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MULTI-OMICS APPROACHES TO MAPPING THE GENE REGULATORY NETWORKS BEHIND ANTHOCYANIN AND NUTRIENT ACCUMULATION IN FRUITS AND VEGETABLES: A COMPREHENSIVE REVIEW

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ABSTRACT

Quality of fruit and vegetables is mainly determined by two traits: anthocyanin pigmentation and mineral micronutrient content. Both influence consumer attraction, antioxidant property and food security, but neither is controlled by a few genes independently. Instead, they each rely on complex gene regulatory networks (GRNs) integrating developmental, hormonal and environmental signals at transcriptional, post-transcriptional and epigenetic levels. Many years of gene-by-gene studies have revealed the key structural and regulatory genes, particularly the MYB-bHLH-WD40 (MBW) complex, which control anthocyanin biosynthesis, but these studies rarely linked upstream signalling to chromatin status, transcription, protein levels and metabolite outputs in a single system. That is starting to change now that high-throughput, multi-omics platforms are less expensive and more widely available. Genome-wide association studies (GWAS), bulk and single-cell and spatial transcriptomics, epigenomic profiling (WGBS, ChIP-seq, ATAC-seq), proteomics and metabolomics/ionomics in combination with statistical and machine-learning approaches e.g. weighted gene co-expression network analysis (WGCNA) and Bayesian network inference, allow one now to reconstruct GRNs at a resolution not available even 10 years ago and for an increasing number of plant species. This review brings together current knowledge of the anthocyanin biosynthetic pathway and its MBW-centred transcriptional logic; summarizes the major omics technologies and GRN reconstruction pipelines applied in fruit and vegetable crops; provides comparative case studies for apple, grape, citrus, strawberry, blueberry, tomato, eggplant, pepper, potato, sweet potato, lettuce, radish and other horticultural species; and adds a section on ionomics and iron, zinc and provitamin A biofortification. Epigenetic regulation, the role of environmental and hormonal signals, the application of GRN knowledge for precision breeding (e.g., CRISPR/Cas editing), and the health relevance of anthocyanin-rich commodities are also discussed.

KEYWORDS: anthocyanin biosynthesis; gene regulatory network; multi-omics integration; MYB-bHLH-WD40 complex; transcriptomics; metabolomics; ionomics; biofortification; fruit quality; CRISPR/Cas genome editing

1. INTRODUCTION

Fruits and vegetables contain the highest levels of vitamins, fibre and health-promoting phytochemicals in our diet, and anthocyanins contribute significantly to that value, playing the dual role of defining product colour and serving as the visual attribute influencing nutraceutical quality. These water-soluble flavonoid pigments are present in almost all types of fruits and vegetables such as apples, grapes, oranges, strawberries, blueberries, eggplant, purple cauliflower, radish, potato and sweet potato and their consumption have been linked to a reduction in the risk of CVD, obesity, type 2 diabetes, and various cancers (Khoo *et al*. 2017; Li *et al*. 2017; Manolescu *et al*., 2019; Noman *et al*., 2025). Individual genes less frequently control either anthocyanin biosynthesis or nutrient accumulation; instead, both characteristics are a product of GRNs that include transcription factor proteins, signalling kinases, chromatin modifiers, small RNAs and metabolic enzymes

working in concert to translate developmental and environmental signals into the pigment profile of a given tissue. The most well-established example of this combinatorial logic is the MYB-bHLH-WD40 (MBW) ternary complex, where antagonistic MYB activators and repressors compete for a common, limited pool of bHLH and WD40 partners to modulate expression of early- and late-stage anthocyanin genes in a tissue, developmental stage and environmental dependent manner (Xu *et al*., 2015; Naing and Kim, 2018; Zhao and Han, 2023). A similar, although much less define, regulatory cascade governs iron, zinc and some other trace minerals extraction from soil, chelation, mobility, and vacuolar storage; centered on nicotianamine synthase, ferritin and metal-transporter family of genes (Connorton and Balk, 2019; Sperotto *et al*., 2012).

For a long time, studies of these pathways relied on single-omics methodologies: candidate-gene expression studies, forward genetics, bulk transcriptomics. They yielded a lot of useful information but were unable to capture systems-level aspects of GRNs (feedback loops, hub-gene centrality), or crosstalk between the anthocyanin and nutrient-transport modules. This began to change with the emergence of next-generation sequencing, mass-spectrometry-based proteomics and metabolomics, ICP-MS-based ionomics, and single-cell/spatial profiling, all of which may now be integrated into a single analytical platform that considers genomic variation, transcript abundance, epigenetic marks, protein abundance, and metabolite or ion concentration (Acharjee *et al*., 2016; Fondi and Lio, 2015; Savoi *et al*., 2017). In conjunction with network-inference tools such as WGCNA (Langfelder and Horvath, 2008) and recently emerged machine-learning-based reconstruction methods, this synthesis is changing the way the regulatory hierarchies governing pigmentation and nutrient density are identified, validated and, eventually, translated into molecular breeding pipelines.

The subsequent sections analyze the biosynthetic and regulatory mechanisms that control anthocyanin accumulation; the omics techniques and bioinformatic tools applied to reconstruct GRNs in horticultural crops; case studies on representative fruit and vegetable taxa; the adaptation of multi-omics concepts to ionomics and biofortification; epigenetic and environmental control; and the application of GRN insights for CRISPR/Cas-mediated precision breeding, concluding with challenges that presently limit the application of multi-omics-guided GRN manipulation to mainstream breeding.

