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Review

Harvesting the Antioxidant Potential of Berry Crops Through Genomic Advances and Modern Breeding Tools

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Abstract

The evolving needs of society, shaped by lifestyle changes and growing health awareness, have intensified interest in sustainable foods with enhanced nutritional benefits. Berry crops are central to this trend, serving as rich dietary sources of bioactive compounds that support human health. Renowned for their high levels of diverse antioxidants, they offer benefits that extend well beyond basic nutrition. This review explores the diversity and global cultivation of major berry crops, including blueberries, raspberries, cranberries, blackberries, and grapes, with emphasis on their nutritional value and antioxidant profiles. It also examines their domestication history, wild relatives, and commercial cultivars, while offering insight into the genetic and phenotypic diversity underpinning their rich chemical composition. Furthermore, the review highlights the use of modern genomic and breeding tools to enhance bioactive compound content. Advances in cultivation and molecular breeding technologies, including transcriptomics, genome-wide association studies (GWAS), and genome editing, offer promising opportunities to maximize the antioxidant potential of these high-value crops, particularly in the face of a changing climate.

Keywords: berry crops; grapevine; antioxidants; nutritional value; breeding; genomic tools

Key Contribution: This review examines the genetic and phytochemical diversity, domestication history, and worldwide cultivation of grapes and other key berry crops, including their wild relatives and commercial cultivars, while emphasizing on their rich antioxidants and nutritional profiles. It also summarizes how modern molecular breeding and genomic tools are being applied to enhance bioactive compound content, to fully exploit the health-promoting potential of these high-value fruits.

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1. Introduction

1.1. A multitude of Berry Crops

Berry crops, grapes among them, include a diverse group of fruits that differ botanically yet share similar cultivation practices, postharvest behavior, and health-promoting properties [1](Kumar et al., 2018). A true berry is a simple fruit that develops from a single ovary and contains multiple seeds within a fleshy pericarp, such as blueberries (*Vaccinium spp.*), cranberries (*Vaccinium macrocarpon*), and grapes (*Vitis vinifera*) [2]. In contrast, fruits such as raspberries (*Rubus idaeus*) and

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blackberries (*Rubus fruticosus*), though commonly referred to as berries, are in fact aggregate fruits, consisting of numerous small drupelets that originate from a single flower with multiple ovaries [3]. Despite this structural difference, all these fruits are commonly grouped under “berry crops” due to their overlapping agricultural, nutritional, and commercial significance [4].

Among major cultivated berries, raspberries (*Rubus idaeus*), native to Europe and northern Asia, yield about 940,000 tons globally, with Russia, Mexico, Serbia, Poland, and the United States as leading producers. Blueberries (*Vaccinium* spp.), native to North America, total about 1.1 million tons, dominated by the United States, Peru, Canada, Chile, and Spain. Cranberries (*Vaccinium macrocarpon*), also of North American origin, produce around 470,000 tons, largely from the United States, Canada, and Chile. In contrast, grapes (*Vitis vinifera*), native to the Near East, far exceed berry crops with over 72.5 million tons, produced mainly in China, Italy, Spain, the United States, and France [5] (Table 1).

Berry cultivation reflects a combination of historical domestication patterns, ecological adaptations, and modern agricultural practices. Raspberries, blackberries, blueberries, and cranberries are primarily associated with temperate or specialized agro-ecological zones, whereas grapes represent one of the most extensively cultivated fruit crops worldwide. A comparative overview of their production statistics, major producing countries, and key soil–climate requirements is presented in the following table [5].

Table 1. Origins, major producer countries, and growing requirements of key berry crops and grapes (FAOSTAT 2023).

Crop	Scientific name	Native origin	Global production	Main producers (tn)	Soil & climate requirements
Raspberry	*Rubus idaeus*	Europe, Northern Asia	~940,000 tn	Russia (219k), Mexico (165k), Serbia (122k), Poland (118k), USA (100k)	Fertile, well-drained soils; cool winters for dormancy and flowering
Blueberry	*Vaccinium* spp.	North America	~1.1 million tn	USA (300k), Peru (230k), Canada (165k), Chile (125k), Spain (70k)	Acidic soils; consistent moisture; frost protection
Cranberry	*Vaccinium macrocarpon*	Northeastern North America	~470,000 tn	USA (300k), Canada (151k), Turkey (12k)	Acidic, water-saturated soils; benefit from regular field renewal
Grape	*Vitis vinifera*	Near East	>72.5 million tn	China (12.5m), Italy (8.1m), Spain 5.9m), USA (5.4m), France (6.2m)	Wide soil tolerance; good drainage; warm, dry summers

1.2. Nutritional and Antioxidant Value of Berries as Pharmaceutical and Nutraceutical Sources

For centuries, fruits such as berries have been valued in traditional medicine and human diets for their health-promoting properties. Despite limited scientific understanding, their historical use was based on empirical knowledge; however, advances in phytochemistry, particularly through metabolomics, have revealed the complex mixture of bioactive compounds responsible for their beneficial effects on human health [6].

Berries are an abundant source of antioxidants, particularly flavonoids. Among these, flavonols such as quercetin and kaempferol present in raspberries, cranberries, and grapes have been reported

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to significantly contribute to their antioxidant capacity and associated health benefits [7]. Quercetin, present in raspberries and cranberries, demonstrates significant anti-inflammatory, antiviral, and antihistamine activities and has been studied as an supportive therapy for arthritis, allergies, and viral infections [8–10]. Anthocyanins, like cyanidin-3-glucoside (C3G), malvidin-3-glucoside (M3G), peonidin-3-glucoside (P3G), and delphinidin-3-glucoside (D3G) are highly concentrated in blueberries, blackberries, and red or purple grapes, where they are primarily responsible for the characteristic pigmentation [8]. Beyond their coloring function, anthocyanins have been extensively studied for neuroprotective and cardiovascular effects, including potential roles in mitigating neurodegenerative diseases such as Alzheimer's and Parkinson's, improving endothelial function, and reducing blood pressure [11,12]. In addition, grapes and cranberries are rich sources of flavan-3-ols, particularly catechin and epicatechin, which are concentrated in the seeds [13]. The tannin group is also well represented in berry crops, with hydrolysable tannins such as ellagic acid detected in abundance [14,15]. These compounds have been associated with cardiovascular protection, antimicrobial and antiviral activities, anti-inflammatory effects, and potential roles in metabolic health and cancer prevention [16]. In addition, condensed tannins, particularly procyanidins, are predominantly found in grape seeds, cranberries, and blackberries, further contributing to the bioactive profile of these fruits [17]. Stilbenoids such as resveratrol and pterostilbene are primarily concentrated in red and purple grapes. Resveratrol and related compounds are multifunctional polyphenols with potent antioxidant and anti-inflammatory properties, acting through the reduction of oxidative stress and modulation of inflammatory pathways, and they have been widely associated with cardioprotective effects [18]. Proanthocyanidins, particularly A-type forms, which are abundant in cranberries, are known for inhibiting bacterial adhesion in urinary tract infections and increasingly investigated for their roles in gut microbiome modulation and vascular health. [19]

Phenolic acids such as chlorogenic, caffeic, and gallic acids are widely distributed in blueberries, grapes, and cranberries [20]. These compounds have been reported to modulate glucose metabolism, enhance insulin sensitivity, and display antiviral activity, thereby playing an important role in the management of metabolic disorders such as diabetes and obesity [21]. Minor bioactive compounds in berries include vitamins C and E, carotenoids such as β-carotene and lutein, and terpenes including limonene, myrcene, linalool, and pinene. These compounds contribute to antioxidant capacity and characteristic aromas, and have been shown to enhance cellular antioxidant defenses, neutralize free radicals, and suppress inflammatory gene expression, thereby protecting against oxidative stress, inflammation, and cellular damage [22]. All the above-mentioned compounds form a robust biochemical platform that is increasingly leveraged in therapeutic and preventive healthcare applications.

Furthermore, micronutrients, such as calcium, magnesium, and iron present in varying amounts across berries play crucial roles in antioxidant defense mechanisms and metabolic homeostasis [23]. Calcium and magnesium contribute to proper enzymatic function and cellular signaling relevant to oxidative stress modulation [24,25] while iron, although required in small amounts, is vital for oxygen transport and redox reactions, and interacts with polyphenols and vitamin C absorption [26]. This synergy between these antioxidants, vitamins, and essential nutrients makes berries a highly nutritious choice for anyone looking to boost their health, reduce inflammation, and protect against chronic diseases [27,28].

Based on the recognized nutritional and pharmaceutical value of the antioxidants of berries, current research is directed toward tools that can increase their accumulation in the fruits. In this context, agronomical techniques, such as priming and grafting, can stimulate antioxidant pathways by improving plant resilience under stress conditions. At the same time, molecular breeding approaches, including marker-assisted selection, QTL mapping, and genome editing, offer targeted opportunities to enhance antioxidant biosynthesis. The integration of these strategies provides a comprehensive framework that connects crop improvement with the development of berry fruits enriched in antioxidants and enhanced health benefits (Figure 1).

