EST-, PCR-, and SSR-based markers for mapping quantitative trait loci (QTL) related to chilling requirement, cold hardiness, and fruit quality in diploid and tetraploid populations (Lisa et al 2012). Concurrently, the L.J. Rowland lab at Maryland established a highbush blueberry EST library, facilitating the identification of cold hardiness genes, including dehydrin family members, through molecular and genomic approaches (Muthalif and Rowland 1994; Dhanaraj et al 2005). Gene expression under cold stress has been further analyzed via DNA microarrays (Dhanaraj et al 2006).

Conclusion

Blueberries are vital to global agriculture and health due to their nutritional and economic value. The genetic diversity within the *Vaccinium* genus offers considerable potential for breeding improved cultivars with enhanced fruit quality, disease resistance, and drought tolerance. Integrating advanced genomic tools with traditional breeding and sustainable cultivation enables targeted introgression of valuable traits from wild *Vaccinium* species through strategic crossing and hybridization. Future research and breeding efforts are essential to develop resilient, high-quality cultivars that meet rising global demand for nutritious blueberries.

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