2. Anthocyanin Biosynthesis and the MYB-bHLH-WD40 Regulatory Complex

Anthocyanin biosynthesis is shunted through the phenylpropanoid-flavonoid pathway. Phenylalanine ammonia-lyase (PAL) begins the sequence by deaminating phenylalanine and the pathway continues via cinnamate-4-hydroxylase (C4H) and 4-coumarate: CoA ligase (4CL) to produce p-coumaroyl-CoA. Chalcone synthase (CHS) combines p-coumaroyl-CoA with three units of malonyl-CoA to produce naringenin chalcone, which is transformed into naringenin by chalcone isomerase (CHI). That intermediate is hydroxylated in a sequential manner by the enzymes flavanone 3-hydroxylase (F3H), flavonoid 3'-hydroxylase (F3'H) and flavonoid 3',5'-hydroxylase (F3'5'H) to form dihydroflavonols, which are further reduced by dihydroflavonol 4-reductase (DFR) to leucoanthocyanidins.Anthocyanidin synthase/leucoanthocyanidin dioxygenase (ANS/LDOX) changes these into the coloured anthocyanidins, and a final glycosylation step catalyzed by UDP-glucose:flavonoid 3-O-glucosyltransferase (UFGT), along with glutathione S-transferase (GST)-mediated vacuolar translocation, stabilizes and secludes the pigment (Holton and Cornish, 1995; Winkel-Shirley, 2001; Koes *et al*., 2005) (Figure 1).

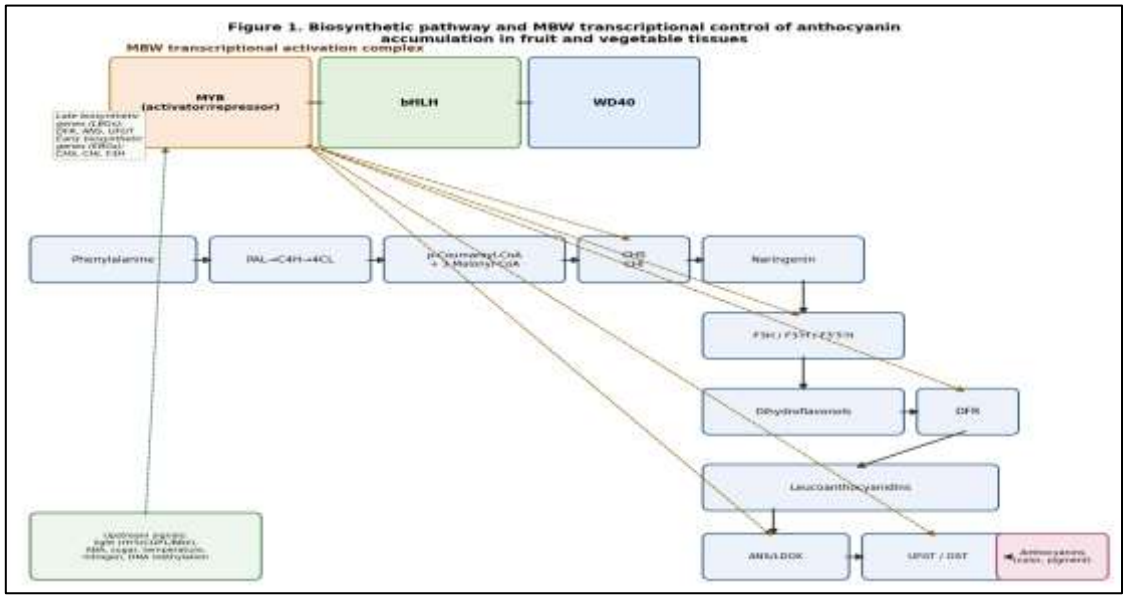

Figure 1. The anthocyanin biosynthesis pathway in fruit and vegetable tissues and its transcriptional regulation by the MBW complex. EBGs = early biosynthetic genes; LBGs = late biosynthetic genes; dashed arrows represent a transcriptional activation by MBW. (Original figure, adapted from Holton and Cornish, 1995; Winkel-Shirley, 2001; Koes *et al*., 2005; Xu *et al*., 2015)

The MBW complex also regulates the majority of the transcriptional regulation at this step. R2R3-MYB transcription factors confer target-gene and tissue specificities, bHLH proteins serve as obligate/facultative MYB partners to enhance MYB binding or elevate transactivation, and WD40-repeat proteins can't bind DNA directly but scaffold the complex and enhance its stability (Xu *et al*., 2015; Feller *et al*., 2011; Zhao *et al*., 2019). A group of activating MYBs-MdMYB10/MdMYB1 in apple, VvMYBA1/VvMYBA2 in grape, FaMYB10 in strawberry, Ruby1 in citrus, and the PAP1/MYB113/MYB114 orthologues in Arabidopsis and relatives appear to mainly switch on late genes such as DFR, ANS and UFGT as opposed to the early ones, which is part of the explanation for why colour phenotypes are so often unlinked to total flavonoid flux (Espley *et al*., 2007; Kobayashi *et al*., 2002; Lin-Wang *et al*., 2010; Butelli *et al*., 2012). Repressive MYBs like MdMYB6 and FaMYB6-like and TgMYB4 which counteract the activation, whether by directly binding late-gene-promoters or through haplotype competition for the bHLH partners the activators require; the outcome on the whole is a RNA rheostat, fine-tuning the pigment intensity (Naing and Kim, 2018).