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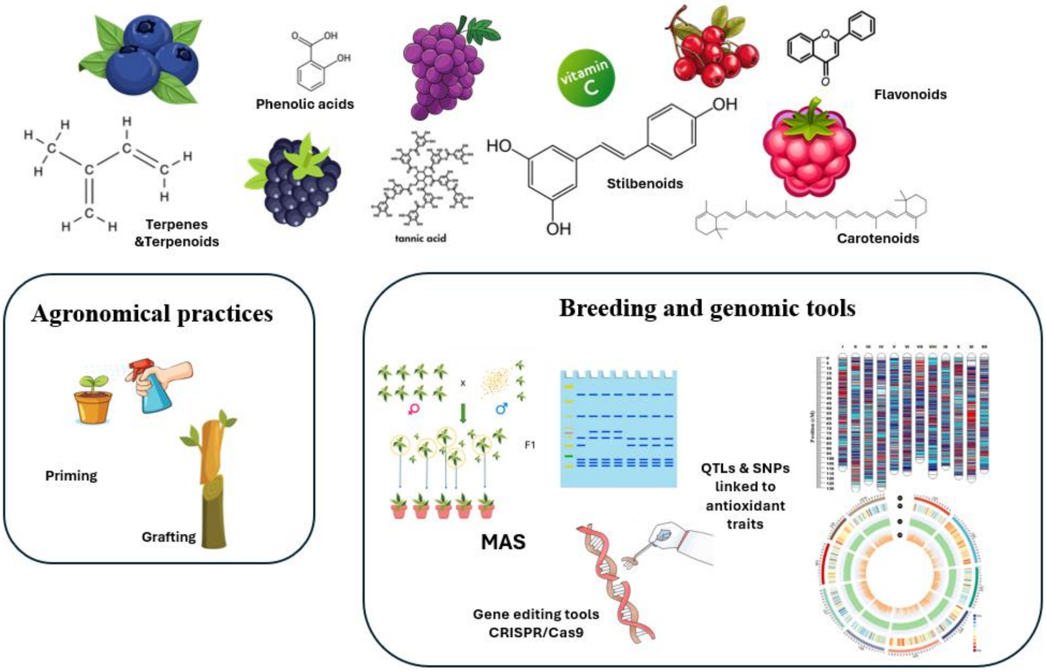

Figure 1. Exploitation of berry crops antioxidant capacity through agronomical practices and molecular breeding methods utilization.

2. Agronomical Practices for Increasing Antioxidant Concentration

2.1. Priming

Environmental changes have intensified abiotic stress in plants, reducing yield and threatening food security [29]. One adaptive mechanism that improves plant resilience is priming, a process that prepares plants to respond more efficiently to future stresses [30].

Priming, also referred to as sensitization or hardening, induces a "primed state" (PS) that enhances the plant's ability to activate defense mechanisms when challenged again [31] This state is triggered by mild pre-exposure to stress or by the application of natural or synthetic agents. Biochemical mechanisms underlying PS include the accumulation of inactive signaling proteins or transcription factors, activation of mitogen-activated protein kinases (MAPKs), changes in redox status, epigenetic modifications, and breakdown of certain cellular compounds [32]. Once primed, plants exhibit enhanced redox balance and faster stress responses, improving resilience and growth [33].

Notably, priming strategies have been shown to enhance the antioxidant capacity of berry crops, thereby improving both stress resilience and nutritional quality. For instance, in blueberries, foliar application of polyamines such as putrescine and spermidine significantly increased antioxidant potential, with the fruit development stage identified as the most favorable period for treatment. Such findings provide valuable guidance for growers to optimize fertilization practices during critical phases of berry growth and maturation [34]. Likewise, in blackberries, priming with *Bacillus amyloliquefaciens* has been used to investigate the role of the flavonol–anthocyanin pathway in the adaptation of plants to biotic stress. This treatment modulated blackberry metabolism and identified key target genes, providing insights for improving plant adaptability and fruit quality [35]. Additionally, a biostimulant derived from *Ascophyllum nodosum,* applied as a foliar spray to raspberry, was found to enhance yield by over 30% while maintaining most nutritional traits. Notably, magnesium content increased in primed fruits, highlighting its potential as a sustainable tool to improve berry yield and quality [36]. Similarly, pre-harvest foliar treatments with ethephon (Eth) at the 'veraison stage' resulted in increased levels of total phenolics and anthocyanin and enhanced antioxidant capacity accompanied by an increased expression of the anthocyanin

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biosynthesis genes, su*ch as chalcone synthase* (*CHS*), *flavanone 3-hydroxylase* (*F3H*), and *flavanol 3-o-glucosyl transferase* (*UFGT*) in ‘Crimson Seedless’ grape berry skins [37]. Moreover, postharvest treatment with melatonin reduced grape berry abscission and decay, enhanced amino acid accumulation, and activated endogenous melatonin and phenolic biosynthesis pathways, highlighting the potential of improving postharvest quality through the modulation of secondary metabolism [38].

2.2. Grafting

Grafting, a technique that is widely used in perennial crops, has been adopted more in berry species such as raspberry and blueberry with the aim of improving plant’s stress tolerance and fruit quality [39]. By combining the root system of a robust rootstock with the scion of a high-yielding or high-quality cultivar, growers can modify physiological traits in a manner that is not possible through conventional breeding. Even though grafting is more commonly linked to disease resistance and soil adaptability, recent studies indicate it may also affect the antioxidant capacity of berry fruits. Changes in nutrient uptake, water-use efficiency, and hormonal signaling can result from physiological interactions between the rootstock and scion. These changes are closely related to the fruit’s generation of secondary metabolites [40].

The accumulation of phenolic compounds, anthocyanins, and flavonoids can be indirectly enhanced by specific rootstocks, according to evidence from grafted raspberry and blueberry systems [39]. Increased photosynthetic activity, a more stable water balance during drought, and improved root-to-shoot nutrient transport, particularly minerals like magnesium and zinc that are essential in antioxidant enzyme function, are frequently linked to these gains [41].

Specifically for grapes, grafting has notably improved antioxidant capacity by adjusting the scion’s physiological and biochemical reactions through rootstock selection. According to the study of Krishankumar et al. (2025), the use of drought-tolerant rootstocks, like Ramsey, R110 and 1103 Paulsen, positively resulted to a significant increase of important antioxidant enzyme activities, including glutathione reductase (GR), ascorbate peroxidase (APX), peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD), under drought stress [42]. Furthermore, Klimek et al. (2024) showed that different rootstocks significantly affected the content of bioactive compounds in Regent grapes [43]. The DPPH radical scavenging measurement in grafted grapes revealed variations in L-ascorbic acid, phenolic acids, total flavonoids, anthocyanins, and tannins, all of which are directly related to antioxidant activity. Together, these findings prove that choosing the right rootstocks greatly increases the antioxidant profile and nutritional value of grapes in addition to increasing their resistance to abiotic stress.

3. Berry Genomics and Breeding for Enhancing Antioxidant Capacity

According to the literature, breeding programs targeting improved tolerance of berries to abiotic stresses such as extreme temperatures, drought, salinity, and iron deficiency have also contributed significantly to enhancing their antioxidant capacity [44]. Building on these health-promoting characteristics, improving the antioxidant content is becoming a central breeding target, guiding current efforts to combine nutritional quality with agronomic performance [45,46]. To achieve these goals, modern breeding strategies integrate advanced genomics and molecular tools [47]. Until now, conventional breeding has been carried out mainly through time-consuming, controlled crossings between selected parents, followed by long-term field evaluations of berry seedlings for traits such as yield, vigor, disease resistance, and fruit quality. Then promising genotypes are clonally propagated to establish new cultivars [47,48].

At the genomic level, high-quality reference genomes have been assembled for several berry crops, facilitating the discovery of gene families involved in secondary metabolite biosynthesis [49,50]. Multi-omic studies have elucidated metabolic pathways and the expression dynamics of regulating genes in response to both developmental and environmental stimuli. Integrated transcriptomic and metabolic profiling reveals that phenolic acid and flavonol biosynthesis in *Rubus*

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chingii Hu (Chinese raspberry) begins at the onset of fruit development. Afterwards, these compounds are systematically accumulated or metabolized into related derivatives [51]. The exploitation of pathways such as those for the phenylpropanoid or the flavonoid biosynthesis are essential to antioxidant production and are tightly coupled with responses to abiotic stresses in berry crops like blackberry [35].

Moreover, Genome Wide Association Studies (GWAS) have improved trait mapping in berries through investigations of the heritability of quality traits and their associations with known QTLs [52]. At the same time, berry breeding increasingly integrates conventional methods with modern molecular approaches, and CRISPR/Cas9-mediated gene editing has already been validated in blueberry, paving the way for precise trait improvement, including antioxidant enhancement without yield penalties [53]. However, GWAS and other genomic tools like CRISPR/Cas9, remain largely unexplored for the exploration and utilization of antioxidant capacity in raspberry breeding.

3.1. Blueberry (Vaccinium corymbosum)

3.1.1. Domestication and Wild Relatives

Blueberry is a widely cultivated native fruit in North America [54]. Domestication began in the early 20th century through extensive interspecific hybridization programs [55]. The genus *Vaccinium* evolved in eastern North America, with biogeographic origins centered in the Appalachian Mountains and Atlantic coastal plains, where natural populations exhibit maximum genetic diversity [56]. Evolutionary relationships within section *Cyanococcus* reveal three main lineages: (1) northern species (*V. corymbosum, V. angustifolium*), (2) southern species (*V. darrowii, V. tenellum, V. virgatum*), and (3) western species (*V. ovalifolium, V. deliciosum*) [57]. Polyploidy evolution is prominent, with diploid ($2n = 2x = 24$), tetraploid ($2n = 4x = 48$), and hexaploid ($2n = 6x = 72$) cytotypes, where allopolyploidy has driven speciation and adaptive radiation across diverse ecological niches [58,59]. Cultivated highbush blueberry (*V. corymbosum*) is tetraploid ($2n = 4x = 48$), with a monoploid genome size of ~2.53 pg [58,60]. Other cultivated groups include lowbush (*V. angustifolium*, diploid) and rabbiteye (*V. virgatum*, hexaploid), with extensive interspecific hybridization among them [61].

Wild blueberry species harbor broad genetic diversity with desirable traits that can be exploited in breeding programs. While most studies on their genetic variability do not directly assess antioxidant capacity, they often provide indirect insights, such as the presence and stress-induced accumulation of metabolites and flavonoids, that are increasingly targeted to enhance the antioxidant potential of cultivated blueberries [62]. More specifically, wild *Vaccinium* species have been recognized as tolerant genetic material with high nutritional value against various abiotic and biotic stressors, such as low chilling requirements in *V. darrowii* [63], and heat and drought tolerance in *V. myrsinites* and *V. darrowii* [64]. Traits like fruit firmness, late ripening, and disease resistance are well-documented in *V. virgatum* [55]. Moreover, diploid southern species such as *V. tenellum, V. elliottii*, and *V. myrsinites* contribute specialized metabolites and stress tolerance [64]. Furthermore, *V. angustifolium* (Lowbush, $2n=24$), a wild-managed species native to northeastern North America, has been recognized as a source of cold hardiness and unique flavonoids [65]. High-quality genome assemblies are now available for *V. darrowii* (~1.06 Gb, >34,000 protein-coding genes) and *V. corymbosum* (‘W8520’ assembly, ~393 Mb), providing essential resources for elucidating the genetic basis of antioxidant capacity in berries and for uncovering the molecular mechanisms underlying its biosynthesis [63,66].