The MBW complex does not constitute the entire regulation. Several other regulators, such as WRKY, NAC, bZIPs (predominantly ELONGATED HYPOCOTYL5, HY5), B-box (BBX), RAV, HD-Zip, and SPL transcription factors, have been shown to direct light, temperature, sugar, and hormone signals into the MBW complex, either by binding to the promoters of structural or MYB-genes directly, or by physically associating with components of the MBW complex (Fang *et al*., 2019; Yao *et al*., 2017; Zhou *et al*., 2015; Gou *et al*., 2011). In strawberry the RAV-family regulator FaRAV1 directly activates FaMYB10 and its targets (Jiang *et al*., 2023), while in the crosstalk between the HD-Zip protein FaANL2 and the repressor FaMYB6-like, they are antagonists in modulating pigment accumulation in ripening fruit. The regulation of canopy light exposure to fruit-skin colour via light-responsive BBX proteins that are upstream of HY5 and activate MYB10/MYB114 transcription has also been shown in apple and pear (Alabd *et al*., 2022a, 2022b). A further post resource also plays a role: in apple, phosphorylation by MPK6 of MdHY5 enhances its DNA-binding ability, a sign that these GRNs are indeed active in both the transcriptional and post-translational level (Zhao and Han, 2023).

Table 1. Representative transcription factors and regulatory hubs governing anthocyanin gene regulatory networks across major fruit and vegetable crops.

Species	Key regulatory gene(s)	Regulatory role	Reference
Apple (Malus domestica)	MdMYB10/MdMYB1, MdMYB6, MdBBX21/22, MdHY5	MYB10 activates late genes; MdMYB6 represses via ANS/GSTF12; BBX-HY5 mediates light induction	Espley *et al*., 2007; Chagne *et al*., 2013
Grape (Vitis vinifera)	VvMYBA1/A2, VvMYBPA1, VvWRKY44/40	MYBA1/A2 control skin-specific UFGT expression; retrotransposon (Gret1) silences white berries	Kobayashi *et al*., 2004; Bogs *et al*., 2007
Citrus (Citrus spp.)	Ruby1 (CsRuby1)	Cold/light-induced via Tcs1 retrotransposon promoter; activated by CBF/ERF factors	Butelli *et al*., 2012; Wang *et al*., 2024
Strawberry (Fragaria spp.)	FaMYB10, FaMYB1, FaRAV1, FaMYB6-like	MYB10 is master activator; RAV1 upstream activator; MYB6-like/MYB1 repressors	Medina-Puche *et al*., 2014; Jiang *et al*., 2023
Blueberry (Vaccinium spp.)	VcMYBL1, VcbHLH1, VcWDL2, MYBPA-type	MBW complex regulated developmentally; MADS-box SQUAMOSA gene contributes	Zhao *et al*., 2019; Jaakola *et al*., 2010
Tomato (Solanum lycopersicum)	SlAN2/SlAN2-like, SlMYB75	Alternative splicing controls anthocyanin-free phenotype; engineered for fruit-wide pigmentation	Colanero *et al*., 2019; Butelli *et al*., 2008
Eggplant (Solanum melongena)	MYB113-like, SmWRKY44, COP1	GWAS-identified MYB/COP1 loci; WRKY44 as co-expression hub	Cericola *et al*., 2014
Potato (Solanum tuberosum)	StAN1, StbHLH1, StWD40	AN1 is dominant regulatory gene of tuber/leaf anthocyanin	Strygina *et al*., 2019
Radish (Raphanus sativus)	RsMYB1	Transposon-induced promoter methylation silences RsMYB1 in red-fleshed radish	Xie *et al*., 2014
Lettuce (Lactuca sativa)	ANS-linked GWAS locus	GWAS-validated regulator of leaf anthocyanin used in genomic design breeding	Xie *et al*., 2014

3. Multi-Omics Technologies for Gene Regulatory Network Reconstruction

Building a biologically meaningful GRN involves multiple lines of evidence DNA sequence variation, transcript abundance, chromatin state, protein levels, metabolite/ion concentration which must be integrated using appropriate statistical methodologies (Figure 2).

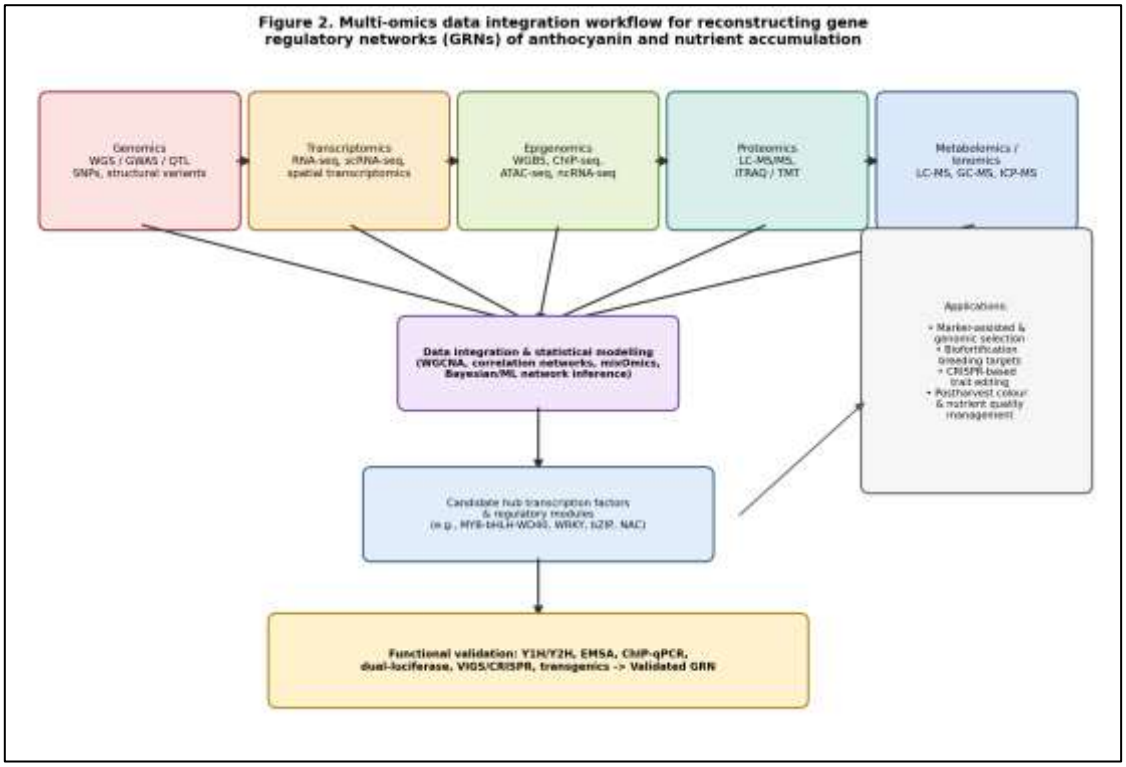

Figure 2. Working process from generation of multi-omics data to statistical integration and identification of hubs for functional validation and subsequent exploitation in breeding for the reconstruction of gene regulatory networks (GRNs) controlling anthocyanin and nutrient accumulation. (Schematic drawing, conceptual framework adapted from Acharjee *et al*., 2016; Fondi and Lio, 2015; Langfelder and Horvath, 2008)

3.1 Genomics, GWAS and QTL Mapping

GWAS developed based on whole-genome resequencing and SNP arrays have identified a myriad of candidate anthocyanin loci, such as MYB113-like and COP1 in eggplant (Cericola *et al*., 2014), MaVHAG3 in mulberry, and ANS-related loci uncovered in a wide lettuce panel for genomic-assisted breeding of high-anthocyanin cultivars. Candidate-gene association analyses in grape berries-based investigations in MYB11, MYBCC and MYC-B variants with skin-anthocyanin content (Cardoso *et al*., 2012). A parallel mineral story, ionomic GWAS in maize, rice and barley has identified QTL for iron, zinc and cadmium accumulation coinciding with transporter and chelator genes, although only a small fraction of these loci currently coincides with functionally validated ionome genes a gap which underscores how much independent validation is still needed (Salt *et al*., 2008; Baxter *et al*., 2008).

3.2 Transcriptomics, Single-Cell and Spatial Profiling

Bulk RNA sequencing continues to be the method of choice for identifying differentially expressed genes among developmental stages, tissues and genotypes, and is the basis of much of what is understood of anthocyanin GRNs in apple, grape, citrus, strawberry and other species (Espley *et al*., 2007; Wang *et al*., 2024). Single-cell and spatial transcriptomics are beginning to uncover the cell-type heterogeneity that bulk methods inherently average over exposing staged ripening programmes along the tomato fruit's longitudinal axis and cell-specific regulatory states in floral and fruit tissue that were simply beyond the reach of previous techniques (Meir *et al*., 2021). Single-cell multi-omics is still a new tool in horticultural species, but it will help to better localize which regulatory modules are active in which parts of a fruit or vegetable tissue.

3.3 Epigenomics: DNA Methylation, Histone Marks and Chromatin Accessibility

Whole-genome bisulfite sequencing confirms that hypermethylation at the MYB10/MYB1 promoter regions can fully suppress anthocyanin production, resulting in striped or non-red bud-sport types in both apple and pear, and that exposure to light induces global remodelling and demethylation at the promoters of anthocyanin genes (Bai *et al*., 2017; El-Sharkawy *et al*., 2015). The ChIP-seq and ATAC-seq provide a direct read-out of transcription-factor binding to DNA and accessibility of chromatin; in mulberry, for example, tandem analysis of ATAC-seq and RNA-seq at the fruit colour transition stage identified enrichment of AP2/ERF, MYB, NAC, LBD and WRKY motifs in open chromatin and constructed a transcriptional network that connects particular hub transcription factors to pigmentation genes (Buenrostro *et al*., 2013; Zhang *et al*., 2008).

3.4 Proteomics, Metabolomics and Ionomics

LC-MS/MS-based proteomics introduces a dimension not attainable by transcriptomics that enables the observation of post-transcriptional and post-translational regulation. Granger-causality analysis of time-course proteomic and metabolomic data in grape berries demonstrated that changes in protein levels can anticipate and foresee flux changes (Wang *et al*., 2018), a case cited to further support the embedding of proteomic data in GRN

rather than treating it as an add-on. Metabolomic (LC-MS, GC-MS) and ICP-MS-based ionomic analyses of mineral content capture the final biochemical phenotype, and support correlation-based retrodiction to upstream transcriptional modules (Alseekh *et al*., 2021; Fernie *et al*., 2004; Lahner *et al*., 2003). The combination of such terminal-phenotype layers with transcriptomic and epigenomic data has been particularly valuable in multi-omics investigations of water-stressed grape berries (Savoi *et al*., 2017), postharvest kiwifruit ripening, black raspberry ripening (where DNA methylation tracked directly to which anthocyanin species accumulated), and Jaboticaba fruit peel colour development, where WGCNA identified a large co-expression module with six anthocyanin structural genes and 98 transcription factors.