Blueberry commercial cultivation focuses on three main groups with distinct antioxidant profiles: a) Northern Highbush (*V. corymbosum*) cultivars dominate global production and include early-season genotypes such as ‘Duke’ and ‘Rubel’ known for high anthocyanin content and excellent antioxidant capacity [67], and mid-season standards like ‘Bluecrop’and ‘Frienship’, recognized for a balanced anthocyanin profile and high phenolic diversity [68]. Late-season cultivars such as ‘Elliott’, are distinguished by elevated proanthocyanidin content and extended shelf-life due to antioxidant stability, high total phenolic content and superior ORAC values [69,70]. According to Stevenson &

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Scalzo (2012), several Northern Highbush cultivars such as ‘Rubel’, ‘Duke’, and ‘Elliott’ consistently exhibit elevated anthocyanin and total phenolic content, reinforcing their value as benchmark genotypes for antioxidant potential. b) Southern Highbush (*V. corymbosum* × *V. darrowii*) hybrids include ‘Jewel’, a mid-season genotype with elevated flavonol content and enhanced UV protection compounds [55], ‘Legacy’ a mid- to late genotype with high total anthocyanins and phenolics and ‘Emerald’, which features a low-chill requirement and unique anthocyanin glycosylation patterns introgressed from *V. darrowii* [69]. c) Rabbiteye (*V. virgatum*) includes early and late-season harvests cultivars, like ‘Tifblue’ which contribute further diversity, serving as industry standards due to its high tannin content and antioxidant stability [55,70,71]. Late-ripening cultivars such as ‘Velluto Blue’ and ‘Centra Blue’ exhibit the highest fruit yields, anthocyanin contents, and estimated total anthocyanin harvestable from a given area [69]. Additionally, Rabbiteye cultivars, such as ‘Ono’, ‘Centurion’, and ‘Southland’, have been highlighted for their superior antioxidant richness, often surpassing both highbush groups in anthocyanin and phenolic content [72]. All the above-mentioned cultivars represent promising candidates for breeding programs focused on phytochemical enhancement regarding antioxidant properties.

3.1.2. Modern Genomic and Breeding Tools for Enhancing the Antioxidant Profile of Blueberries

Several genes and gene families have been studied in relation to the antioxidant capacity of blueberries, and their roles have been linked to genotypes with desirable traits and high nutritional value. For instance, the *MATE* transporter gene family, which encodes transport proteins that facilitate the movement of anthocyanins across cell membranes during fruit ripening, has been characterized as *V. corymbosum* [73]. Additionally, genes involved in the flavonoid biosynthetic pathway include those encoding CHS, chalcone isomerase (CHI), F3H, flavonoid 3′-hydroxylase (F3′H), flavonoid 3′,5′-hydroxylase (F3′5′H), dihydroflavonol 4-reductase (DFR), anthocyanidin synthase (ANS), (UFGT), and leucoanthocyanidin reductase (LAR), are reported to be upregulated during fruit ripening and correlate with anthocyanin accumulation [74,75]. Notably, *VcGSTF* genes show high expression during ripening and are implicated in anthocyanin stabilization and vacuolar transport [74]. In research by Liu et al. (2021), a genome wide analysis of the *Caffeic Acid O-Methyltransferase (COMT)* gene family was conducted in *V. corymbosum*, identifying 92 *COMT* genes involved in phenylpropanoid metabolism [75]. Moreover, several gene families encoding enzymes involved in the phenylpropanoid pathway, such as phenylalanine ammonia lyase (PAL), anthocyanidin reductase (ANR), cinnamate-4-hydroxylase (C4H), 4-coumarate:CoA ligase (4CL), flavonol synthase (FLS), hydroxycinnamoyl-CoA:shikimate hydroxycinnamoyl transferase (HCT), have been identified in blueberry under stress conditions and are associated with antioxidant responses [76,77]. Terpene synthases (TPSs) have also been characterized in blueberries, contributing to volatile profiles and secondary metabolite diversity relevant to antioxidant potential [78]. Table 2 summarizes the genes and their corresponding enzyme classes investigated in blueberries with respect to antioxidant capacity. Despite the availability of high-quality genome assemblies, the precise roles, annotations, and functional characterizations of numerous genes in blueberry remain unresolved and continue to be active areas of research.

Table 2. Genes analyzed for their functional involvement in the antioxidant capacity of blueberries.

Enzyme Class	Gene	Functional Role	Reference
Multidrug and Toxic Compound Extrusion Transporters (MATE)	*VcMATE2* *VcMATE3* *VcMATE5* *VcMATE7* *VcMATE8* *VcMATE9*	Facilitate anthocyanin movement across cellular membranes during ripening	Chen et al., 2015 [73]
Glutathione S-transferases (GSTs)	*VcGSTF8* *VcGSTF20*		Zhang et al., 2024 [74]

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	VcGSTF22	Highly expressed during fruit ripening; strong correlation with anthocyanin accumulation	
O-methyltransferases (COMTs)	*VcCOMT40* *VcCOMT92*	Highly expressed during fruit development; involved in lignin biosynthesis and anthocyanin modification	Liu et al., 2021 [75]
Flavonoid Biosynthesis Enzymes	*VcCHS*	Initiates flavonoid biosynthesis; upregulated during ripening	Chu et al., 2024; Zhang et al., 2025 [79,80]
	VcCHI	Converts chalcone to naringenin; active in ripening fruit	
	VcF3H	Hydroxylates flavonoids contribute to anthocyanin diversity	
	VcF3′H	Adds hydroxyl group; modifies anthocyanin structure	
	VcF3′5′H	Adds hydroxyl groups for delphinidin-type anthocyanins	
	VcDFR	Converts dihydroflavonols to leucoanthocyanidins	
	VcANS	Synthesizes anthocyanidins; active during ripening	
	VcUFGT	Glycosylates anthocyanins for stability and vacuolar transport	
Flavonol Synthase (FLS)	*VcFLS homologs*	Produces flavonols like quercetin and kaempferol in fruit skin	
Leucoanthocyanidin Reductase (LAR)	*VcLAR*	Synthesizes catechin; contributes to proanthocyanidin biosynthesis	Günther et al., 2020 [77]
Anthocyanidin Reductase (ANR)	*VcANR1*	Produces epicatechin; active in fruit tissues	

Advanced omic approaches such as transcriptome sequencing (RNA-seq) offer powerful insights into antioxidant-related genes, their expression dynamics, and their contribution to key metabolic traits in berry crops. RNA-seq analysis of 196 F1 genotypes, developed from the crosses of the cultivars ‘Draper-selection-44392” and ‘Jewel’, differing in anthocyanin glycosylation and acylation identified several differentially expressed genes (DEGs). For the major-effect QTL on chromosome 4 controlling glycosylation, three strong candidate genes were detected, including the UDP-glucosyltransferase *(Vcev1_p0.Chr04.11988)* and two endotransglycosylation *(Vcev1_p0.Chr04.11996 and Vcev1_p0.Chr04.11998)*. Among them, *Vcev1_p0.Chr04.11988* was significantly up-regulated in high-glycosylated genotypes and validated by qRT-PCR, supporting its role as a key regulator of anthocyanin glycosylation. For acylation, a major locus on chromosome 2 contained two acyltransferase genes (*Vcev1_p0.Chr02.03371* and *Vcev1_p0.Chr02.03383*), both up-regulated in high-acylated genotypes [81].

The chromosome-scale, haplotype-resolved assembly of tetraploid highbush blueberry (Draper’) provided a framework for annotating candidate genes, including UGTs, acyltransferases, and HCT, many colocalizing with antioxidant QTLs [66]. Multi-year QTL mapping in a V. corymbosum var. caesariense × V. darrowii population identified a major chlorogenic acid locus on Vc02 overlapping HCT/UGCT, and 67 QTLs for anthocyanins across Vc01–Vc12 explaining 11–67% of variance. Major hotspots included 34 QTLs on Vc04 (~115 cM) linked to glucoside-enriched anthocyanins and 15 QTLs on Vc01 (18–35 cM) linked to reduced arabinose/galactose conjugates, with additional loci on Vc02 and Vc08–Vc12 [82]. GWAS with an 89K SNP array identified 47 SNP–trait associations for flavonoid metabolites, revealing novel biosynthetic genes and regulatory

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networks shaping antioxidant diversity [81]. These studies highlight the polygenic control of antioxidant metabolism in polyploid blueberry.

3.2. Red Raspberry (*Rubus idaeus* L.)

3.2.1. Domestication and Wild Relatives

Red raspberry, a member of the Rosoideae subfamily (Rosaceae), comprises over 740 described species worldwide, with two recognized diploid subspecies: the European *R. idaeus* subsp. *idaeus* and the North American *R. idaeus* subsp. *strigosus* [83,84]. Cultivated raspberries are diploid (2n = 2x = 14), with domestication involving both subspecies across more than 2,000 years of cultivation in Europe and approximately 150 years in North America [85,86]. Evolutionary origins trace to Eurasian forests, with natural distribution extending from Western Europe through Siberia to eastern Asia, and separate colonization of North America during Pleistocene glacial periods [87]. Phylogenetic analysis using chloroplast genome sequences places raspberry within subgenus *Idaeobatus*, with sister relationships to *R. corchorifolius* and close affinity to Korean raspberry (*R. crataegifolius*) [88]. Biogeographic reconstruction indicates Eurasia as the center of diversification.