4. Approaches to Gene Regulatory Network Inference and Functional Validation

Statistical and computational inference that converts correlative multi-omics data into testable regulatory hypotheses (Figure 3). WGCNA is still the most frequently employed approach in multi-omics studies applied to horticulture: genes are clustered into co-expression modules and each module's eigengene is correlated with phenotypic traits to identify candidate hub genes. The strategy has revealed hub regulators in the anthocyanin networks of maize pericarp, blood orange, *Coffea arabica* peel, chrysanthemum and eggplant, among other systems (Langfelder and Horvath, 2008).

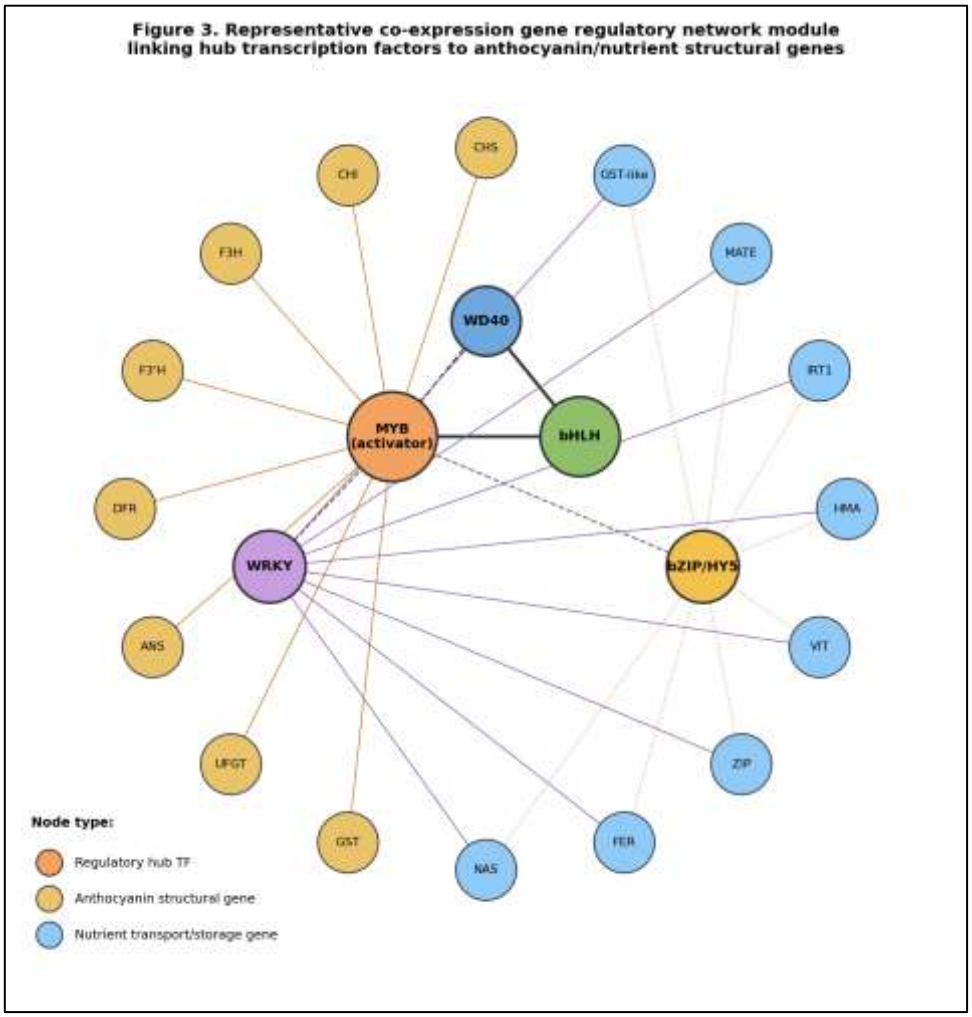

Figure 3. A representative co-expression gene regulatory network module linking hub transcription factors (MYB, bHLH, WD40, WRKY, bZIP/HY5) to anthocyanin structural genes and nutrient transport/storage genes, illustrating the hub-and-spoke topology typical of WGCNA-derived modules. (Original figure, adapted from the WGCNA framework of Langfelder and Horvath, 2008)

Besides WGCNA, inference methodologies that integrate chromatin accessibility superimposing ATAC-seq/ChIP-seq footprints onto expression data further focus the hunt for direct transcription factor-target interactions. Gradient-boosting and deep-learning frameworks developed for mammalian systems, e.g. SCING and maxATAC, are starting to be extended for GRN inference from single-cell and chromatin-accessibility data, and they tend to outperform pure correlation-based methods at comparable scale. However, regardless of the inference method, predictive regulatory edges must still be confirmed experimentally. That validation usually involves yeast-one hybrid (Y1H) and two hybrid (Y2H) assays, EMSA, ChIP-qPCR, dual-luciferase reporter assays, virus induced gene silencing (VIGS), as well as stable or transient overexpression or CRISPR-based knock out performed in the species under investigation or in heterologous systems like tobacco and Arabidopsis, as

shown for functional verification of MdMYB66-MdF3H, FaRAV1-FaMYB10 and AtrANS-anthocyanin pathway interactions.

5. Comparative Case Studies in Fruit Crops

5.1 Pome and Stone Fruits

In apple, transcriptomic data combined with genome resequencing identified a mini-satellite repeat located upstream of MdMYB10 that mediates the gene's own autoregulation and is the molecular basis for red-fleshed phenotypes (Espley *et al*., 2009; Chagne *et al*., 2013; Zhang *et al*., 2019). Both ripening-associated repressors MdMYB6 and MdMYB16 can bind to the promoters of sugar-transport genes including MdTMT1, which provides a connection between anthocyanin accumulation and soluble-sugar metabolism.

5.2 Grape and Small/Berry Fruits

The colour of a grape berry is largely determined by the VvMYBA1/VvMYBA2 locus, which is disrupted in white cultivars by the insertion of the Gret1 retrotransposon (Kobayashi *et al*., 2004), and the proanthocyanidin and the flavonol branches are controlled independently by VvMYBPA1/PA2 and VvMYBF1 (Bogs *et al*., 2007). To our knowledge, this is the most comprehensive study combining transcriptomics and targeted metabolomics in water-deficit. The VvMYB15-VvWRKY40 and VvMYBA1-VvWRKY44-VvMYB308 modules provide a striking example of how WRKY proteins can further mediate stress and developmental signals through the core MBW module. Blueberry features a similar MBW complex VcMYBL1, VcbHLH1, VcWDL2 along with a SQUAMOSA MADS-box regulator that controls developmentally regulated anthocyanin accumulation (Zhao *et al*., 2019; Jaakola *et al*., 2010). WGCNA-based hub analysis has brought out similar signatures in Aronia melanocarpa (AmMYB10) and mulberry, where a haplotype-resolved genome assembly combined with GWAS pinpointed a vacuolar H^+-ATPase subunit gene (MaVHAG3) associated with purple pigmentation, and where ATAC-seq/RNA-seq integrative approaches unveiled the chromatin-level network operating during the colour turning stage.

5.3 Strawberry

Tractable transient transformation systems in strawberry have rendered it a model species for the analysis of anthocyanin GRNs. FaMYB10 is the master activator, triggered independently by light and by ABA signaling through the FaNCED1/FaCHLH-mediated ABA biosynthesis and perception (Aharoni *et al*., 2001; Medina-Puche *et al*., 2014). FaRAV1 activates FaMYB10 and its biosynthetic-gene promoters directly (Jiang *et al*., 2023); a complex accessory network modulates it: FaMYB6-like and FaMYB44.2 act as feedback repressors and link anthocyanin accumulation to sucrose metabolism as well by forming complexes with FabHLH3 and binding to the promoters of sucrose-phosphate-synthase; and FaANL2, a HD-Zip protein, represses FaMYB10 in a light- and hormone-dependent way. Homoeolog-specific CRISPR/Cas9-mediated mutagenesis of MYB10-1B in octoploid strawberry has provided such direct causal evidence by producing white-fruited lines with the co-suppression of CHS, DFR and ANS.

5.4 Citrus

Pigmented citrus-like blood orange results from independent insertions of a Copia retrotransposon upstream of the Ruby1 MYB gene that drive cold-dependant transcriptional activation (Butelli *et al*., 2012; Huang *et al*., 2018, 2019). Recent data demonstrate that cold-induced ethylene response factors also directly activate Ruby1 through this retrotransposon-derived promoter, thereby integrating temperature sensing into the citrus anthocyanin GRN (Wang *et al*., 2024). The fruit specific beta-cyclase 2 gene has also been targeted by CRISPR/Cas9 editing to stack anthocyanin and lycopene pigmentation within a single variety.

5.5 Tomato and Other Solanaceous Fruits

Alternative splicing of the cryptic intron I2 in the R2R3-MYB gene SlAN2-like is the reason why cultivated tomato fruit naturally lacks anthocyanins (Colanero *et al*., 2019). By introgressing wild Solanum alleles, or ectopically expressing the snapdragon-derived Delila and Rosea1-type MYB/bHLH factors, purple tomato enriched in anthocyanin with enhanced antioxidant capacity was successfully obtained (Butelli *et al*., 2008; Zhang *et al*., 2015). Ripe tomato fruit colour is also, independently, determined by a carotenoid mainly lycopene that is regulated by a hierarchical transcriptional network mediated by SlAP2a/SlAP2c, EIN3/EIL and ethylene-responsive factors regulating histone acetylation at lycopene-pathway genes. This is a good reminder that GRNs for fruit colour frequently may show interplay between the flavonoid and carotenoid pathways of secondary metabolism (Duduit *et al*., 2022).

6. Comparative Case Studies in Vegetable Crops

Vegetable crops are subject to the same MBW-containing regulatory logic described above, but they frequently execute it in root or non-fruit tissues, demanding tissue-specific regulatory adaptations.

Table 2. Multi-omics case studies of anthocyanin gene regulatory networks in representative vegetable and vegetable-fruit crops.