Wild relatives and inter-subspecific hybrids (*R. idaeus* × *R. strigosus*) are maintained in germplasm banks and serve as crucial donors of antioxidant-related traits, with genomic analyses confirming synteny across the seven chromosomes [84,86,89]. Species diversity within raspberry germplasm includes multiple interfertile taxa. The *R. idaeus* complex comprises the European subspecies *idaeus* (2n=2x = 14), a source of high sugar content, and diverse flavor profiles [90], and the North American subspecies *R. strigosus* (2n =2x = 14), which contributes cold hardiness, disease resistance, and unique anthocyanin profiles [86,91]. Related species provide specialized traits: *R. occidentalis* (black raspberry, 2n=2x = 14) is notable for exceptional anthocyanin content and unique cyanidin-3-rutinoside accumulation [92]. Wild population diversity studies using SSR and SRAP markers reveal significant population structure across natural ranges, with diversity centers in the Caucasus region (European populations) and the Pacific Northwest (North American populations), both serving as priority collection sites [87,93]. Germplasm repositories maintain over 1,500 accessions globally, with core collections at USDA Corvallis (~450 accessions), Agriculture Canada (~320 accessions), and major European collections, representing maximum allelic diversity for breeding applications [84].

Commercial raspberry production focuses on antioxidant-rich cultivars such as ‘Haida’, ‘Chilli wack’ and ‘Meeker’, spanning different ripening seasons. European-derived cultivars are reported with several important commercial traits such as high anthocyanin content, excellent antioxidant stability during storage [94], elevated ellagitannin levels and superior ORAC values [95]. Several early-season cultivars also contain high total phenolic content and spine-free canes that facilitate harvest [96]. North American selections include genotypes with cold-hardy characteristics notable for unique anthocyanin glycosylation patterns and high vitamins, as well as others which combine disease resistance with elevated flavonol content and extended shelf-life due to antioxidant stability [94]. Interspecific hybrids (*R. idaeus* × *R. occidentalis*) contribute to genetic diversity, providing genotypes with exceptional anthocyanin profiles (including cyanidin and pelargonidin derivatives) and dual-purpose potential for both fresh consumption and processing [92]. Beyond established cultivars, advanced breeding lines specifically selected for enhanced antioxidant profiles exhibit 40–60% higher total phenolic content than standard cultivars, with specialized metabolite compositions tailored for functional food applications [95,97]. Notably, cultivars such as ‘Saanich’, ‘KiwiGold’, and ‘Summit’ have demonstrated superior antioxidant activity and mineral content, with ‘Saanich’ showing exceptional iron and manganese levels and ‘KiwiGold’ ranking among the highest in potassium and phenolics [45]. Additionally, non-commercial cultivars like ‘Adelita’, ‘Glen Dee’, ‘Himbo Top’, ‘San Rafael’, and ‘Tula Magic’ have shown promising antioxidant potential and polyphenol content, suggesting their suitability for future breeding programs targeting nutritional enhancement [98]. Recent evaluations of cultivars grown in northern Mexico further highlight ‘Red

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Autumn Bliss' for its high anthocyanin concentration and antioxidant capacity, and 'Yellow Autumn Bliss' for its exceptional levels of total phenolics, ellagic acid, ascorbic acid, and polyphenolic summation, reinforcing their value in antioxidant-focused breeding strategies [99].

3.2.2. Modern Genomic and Breeding Tools for Enhancing the Antioxidant Character of Raspberry

Raspberry antioxidant quality is regulated by constitutive genes that sustain baseline metabolite production during fruit development. Three polyketide synthase (PKS) genes (*PKS1*, *PKS2*, and *PKS3*) have been characterized from raspberry cell cultures, with *RiPKS1* encoding a typical chalcone synthase (CHS) active in naringenin production, *RiPKS3* producing mainly p-coumaroyltriacetic acid lactone (CTAL), *RiPKS2*, an inactive form, and a newly identified, PKS4, with a crucial role in enhancing ketone levels in raspberry leaves and fruit [100,101]. Another study investigated antioxidant-related gene expression during fruit development in yellow, red, and black raspberries, revealing cultivar- and stage-specific patterns. Genes encoding antioxidant enzymes (SOD, POD, Peroxiredoxin-PRDX, CAT) and flavonoid/anthocyanin biosynthesis enzymes (PAL, FLS, ANR, LAR, UFGT*)* were found differentially expressed, with expression generally peaking at ripening stages. Transcription factor genes belonging to the *basic Helix–Loop–Helix* (*bHLH), myeloblastosis (MYB)*, and *WRKY* families also displayed distinct expression profiles, demonstrating their regulatory role in antioxidant metabolism, [102]. Baseline phenolic acid biosynthesis relies on phenylpropanoid pathway genes such as *PAL, C4H*, and *4CL,* which have been characterized in raspberry [51,103]. All the above-mentioned studies provide a valuable reference for understanding the molecular basis of antioxidant regulation in raspberry fruit. However, only a limited number of these genes have been functionally annotated and characterized (Table 3), and further research is required to exploit their roles in enhancing the antioxidant capacity of raspberries.

Table 3. Genes analyzed for their functional involvement in the antioxidant capacity of raspberries.

Enzyme Class	Gene	Functional Role	Reference
Polyketide synthase (PKS)	*RiPKS1*	Encodes a typical chalcone synthase (CHS) active in naringenin production	Zheng et al., 2001 & Kassim et al., 2009 [100,101]
	RiPKS2	Characterized from raspberry cell cultures but found inactive due to specific amino acid exchanges	
	RiPKS3	Produced mainly p-coumaroyltriacetic acid lactone (CTAL)	
	RiPKS5	Catalyzes first step in flavonoid biosynthesis	
4-coumarate:CoA ligase (4CL)	*Ri4CL1*	Catalyzes activation of 4-coumarate to CoA esters used in phenylpropanoid pathway	Woodhead et.al, (2010) [103]
	Ri4CL2		
	Ri4CL3		
Anthocyanidin synthase (ANS)	*RiANS*	Conversion of leucocyanidins to anthocyanidins	
Lipoxygenase (LOX)	*RiLOX*	Involved in lipoxygenase pathway	
Terpene synthase (TPS)	*RiTerpSynth*	Involved in monoterpene biosynthesis	
3-hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) reductase	*ERubLR_SQ8.1_H09*	Involved in mevalonic acid pathway	

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Isopentenyl-diphosphate delta-isomerase (IDI/IPI)	*ERubLR_SQ13.1_F09*
Aconitase (ACO)	*ERubLR_SQ13.2_C12*

Genomic resources for raspberry are rapidly expanding and include a chromosome-scale diploid reference genome (~240 Mb, 2n = 2x = 14) assembled using PacBio long reads and Hi-C scaffolding, comprising 32,831 predicted genes with comprehensive functional annotation [89]. The genome architecture reveals seven linkage groups with high synteny to *Fragaria* and other Rosaceae species. Comparative genomics highlights gene family expansions in secondary metabolite biosynthesis, particularly phenylpropanoid-related genes—as well as disease resistance genes. Prominent tandem gene clusters encode flavonoid biosynthetic enzymes, terpene synthases, and glycosyltransferases. QTL mapping and SNP-based studies have identified loci associated with anthocyanin composition and antioxidant traits [104,105]. A review study by McCallum et al., (2018), in raspberry further reported QTLs directly associated with anthocyanin traits, antioxidant capacity, and phenolic content [106]. These include QTLs for anthocyanin composition, such as specific glycosides of cyanidin and pelargonidin, and the mapping of candidate genes underlying anthocyanin/phenolic biosynthesis (structural and regulatory genes/transcription factors such as *bHLH, MYB*, and *basic-leucine zipper transcription factor-bZIP*), which often colocalize with QTLs for pigmentation and anthocyanin-related traits. Investigation of gene relative expression as well as transcriptome resources provide datasets related to antioxidant characteristics at diverse developmental stages, tissues, and environmental conditions, supporting functional genomics and breeding applications [89,102].

3.3. Blackberry (Rubus occidentalis/Rubus fruticosus agg.)

3.3.1. Domestication and Wild Relatives

Blackberry (*Rubus* subgenus *Rubus*) represents a taxonomically and genetically complex crop group characterized by polyploidy, hybridization, and broad ecological adaptation. The cultivation history spans more than 150 years, though systematic breeding only intensified from the 1960s onward [107,108]. Evolutionary origins are geographically diverse: European species such as the *R. fruticosus* aggregate contributed to Old World germplasm, while *R. allegheniensis* and *R. argutus* formed the basis of North American breeding programs [84]. The species exhibit remarkable cytogenetic diversity, with chromosome numbers ranging from diploid (2x = 14) to dodecaploid (12x = 84), though most commercial cultivars are tetraploid (2n = 4x = 28) or hexaploid (2n = 6x = 42) [108]. Domestication has relied heavily on interspecific hybridization between taxonomically distinct lineages, generating highly heterozygous populations and enabling transgressive segregation for traits such as flavor, yield, and antioxidant content [107]. Genomic resources now support the study of this complexity, with *R. occidentalis* (black raspberry, ~290 Mb) sequenced using PacBio and Hi-C technologies [89], while the *R. argutus* 'Hillquist' genome (~270–298 Mb) offers a high-quality chromosome-scale reference for dissecting antioxidant-related traits in polyploid backgrounds [86].

Wild *Rubus* relatives remain an essential reservoir of antioxidant and adaptive diversity. The *R. fruticosus* aggregate, comprising more than 400 microspecies (agamospecies) maintained through apomixis, provides stable sources of anthocyanins, ellagitannins, and pathogen resistance [84]. European populations exhibit anthocyanin concentrations as high as 589 mg/100 g FW and unique ellagitannin profiles, though this value applies to select *R. fruticosus* accessions used in food colorant studies—not necessarily wild populations broadly [92]. North American species contribute complementary adaptive traits: *R. allegheniensis* combines cold hardiness and unique terpene profiles; *R. argutus* contributes heat tolerance, extended harvest windows, and high phenolic diversity; and *R. ursinus*, the Pacific dewberry, imparts exceptional flavor and rare anthocyanin glycosides [109]. Further genetic breadth derives from Mexican wild species with tropical adaptation and continuous fruiting potential, though direct citation is limited and may be supported by regional germplasm

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surveys or USDA reports. Asian taxa such as *R. corchorifolius* and *R. phoenicolasius* contribute disease resistance, stress tolerance, and novel bioactive compounds [110]. Comparative studies using AFLP markers reveal that North American blackberry cultivars have a narrower genetic base compared with European wild populations, underscoring the need to broaden germplasm pools in breeding programs [111]. Conservation initiatives now safeguard over 800 accessions globally, capturing diverse lineages to ensure sustainable exploitation of antioxidant-related traits [112].