Crop	Tissue studied	Omics approach(es)	Key regulatory finding
Eggplant (Solanum melongena)	Fruit epidermis, vegetative tissue	GWAS (MAGIC population), LD/association mapping	MYB113-like and COP1 loci associated with pigmentation; SmWRKY44 identified as WGCNA hub gene

Crop	Tissue studied	Omics approach(es)	Key regulatory finding
Pepper (Capsicum spp.)	Fruit	GWAS, QTL mapping	A-locus MYB linked with fs10.1 fruit-shape QTL; candidate genes for agronomic/pigment traits
Potato (Solanum tuberosum)	Tuber, leaf	Transcriptomics, metabolomics, TF overexpression	StAN1 (with StbHLH1/StWD40) is master regulator; miR828 associated with skin/flesh colour
Sweet potato (Ipomoea batatas)	Tuberous root	Comparative transcriptomics	IbMYB1/IbbHLH2/IbWRKY activators counteracted by IbMYB27/IbMYBx-ZZ repressors
Radish (Raphanus sativus)	Taproot	Transcriptomics, epigenetics	Transposon-induced RsMYB1 promoter methylation silences red-flesh phenotype
Lettuce (Lactuca sativa)	Leaf	Whole-genome resequencing, GWAS (811 accessions)	ANS-linked QTL validated and deployed in genomic design breeding of high-anthocyanin cultivar
Purple cauliflower/Brassica (Brassica spp.)	Curd/stem tissue	Transcriptomics, biochemical profiling	MYB-dependent activation of anthocyanin pathway in purple tumorous stem mustard
Coffee (Coffea arabica)	Fruit peel	Metabolomics + transcriptomics + WGCNA	Structural genes (3AT, BZ1, lcyE) and MYB/NAC/bHLH TFs co-regulate anthocyanin and carotenoid pigmentation

A common theme in these vegetable systems is the presence of MBW-equivalent activator complexes AN1/StAN1, IbMYB1, RsMYB1, SmMYB113-like which are negatively regulated by tissue or condition-specific repressor MYBs and further influenced by transposable-element insertions and promoter methylation. This suggests that epigenetic silencing is a highly conserved mechanism for regulating anthocyanin GRN output in a manner independent of coding-sequence variation.

7. Multi-Omics Insights into Nutrient (Mineral) Accumulation and Biofortification

The ionome- the full complement of mineral and trace elements found within a plant tissue can now be measured at high throughput using ICP-MS, and can be used for GWAS and QTL mapping of traits related to elemental accumulation similar to how anthocyanin pigmentation traits have been mapped (Lahner *et al*., 2003; Baxter *et al*., 2008; Salt *et al*., 2008). Transport and chelation networks of iron and zinc are controlled by a complex of genes encoding for nicotianamine synthase (NAS), ferritin (FER), ZIP-family zinc transporters, vacuolar iron transporters (VIT), heavy-metal ATPases (HMA), and IRT1-type iron-regulated transporters whose expression is coordinately regulated to control the efficiency with which minerals are absorbed, transported via the xylem/phloem, and deposited into edible tissue (Connorton and Balk, 2019; Connorton *et al*., 2017; Bashir *et al*., 2011; Sperotto *et al*., 2012).

This GRN-level knowledge of transporter networks has nourished agronomic, conventional-breeding and transgenic strategies to biofortification alike. Overexpression of NAS and ferritin genes has also increased the levels of iron and zinc in potato tubers and rice endosperm, and GWAS loci co-locating with known ionome-gene orthologues has fast tracked marker assisted selection for high-iron and high-zinc cultivars in rice, wheat and maize (White and Broadley, 2009; Bouis and Saltzman, 2017). Work in maize with multi-omics approaches has demonstrated that phosphorus fertiliser dramatically alters the whole-plant ionome, with genotype-by-environment interactions having a strong effect on the efficiency of zinc translocation yet another indication that nutrient GRNs, just like anthocyanin GRNs, are plastically responding to the environment conditions rather than behaving as fixed circuits.

Table 3. Representative gene network nodes linking multi-omics evidence to mineral nutrient accumulation and biofortification traits.

Nutrient/trait	Key gene(s)/network node(s)	Omics evidence	Representative reference
Iron accumulation	NAS, FER, IRT1, VIT, HMA	GWAS, ionomics (ICP-MS), transgenics	Connorton and Balk, 2019; Bashir *et al*., 2011
Zinc accumulation	ZIP transporters, NAS	Ionomic GWAS, QTL mapping	White and Broadley, 2009; Salt *et al*., 2008
Provitamin A (carotenoids)	PSY, PDS, LCYB/LCYE, CYP97 family	Transcriptomics, targeted metabolomics	Duduit *et al*., 2022; Zhang *et al*., 2015
Anthocyanin-nutrient co-regulation	MYB-HY5-ABA cross-talk nodes	Multi-omics (transcriptome + metabolome + ionome)	Savoi *et al*., 2017

8. Epigenetic and Environmental Integration into Anthocyanin and Nutrient GRNs

DNA methylation, histone modification and small RNA-mediated silencing (Figure 2) are now considered to be basic elements rather than a peripheral superstructure of anthocyanin GRNs. Promoters of MYB10/MYB1 are hypermethylated in striped and bud-sport and yellow fruit phenotypes in apple and pear, respectively, while genome-wide dynamic RNA-directed DNA methylation (RdDM) and active demethylation reprogram the methylome in response to light mediating the expression of PyUFGT, PyDFR, and other structural genes even the MYB10 promoter itself is not the target of this process (Bai *et al*., 2017; El-Sharkawy *et al*., 2015). In black raspberry, system wide methylation changes during ripening have been linked directly to which anthocyanin species accumulate through multi-omics profiling, and in radish a transposon insertion and subsequent promoter methylation of RsMYB1 completely shuts down the anthocyanin GRN, resulting in white-fleshed roots (Xie *et al*., 2014).