Commercial blackberry cultivars display marked variation in antioxidant composition, reflecting their diverse pedigrees. Erect thorny selections have been reported with high total phenolic content and anthocyanin levels, showing excellent ORAC values and storage stability [113]. Thornless erect cultivars display high ellagitannin accumulation, malvidin-rich anthocyanin profiles, and shelf-life extension, while semi-erect genotypes achieve total antioxidant capacities up to 11,200 μmol TE/100 g, with consistent phenolic retention under stress [95]. Trailing cultivars from the Pacific Northwest dominate the processing and fresh markets due to their distinctive flavor, anthocyanin diversity, and superior postharvest vitamin C retention [94,108]. Recent metabolomic and pharmacological profiling identified several high-antioxidant cultivars such as ‘Kiowa’, which exhibits the highest antioxidant and anti-inflammatory activity, driven by high levels of quercitrin, luteolin, and cyanidin 3-O-glucoside. Cultivar ‘10-5n-2’ contains strong total anthocyanin and polyphenol content, while ‘Chester’ is noted for strong fruit firmness and high antioxidant retention; and ‘Shuofeng’ combines high yield with excellent antioxidant capacity. Additional cultivars such as ‘Thorn Free’, have been reported with notably high cyanidin-3-glucoside and total phenols concentrations as well as ‘Loch Ness’, which exhibits very high total phenols and strong antioxidant capacity, further exemplify the nutritional potential of thornless selections [114], while ‘TripleCrown’ and ‘Encore’ exhibit exceptionally high antioxidant activity, with DPPH values of 184.43 and 316.02 μg/ml, respectively, and strong FRAP values, highlighting their potential for breeding antioxidant-rich genotypes [45]. Complementing cultivated germplasm, wild relatives such as *R. ulmifolius, R. perrobustus, R. wimmerianus, R. pedemontanus,* and *R. grabowskii* also display exceptionally high phenolic content in fruits and leaves, offering unique opportunities for bioactive compound enrichment in breeding [115].

3.3.2. Modern Genomic and Breeding Tools for Enhancing the Antioxidant Character of Blackberry

Blackberry fruit quality depends on constitutive antioxidant production systems that operate throughout normal fruit development. In the study of Huang et al. (2023), the authors investigated the molecular basis of antioxidant accumulation during fruit ripening in blackberry by monitoring the expression of structural and defense-related genes [116]. They collected blackberry fruits at different developmental stages (green, red, and fully black) and quantified the gene expression through transcriptome profiling combined with quantitative RT-PCR. They reported that genes such as *PAL, CHS, CHI*, and *F3H* exhibited higher expression at early developmental stages, establishing the metabolic framework for downstream flavonoid biosynthesis. In contrast, the late anthocyanin biosynthetic genes, namely *DFR, ANS,* and *UFGT*, showed pronounced up-regulation during the red-to-black transition, coinciding with the rapid accumulation of anthocyanins. Additionally, antioxidant defense enzymes including SOD, CAT, APX, and POD, were significantly induced at the late ripening stage, reflecting their role in mitigating oxidative stress during fruit maturation [116]. Table 4 presents the genes that have been functionally annotated and characterized till today according to the international literature findings.

Table 4. Genes analyzed for their functional involvement in the antioxidant capacity of blackberries.

Enzyme Class	**Gene**	**Functional Role**	**References**
Phenylalanine ammonia-lyase (PAL)	*RuPAL*	Converting L-phenylalanine to trans-cinnamic acid, providing precursors for flavonoids, lignin, and phenolic acids	Vogt et al., 2010 [117]

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Chalcone synthase (CHS)	*RuCHS*	Catalyzes the formation of naringenin chalcone	Dao et al., 2011 [118]
Chalcone isomerase (CHI)	*RuCHI*	Catalyzes the stereospecific isomerization of chalcones into flavanones	Jez et al., 2000 [119]
Dihydroflavonol 4-reductase (DFR)	*RuDFR*	Reduces dihydroflavonols to leucoanthocyanidins	Petit et al., 2007 [120]
Anthocyanidin synthase (ANS)	*RuANS*	Oxidizes leucoanthocyanidins to anthocyanidins	Saito et al., 2013 [121]
UDP-glucose:flavonoid 3-O-glucosyltransferase (UFGT)	*RuUFGT*	Glycosylates unstable anthocyanidins at the 3-hydroxyl position	Kobayashi et al., 2001 [122]

Genomic resources for blackberry are rapidly advancing, with recent efforts focused on developing chromosome-scale reference genomes for diploid *R. argutus* (‘Hillquist’, ~298 Mb) and tetraploid BL1 (~919 Mb), assembled using PacBio, Oxford Nanopore, and Hi-C technologies [123,124]. These assemblies reveal complex subgenome relationships shaped by ancient polyploidization and diploidization, with gene family expansions in secondary metabolite biosynthesis—particularly phenylpropanoid and flavonoid pathways—and stress-response genes. Transcriptomic datasets across fruit developmental stages and tissue types support functional annotation and breeding applications [125]. Resequencing of seven tetraploid cultivars uncovered candidate genes for antioxidant traits and fruiting habits [124]. Early diversity studies using AFLP markers laid the foundation for pangenome initiatives, suggesting an accessory genome comprising 15–20% of total genes enriched for secondary metabolism and environmental response [126]. Likewise, Chizk et al. (2023), performed a large-scale GWAS on 300 tetraploid blackberry genotypes to identify genetic factors controlling fruit firmness and red drupelet reversion (RDR). Their findings demonstrated that postharvest quality traits are moderately heritable and influenced by multiple small-effect QTLs, including loci linked to polygalacturonase, pectin methylesterase, and glucanase related genes. Overall, the research highlighted that postharvest quality is genetically complex but provided candidate loci and datasets for future genomic selection [127].

3.4. Cranberry (Vaccinium macrocarpon Ait.)

3.4.1. Domestication and Wild Relatives

American cranberry is a diploid species (2n = 2x = 24) native to acidic bog ecosystems of North America, cultivated primarily in the United States, Canada, and Chile for its high polyphenol content and associated health benefits. It represents one of only three widely cultivated fruit crops native to North America, with domestication dating back approximately 200 years ago [54,128]. Natural populations span acidic wetlands from Newfoundland to North Carolina and west to Minnesota, with genetic diversity centers in Wisconsin, Massachusetts, and New Jersey bog regions [128]. Phylogenetic analyses using chloroplast markers indicate that *V. macrocarpon* diverged from other cranberry species 5–10 million years ago, followed by population expansion after Pleistocene glacial retreats [129,130]. Early domestication involved selection from wild bog populations, showing minimal genetic bottlenecks compared to other crops. Modern breeding began in the 1950s, initially targeting fruit quality and yield, and later expanded to include enhanced antioxidant content and anthocyanin profiles, as confirmed by genotyping and metabolomic studies of breeding cycles [131,132].

Cranberry genetic resources include both wild populations and related species. Wisconsin bog populations harbor the largest genetic diversity, with over 150 distinct genotypes serving as sources of disease resistance and unique antioxidant profiles [128,133]. Massachusetts populations provide cold hardiness traits and elevated proanthocyanidin content [134], while New Jersey Pine Barrens populations contribute heat tolerance, unique A-type proanthocyanidin variants, and extended

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harvest characteristics [54]. Canadian populations exhibit extreme cold tolerance, specialized flavonoid profiles, and late season ripening traits [135]. Related species, including *V. oxycoccos* (small cranberry) and *V. microcarpum*, are genetically distinct wild relatives of *V. macrocarpon*. They contribute specialized traits such as cold hardiness, disease resistance, high anthocyanin content, unique glycosylation patterns, and stress tolerance [54]. Microsatellite marker analyses of wild cranberry (*V. macrocarpon*) clones from Newfoundland and Labrador revealed significant population structure correlated with geography, along with high allelic richness and diverse antioxidant properties, highlighting these Canadian populations as priority resources for cranberry conservation and breeding [135]. The USDA's National Clonal Germplasm Repository (NCGR) maintains over 300 cranberries and cranberry-wild relative accessions (including ~146 V. macrocarpon) in its *Vaccinium* collection. Genetic studies of cultivars and accessions have identified dozens of unique genotypes among these accessions for example, 64 unique genotypes out of 271 plants in 77 accessions confirming substantial genotypic diversity in the repository [136] . Recent chromosome-level genome assemblies for *V. macrocarpon* and *V. microcarpum* further provide foundational resources for trait and antioxidant biosynthesis analyses [133].

Commercial cranberry cultivars are selected for enhanced antioxidant profiles across early, mid, and late harvest seasons. Early-season cultivars are noted for high anthocyanin and total phenolic content, excellent processing quality, elevated proanthocyanidin levels, and superior antioxidant stability [134]. Heritage cultivars such as 'Early Black' and 'Howes' also fall into this category, showing strong antioxidant capacity and minimal genetic bottlenecks, with 'Howes' exhibiting exceptionally high anthocyanin concentrations and 'Early Black' known for stable phenolic content across ripening stages [137]. Mid-season cultivars such as 'Stevens' serve as industry standards with balanced antioxidant profiles and high A-type proanthocyanidin content, while others like 'Ben Lear' exhibit elevated total phenolics, unique anthocyanin glycosylation patterns, and strong cold hardiness. 'McFarlin', another heritage cultivar, contributes moderate antioxidant levels and maintains high genetic diversity, making it valuable for breeding and conservation. Late-season cultivars are recorded with exceptional anthocyanin concentrations and the highest observed ORAC values, with selections like 'Bergman' noted for elevated flavonol content and superior antioxidant retention during processing [138]. Additional high-antioxidant cultivars include 'Crimson Queen', known for its high anthocyanin and proanthocyanidin content with excellent color stability; 'Mullica Queen', which demonstrates elevated total phenolics and strong antioxidant retention during processing; 'Scarlet Knight', rich in flavonols and anthocyanins and bred for functional food applications; 'HyRed', valued for its deep red pigmentation and high ORAC values; and 'Demoranville', which combines high proanthocyanidin levels with improved disease resistance [132]. Specialized selections provide deep red pigmentation, diverse anthocyanin profiles, enhanced proanthocyanidin content, and improved disease resistance, making them ideal for premium fresh markets and functional food applications [137]. Finaly 'Kalnciema Agrā' and 'Kalnciema Tumšā' exhibit high proanthocyanidin and triterpenoid content, contributing to strong antioxidant activity. 'Searles' is notable for elevated proanthocyanidins, while 'Black Veil' stands out with high flavonol levels. 'Red Star' combines high chlorogenic acid with triterpenoids, and 'Pilgrim', despite lower anthocyanins, shows significant antioxidant capacity due to its triterpenoid content [137,139].

3.4.2. Modern Genomic and Breeding Tools for Enhancing the Antioxidant Character of Cranberry

Cranberries are rich in phenolic compounds, particularly flavonoids and proanthocyanidins, which have been widely studied for their diverse biological activities [140]. Breeding strategies have primarily focused on traits prioritized by the industry, such as fruit quality characteristics—including firmness, size, and anthocyanin content—as well as resistance to fruit rot, which are considered the most desirable attributes in new cranberry cultivar releases [141]. Several studies suggest that advanced DNA-based selection strategies could significantly benefit the global cranberry industry [141], while others emphasize that integrating genetic diversity analysis with antioxidant profiling using DNA markers (ISSR, EST-SSR, EST-PCR) is crucial for conserving wild cranberry germplasm

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and identifying diverse, antioxidant-rich genotypes for future breeding and crop improvement [135]. Vorsa et al. in 2019 reviewed the biology, domestication history, key traits, and breeding challenges of American cranberry, highlighting recent molecular advances and proposing MAS as a crucial tool for future genetic improvement [142]. They noted QTLs identified for important traits like productivity, berry size and anthocyanins content pointing the value of molecular tools and MAS to overcome traditional breeding limitations and accelerate genetic improvement. Debnath et al. (2012) used DNA-based markers, particularly ISSRs, to analyze genetic diversity in wild and cultivated berry crops, showing that these markers effectively differentiated genotypes and are useful for cultivar identification and breeding, while also reporting wide variation in antioxidant activity that did not align with molecular diversity patterns, highlighting the need to combine both approaches for germplasm conservation and crop improvement [143].

Few studies have applied GWAS in cranberry breeding, identifying marker–trait associations for key agronomic traits such as average fruit weight and fruit rot resistance [144]. Other research has uncovered extensive gene presence–absence variation related to disease resistance and fruit quality, with these resources now publicly available to accelerate molecular breeding in *Vaccinium* crops [131]. However, breeding efforts specifically targeting fruit antioxidant improvement remain largely unexplored in cranberries. Moreover, while genetic tools such as CRISPR/Cas9 have begun to be applied in other berry species like blueberries [145,146], these efforts have primarily focused on traits unrelated to antioxidant capacity. To date, no reports exist on the application of such genome-editing tools in cranberry.

3.5. Grapevine (Vitis vinifera L.)

3.5.1. Domestication and Wild Relatives

V. vinifera is one of the most economically important crops cultivated worldwide. Global vineyard coverage spans about 7.2 million hectares, producing 77.8 million tons of grapes annually of which 47.4% is used for winemaking, 44.5% for fresh consumption, and 8% for dried grapes (OIV). Grape species are classified into two subgenera: Muscadinia, (2n = 40) and the larger subgenus Euvitis (including V. vinifera), with a basic chromosome number of 2n = 38. Grapevine domestication dates back approximately 8,000–11,000 years and archaeological and genetic evidence points to Western Asia (a region between the Black Sea and Iran) as the primary center of origin, though multiple domestication centers have also been proposed [145–147]. The Vitis genus comprises 60–80 species characterized by exceptionally high genetic diversity. Ampelographic and genetic analyses suggest that between 6,000 and 10,000 V. vinifera cultivars are currently cultivated worldwide [148]. Global germplasm inventory repositories, such as the Vitis International Variety Catalogue (VIVC), have catalogued thousands of cultivars and wild accessions, providing valuable resources for elucidating phylogenetic relationships and trait variation.

Wild *Vitis* relatives exhibit important traits not found in *V. vinifera* that have been associated mainly with resistance/tolerance to biotic/abiotic stresses [149]. Historically, European *V. vinifera* grapevines were grafted onto American varieties derived from wild *Vitis* species to combat the catastrophic infestation of the soil born aphid phylloxera (*Daktulosphaira vitifoliae)* [150]. Moreover, over the years, breeding efforts utilizing crosses of *V. vinifera* with wild species such as *V. arizonica* and *Muscadinia rotundifolia* managed to develop lines with resistance to devastating bacterial and fungal diseases like ‘Pierce’s disease-PD’, downy mildew (*Plasmopara viticola*) and (powdery mildew (*Erysiphe necator*), respectively [151].

In the context of antioxidant capacity, the wild relative *Muscadinia rotundifolia* (Michx.) Small, a grape species native to the southeastern United States, has drawn much interest recently from the food, pharmaceutical, and nutraceutical industries owing to their unique chemical composition and nutritional value [152]. Muscadine grapes are rich in distinctive primary and secondary metabolites, including notable phenolics like gallic acid, ellagic acid, proanthocyanidins, catechins, quercetin, resveratrol, and myricetin. Muscadine-derived bioactive compounds exhibit strong antioxidant,

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anticancer, antimutagenic, antimicrobial, and anti-inflammatory properties, and thus hold tremendous health promoting potential [153]. They are also essential contributors to grape quality traits and their wide flavor diversity.

3.5.2. Modern Genomic and Breeding Tools for Enhancing the Antioxidant Character of Grapevine

The grapevine was among the first fruit crops to be sequenced, with the reference genome of the near-homozygous Pinot Noir PN40024 line published by Jaillon et al. (2007) [154] . The diploid genome is made up of 19 chromosomes and covers about 487 Mb. The assembly has been improved to chromosome-scale resolution by further updates that included BAC-by-BAC sequencing, Illumina short reads, and long-read data [155]. Gene families engaged in secondary metabolism were revealed by annotation, particularly those associated with the biosynthesis of terpene and polyphenol compounds, which include antioxidants like anthocyanins and stilbenes (e.g., resveratrol) [156]. These genomic resources have allowed identification of key genes such as *VvDXS1, VvbZIP61* (terpene synthesis) *VvCHS, VvMYBA1* and *VvSTS* (polyphenol synthesis) facilitating marker-assisted breeding and functional genomics in grapevines. Table 5 presents the genes that have been functionally annotated and characterized regarding their association with antioxidant levels.

Advances in DNA sequencing technologies have yielded enhanced grapevine reference genome assemblies [157] and a non-reference pangenome derived from wild grapevine accessions [158]. These genomic resources provide powerful tools for elucidating the molecular basis of key morphological, physiological, and biochemical traits that influence yield, fruit quality, and stress tolerance. Notably, the recent development of a comprehensive grapevine pangenome reference map built from resequencing data of 466 cultivars and capturing an extensive array of both short variants and structural variants (SVs)—is expected to shed light on complex polygenic trait associations and accelerate genomic-assisted breeding efforts [159].

Transcriptomics and metabolomics are increasingly employed to investigate molecular processes in grapevines and have been focusing either on plant and fruit development or on interactions with abiotic and biotic factors. Integrating these approaches has become critically important for understanding key physiological and metabolic pathways [160]. Insights gained from such analyses can be directly applied in breeding programs, using genes linked to improved fruit quality—such as those enhancing the accumulation of health-promoting compounds like antioxidants—as molecular markers for crop improvement.

Over the past two decades, a wealth of transcriptome and metabolome data has been produced which greatly aid the investigation of grapevine berry physiology, secondary metabolism, and quality traits [160–164]. Integrated "omics" datasets can be compiled into user-friendly resources for the research community, enabling exploration of potential gene–gene and gene–metabolite interactions that can later be validated through hypothesis-driven experiments. One such resource is the Grapevine Transcriptomic and Metabolomic Integrated Database (TransMetaDb), available through the Vitis Visualization (VitViz) platform (https://tomsbiolab.com/vitviz which provides a valuable tool for linking antioxidant potential with berry development and metabolism [164].

Table 5. Genes analyzed for their functional involvement in the antioxidant capacity of grapevine.

Enzyme Class	**Gene name**	**Functional Role/ Association with antioxidant levels**	**References**
1-deoxy-d-xylulose-5-phosphate synthase (DXS)	*VvDXS1*	Rate-limiting enzyme of the MEP pathway; Major determinant of monoterpenoid content	Yang et al., 2017 [165] Zhang et al., 2025 [167]
Basic-leucine zipper transcription factor	*VvbZIP61*	Monoterpene metabolism Associated with increased levels of monoterpenes	Zhang et al., 2023 [166]

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Isopentenyl pyrophosphate synthases (IPPS) (geranylgeranyl pyrophosphate synthase)	*VvGGPPS-LSU*	Enzyme of the MEP terpene biosynthesis pathway; Associated with increased accumulation of monoterpenoid and norisoprenoid levels.	Zhang et al., 2025 [167]
WRKY transcription factors	*VvWRKY24*	A key regulator of isoprenoid metabolism. Associated with enhanced levels of β-damascenone, an isoprenoid important for berry and wine aroma	Wei et al., 2025 [168]
MYB transcription factors	*VvMYBA1*	A key positive regulator of anthocyanin biosynthesis	Liu et al., 2023 [169]
Chalcone synthase (CHS)	*VvCH2*	A key enzyme in the phenylpropanoid metabolism committed to the synthesis of flavonoids	Lai et al., 2025 [170]
Stilbene synthases (STS)	*VvSTS*	A key enzyme of the phenylpropanoid pathway committed to the synthesis of resveratrol	Lai et al., 2025 [170]

In grapevine, antioxidants comprise large classes of compounds, including secondary metabolites involved in shaping the aroma and flavor of grapes and wine (e.g., terpenoids), compounds related to health benefits (e.g., polyphenols, resveratrol, anthocyanins), and compounds determining the responses to biotic and abiotic stresses (e.g., stilbenes, anthocyanins, terpenes) [171]. Despite the importance of antioxidant content as a key quality trait in grapevine breeding, the genetic basis underlying the biosynthesis and accumulation of aroma/flavor-related terpenoid compounds or phenolic, flavonoid and anthocyanin molecules remains obscure due to the complexity of the respective metabolic pathways that involve a multitude of enzymes and gene networks [172]. Moreover, reliable molecular markers linked to these traits are largely lacking, posing a challenge for the implementation of marker-assisted selection (MAS) in antioxidant-enhancement breeding and pointing to the necessity of adopting modern genomic tools and molecular breeding strategies [173].

- *Antioxidants related to the aromatic profile of grapes and wine*

The complex bouquet of grape aroma is attributed to a diverse array of volatile organic compounds, such as benzenoids, esters, norisoprenoids, and terpenoids [174,175]. Distinct classes of terpenoids like mono- and sesquiterpenes contribute significantly to the characteristic aromatic profiles of individual grapevine cultivars. For example, monoterpenoid alcohols such as linalool, nerol, and geraniol are key contributors to the floral and citrus-like aromas typical of highly aromatic varieties such as *Muscat* and *Gewürztraminer*. On the other hand, sesquiterpenes, such as rotundone, are associated with spicy, peppery notes, particularly characteristic of the *Syrah* cultivar [176]. Despite numerous investigations, thus far, the genetic and regulatory aspects of terpenoid production have not been fully uncovered due to the complexity of the particular biosynthetic pathway. Monoterpenoids and sesquiterpenes are synthesized via the mevalonate pathway (MVA) and the methylerythritol phosphate (MEP) pathway which are compartmentalized into the cytoplasm and plastids, respectively (Figure 2). The 1-deoxy-d-xylulose-5-phosphate synthase (DXS) and HMG-CoA reductase (HMGR) are the major contributors to metabolic flux control of the pathways, respectively whereas terpene synthases (TS) functioning in the final steps are associated with the synthesis of distinct terpenoid molecules from prenyldiphosphate precursors [177]. A large family of terpene synthases (TS) encoded by 69 genes identified in the grapevine genome is considered to play critical roles in terpenoid biosynthesis [178]. Structurally diverse terpene synthases acting on

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prenyldiphosphate precursors are considered to drive the wide range of terpenes produced in grapes. These terpenes can then undergo secondary modifications, such as glycosylation and oxidation, further expanding the diversity of grape and wine aromas and flavors [177]. In addition, transcription factors such as members of the NAC, MYB, and AP2/ERF families, have been suggested to play roles in the transcriptional regulation of terpene synthesis genes in grapevine [178]. However, the functional characterization of most TS genes/enzymes or transcription factor genes has been limited. Similarly, the oxidation, reduction, and glycosylation processes that lead to the production of monoterpenoid derivatives -also key aroma compounds- are poorly understood [167].

Early QTL mapping and GWAS studies demonstrated that a major QTL linked to aroma profiling and monoterpenoid content was associated with the *VvDXS1*gene**.** A series of causal SNPs were identified mainly in the coding region of the gene affecting the catalytic activity of the DXS1 enzyme, as well as in the regulatory region affecting gene expression levels [165,179–182]. Thus, *DXS1* was proposed as an effective molecular marker for an aroma-related quality trait; nevertheless, various aromatic varieties and their aroma/flavor variabilities were found unrelated to *DXS1*, highlighting the need for further gene mining and development of additional genetic markers.

An integrative approach combining QTL mapping, transcriptomic analysis, and calli transformation revealed additional genes associated with monoterpenoid composition and abundance [166]. The F1 progeny of a cross between an aromatic (Beifeng) and a neutral (3–34) grape variety identified stable QTLs associated with 11 monoterpenes. In parallel, RNA seq analysis from 34 diverse cultivars with distinct aromatic profiles, indicated a significant correlation between the expression of genes mapping to these QTLs and monoterpenoid content of grapevine berries. One of those was identified as the *VvbZIP61* gene encoding a basic-leucine zipper transcription factor known to regulate various plant processes. *VvbZIP61* implication in monoterpenoid metabolism was further supported by its overexpression in *V. amurensis* calli, which led to a significant increase in the accumulation of several monoterpene compounds [166].

To identify additional candidate genes associated with monoterpenoid levels, Zhang et al. (2025) employed GWAS on an F1 population derived from crosses between a typical muscat variety (Muscat of Alexandria) and a nonaromatic variety (Christmas Rose). A range of significant SNPs were identified, all located on chromosome 5, forming a monoterpenoid-related marker cluster. 25 lead SNPs appearing stable over a two-year period were validated in a germplasm population of 97 diverse *Vitis* species and were found highly associated with monoterpenoid levels. The underlying genes encode the DXS enzyme, a glycoside hydrolase, an ABC transporter, and a geranylgeranyl pyrophosphate synthase-large subunit (*VvGGPPS-LSU*) involved in the terpene synthesis pathway. Notably, the functionality of the latter in increasing monoterpenoid levels was verified in vivo through transformation and overexpression in transgenic calli, reinforcing the reliability of the marker cluster. Importantly, the 25 lead SNP markers were converted to a KASP marker set, enabling direct molecular breeding applications [167].

Besides terpenoid compounds, norisoprenoids, which are derived from the oxidative cleavage of carotenoids, constitute another class of volatile aroma compounds with major contribution to the floral and fruity notes of grape berries and wine. Among them, β-damascenone is the most abundant norisoprenoid found in grapevine [183]. A recent study reported that a WRKY transcription factor, ViWRKY24, plays a role as a key regulator of β-damascenone accumulation in grape berries. Functional characterization through overexpression and gene silencing in grape leaves, berries, and calli revealed that *VviWRKY24* modulates norisoprenoid metabolic flux and alters norisoprenoid composition, specifically enhancing β-damascenone levels [168].

The studies described above will illuminate further the regulation of terpenoid and isoprenoid metabolic pathways and facilitate grapevine improvement as it pertains to antioxidant related aroma/flavor traits.

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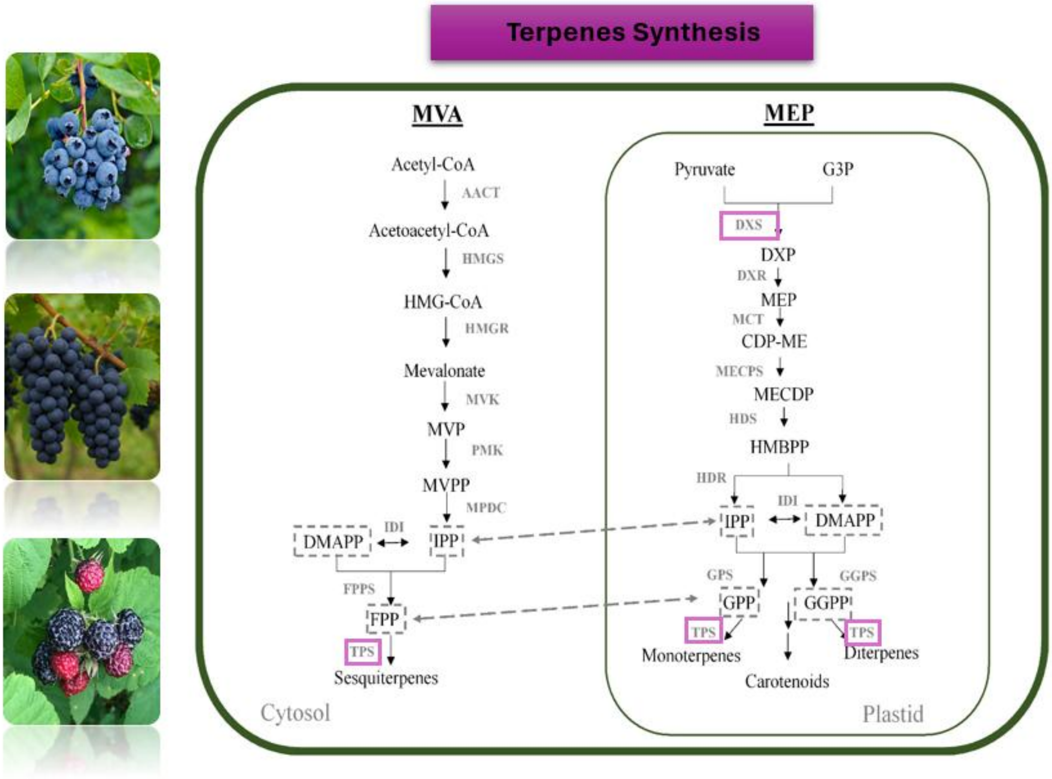

Figure 2. A general model of the mevalonate (MVA) and methylerythritol phosphate (MEP) pathways, operating in the cytosol and plastid, respectively, generate the prenyldiphosphate precursors required for terpene biosynthesis; downstream metabolic products and respective enzymes are indicated. AACT, acetyl-CoA acetyltransferase; CDP-ME, 4-diphosphocytidyl-2-C-methyl-D-erythritol; CDP-MEP, CDPME 2-phosphate; CMK, 4-(cytidine 5′-diphospho)-2-C-methyl-D-erythritol kinase; DMAPP, dimethyl allyl diphosphate; DXP, 1-deoxy-D-xylulose 5-phosphate; DXS, DXP synthase; DXR, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; FPP, farnesyl diphosphate; FPPS, FPP synthase; G3P, glyceraldehyde 3-phosphate; GGPP, geranylgeranyl diphosphate; GGPPS, GGPP synthase; GPP, geranyl diphosphate; GPPS, GPP synthase; HDR, hydroxymethylbutenyl diphosphate reductase; HDS, 4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase; HMBPP, (*E*)-4-hydroxy-3-methylbut-2-en-1-yl diphosphate; HMG-CoA, hydroxymethylglutaryl-CoA; HMGR, HMG-CoA reductase; HMGS, HMG-CoA synthase; IDI, isopentenyl pyrophosphate isomerase; IPP, isopentenyl diphosphate; MCT, 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase; MECPD, 2-C-methyl-D-erythritol 2,4-cyclodiphosphate; MECPS, MECPD synthase; MVK, mevalonate kinase; MPDC, mevalonate diphosphate decarboxylase; MVP, mevalonate 5-phosphate; MVPP, mevalonate 5- pyrophosphate; PMK, phosphomevalonate kinase; TPS, terpene synthase (modified from Bosman et al., [177]).

-Antioxidants related to phenolic compounds and health benefits

In the context of the rising interest in nutraceuticals and phenolic antioxidants found in the skin, flesh, and seeds of grapevine berries, Muscadine grapes (*Muscadinia rotundifolia* Michx.), a native grape of the southeastern United States, have been gaining much attention in recent years. Muscadine grapes have distinctive flavor and aroma properties and are a major source of essential oils, vitamins, minerals, and antioxidants. Notably, they accumulate significantly higher levels of bioactive polyphenolic compounds compared to other grape species [184,185], enhancing their value both nutritionally and commercially.

Antioxidant activity is higher in skin than pulp and increasing the antioxidant capacity of muscadine berry skin has been an important breeding target. Although many studies have reported on the phenolic content and antioxidant activities in muscadine berries [186], the genetic basis of varied antioxidant profiles has not been studied until recently. Transcriptomic analysis of two

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contrasting muscadine genotypes differing in phenolic content and antioxidant capacity revealed distinct transcript profiles across berry development that was accompanied by differential total phenolic/total flavonoid contents and antioxidant activities [187]. Differential gene expression was witnessed predominately at the veraison stage and involved genes coding for key enzymes in the polyphenol biosynthetic pathway which were found significantly upregulated in the C5 genotype pointing to genotype-specific regulation. On the other hand, genes of the non-flavonoid pathway—*stilbene synthases (STS1, STS2, STS3)*— exhibited higher expression levels at the veraison and ripe berry stages in the C6 genotype, indicating, enhanced, genotype-specific production of stilbenoid compounds in the late berry developmental stages [187]. Similarly, RNA-seq was employed to perform comparative transcriptomic analysis of phenolic pathway associated genes in two muscadine grape varieties, Paulk and Supreme, during berry development. Differential gene expression was observed for *VvPAL* and *VvCHS* which manifested in a genotype- and tissue-specific manner. Conversely, *VvMYBA1* and *VvMYBCS1* were significantly upregulated in veraison and mature grape berries in both varieties, suggesting a positive contribution to anthocyanin accumulation [188]. These findings underscore the importance of specific metabolic genes and transcriptional regulators in shaping antioxidant biosynthesis, providing valuable targets for breeding programs aimed at enhancing antioxidant capacity and fruit quality.

To elucidate the genetic architecture of antioxidant quantitative traits in Muscadinia grape, Park et al. (2022) conducted a multi-locus GWAS analysis to identify genomic loci associated with total phenolic and flavonoid content and the quantitative property of their antioxidant activity [173]. 350 genotypes were analyzed through a combination of phenotypic (phenolic content determination and antioxidant activity), berry skin transcriptomic, and GWAS analyses. 12 Quantitative Trait Nucleotides (QTNs) were significantly correlated with four antioxidant-related traits, and candidate genes were found to be positively and negatively associated with antioxidant activities. Among the positively correlated candidate genes, a UDP-glycosyltransferase was identified as a strong candidate significantly correlating with high antioxidant values. UDP-glycosyltransferases are known to be involved in flavonoid and anthocyanin biosynthesis, indicating that the muscadine gene identified in this study may play an important role in enhanced antioxidant capacity. Notably, the gene coding for 4-hydroxy-4-methyl-2-oxoglutarate aldolase was negatively associated with antioxidant values. This gene is involved in the degradation pathway of gallic acid—a key phenolic compound—in bacteria [188], suggesting that it may act as a negative regulator of phenolic antioxidant activity in muscadine grapes. In another study, the role of the VvMYBA1 transcription factor in anthocyanin biosynthesis was further elucidated using a combination of CRISPR/Cas9-mediated knockout of *VvBBX44*, a gene encoding a repressor of *VvMYBA1*, and transient transformation of ‘Jingxiu’ grapevine berries. Loss of *VvBBX44* function led to increased anthocyanin accumulation, whereas overexpression of *VvMYBA1* increased *VvBBX44* expression [189]. These results demonstrate that *VvMYBA1* and *VvBBX44* act together in a regulatory feedback loop to fine-tune anthocyanin biosynthesis, maintaining anthocyanin levels in the desired range.

Along these lines, a notable recent study has reported on the use of targeted editing through CRISPR/Cas9 technology to induce a metabolic shift from the flavonoid to the stilbenoid pathway, thereby promoting resveratrol biosynthesis and accumulation in *V. davidii* transgenic cells [170]. This approach leveraged the fact that both resveratrol (a stilbenoid compound) and flavonoids are derived from the phenylpropanoid biosynthesis pathway, where stilbene synthase (STS) and chalcone synthase (CHS) compete for the same substrate to produce stilbenoids and flavonoids, respectively (Figure 3). CRISPR/Cas9-based targeted editing of the *VdCH2* gene resulted in efficient CHS2 knock-out leading to suppression of the flavonoid pathway and reduction in flavonoid levels, but to a significant increase in stilbenoid compounds such as resveratrol and its derivatives. These findings offer novel strategies for enhancing targeted antioxidant content in grapevine through redirecting metabolic flux from the flavonoid to the stilbenoid pathway and present an effective alternative to the challenging and costly extraction of resveratrol, a highly valuable antioxidant with beneficial antioxidant properties [170,190].

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The findings above set the basis for a deeper understanding of polyphenol accumulation and antioxidant activity and pave the way for enhancing the nutraceutical value of berries through modern breeding and metabolic engineering approaches.

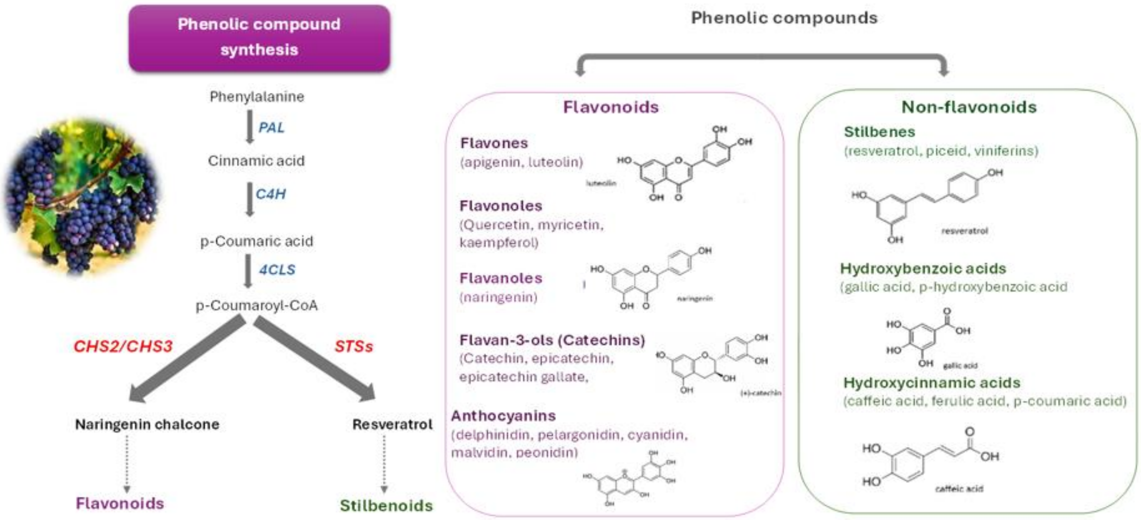

Figure 3. A general model of the phenylpropanoid pathway; Targeted genome editing of *chalcone synthase* (*CHS*) using CRISPR/Cas9 reduces flux into the flavonoid branch of the phenylpropanoid pathway. This shift enhances substrate availability for *stilbene synthase* (*STS*), thereby promoting resveratrol biosynthesis and accumulation in transgenic cells. Both flavonoids and stilbenoids originate from the same precursor, p-coumaroyl-CoA, highlighting the competitive relationship between the two pathways.

4. Conclusions

Berry crops are highly valued for their rich antioxidant capacity, largely attributed to anthocyanins, flavonols, proanthocyanidins, vitamins, and carotenoids, which play important roles in reducing oxidative stress and lowering the risk of chronic diseases in humans. Until recently, breeding programs rarely focused directly on antioxidant traits. Instead, breeding for antioxidants was achieved indirectly, mostly through the selection of genotypes with increased tolerance to environmental stresses. Despite the availability of high-quality genome assemblies for several berry species, the specific roles, annotation, and functional characterization of many genes underlying antioxidant biosynthesis remain insufficiently explored. However, due to the rising demand for functional foods, the targeted breeding of berries for enhanced antioxidant potential has become an emerging priority. This shift is supported by the exploitation of the wide genetic diversity found in wild relatives, which serve as reservoirs of valuable traits, and by the integration of modern molecular and genetic breeding tools, including QTL mapping, marker-assisted selection, genomic selection, and CRISPR/Cas9-based gene editing [191]. The combination of advanced molecular and genetic breeding tools provides powerful opportunities to accelerate the development of cultivars that combine high nutritional quality with resilience and productivity, contributing both to human health and to sustainable berry production systems.

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