Histone modifications including H3 acetylation and H3K4 trimethylation are associated with transcriptional activation of MdMYB1 alleles in response to light and temperature signals that induce pigmentation, whereas SPL transcription factors regulated by microRNA miR156 destabilize the MBW complex, reducing anthocyanin biosynthesis during floral and fruit maturation (Gou *et al*., 2011). Taken together, these results suggest that the GRNs controlling the biosynthesis of anthocyanins, and by extension nutrients, are not hardwired. These are circuits that are constantly being reshaped by light quality and duration, ambient and diurnal temperature, water and nutrient status, sugar signalling and developmental stage variables that any multi-omics GRN model seeking to predict breeding outcomes will have to explicitly model.

9. Genome Editing and Biotechnological Translation of GRN Knowledge

CRISPR/Cas-based genome editing has now become the main strategy for testing GRN hypotheses and for directly engineering traits in horticultural crops. Targeted disruption of FveMYB10 and FveCHS in diploid and octoploid strawberry resulted in predictable reductions in anthocyanin levels, and homoeolog-specific editing of MYB10-1B resulted in white-fruited strawberry lines with coordinated down-regulation of CHS, DFR and ANS the first direct causal evidence for the FaMYB10-centred network. In citrus, dual-sgRNA CRISPR/Cas9 editing of the fruit-specific beta-cyclase 2 gene have been exploited to generate varieties that produce both anthocyanin and lycopene pigmentation, a trait that could not be produced through conventional breeding due to sexual incompatibility between the candidate parental genotypes.

In addition to basic loss-of-function editing, CRISPR activation systems such as CRISPR-Act3.0 enable multiplexed and dosage-dependent up-regulation of anthocyanin and other secondary-metabolites pathway genes as demonstrated, e.g., in pear calli providing a gain-of-function approach to biofortification that is complementary to traditional overexpression transgenics. Anthocyanin-marker aided CRISPR (AAC), which integrates pathway activation with a CRISPR/Cas9 cassette as a visible transformation marker, has facilitated the obtainment of transgene-free edited rice plants, a more practical benefit from knowledge on anthocyanin GRN (He *et al*., 2020). On the nutrition front, transgenic co-expression of the nicotianamine synthase and ferritin genes has been used to accomplish iron and zinc biofortification in potato tubers concurrently, suggesting that GRN-based multigene stacking can surpass single-gene approaches for intricate nutrient traits.

10. Nutritional and Health Significance of Anthocyanin- and Nutrient-Enriched Produce

The case for engineering anthocyanin and nutrient-rich produce is not only based on agronomy or molecular biology, but also supported by a large body of evidence from nutritional and clinical studies. Anthocyanins have antioxidant, anti-inflammatory, and anti-apoptotic properties relevant to the prevention and treatment of cardiovascular disease, obesity, type-2 diabetes, and some cancers, and epidemiological studies have reported an association between higher anthocyanin intake and improved cardiometabolic and cognitive outcomes (Li *et al*., 2017; Khoo *et al*., 2017; Manolescu *et al*., 2019). Clinical and preclinical studies have additionally shown that anthocyanins and their gut-microbial metabolites affect inflammatory markers, lipid profiles, and oxidative-stress biomarkers, and that the bioavailability and metabolism of anthocyanins are influenced by the glycosylation and acylation pattern of the parent anthocyanidin (Noman *et al*., 2025).

Iron, zinc and provitamin A deficiencies are still among the most prevalent forms of malnutrition globally, affecting disproportionately women in the reproductive age and young children residing in low- and middle-income countries. Iron- and zinc-biofortified crops produced through conventional breeding and transgenic approaches as well as agronomic biofortification have demonstrated population-level composition and efficacy in biofortified crops for iron and zinc is proof that multi-omics-aided mining of GRN hub genes for nutrient transport and accumulation has real, translatable public-health value that surpasses basic science (Bouis and Saltzman, 2017; Vasconcelos *et al*., 2017).

11. Challenges and Future Perspectives

Several theoretical and technical challenges remain to bridge between the current state of the field and the stage of routine multi-omics GRN reconstruction in horticultural breeding. The first is data heterogeneity: platform-related biases within genomics, transcriptomics, proteomics and metabolomics make it challenging to combine data in a statistically meaningful way, and standardized pipelines tailored for horticultural crops are much less advanced than those for model species or human disease. Approximately 1/3rd of GRNs published are causal as

opposed to correlative WGCNA and similar co-expression approaches do identify statistically associated hub genes, but they cannot distinguish direct regulatory relationships from indirect, co-regulated, or 'bystander' ones. This means validation downstream via Y1H/Y2H, ChIP and CRISPR-based perturbation is an unavoidable bottleneck, "rate-limiting step" in their parlance. Third, the single-cell and spatial multi-omics technologies that are truly transformative in model organisms are currently challenging and expensive to implement in the polyploid, heterozygous, and frequently poorly annotated genomes that define most horticultural crops for example the octoploid strawberry and hexaploid sweet potato, as well as many clonally propagated fruit species.
A fourth caveat is that the majority of GRN analyses so far have focused on a single omics layer, or at best a double omics layer transcriptome-metabolome dataset. Truly integrative approaches at the level of genomic variation, chromatin accessibility, transcriptome, proteome and metabolome/ionome data integrated in a single statistical framework are still not very common, and precisely such type of integration would be needed to dissect causal hierarchies and cross-talk between the anthocyanin and nutrient-transport networks that were outlined during this review. Fifth, the pathway from validated GRN hub genes to elite cultivars still relies on robust, genotype-independent transformation and regeneration methodologies a bottleneck that remains for many woody perennial fruit crops.
Looking ahead, several trends are expected to drive advancement of the field collectively: the adoption of machine-learning and deep-learning GRN inference methods, which can integrate a variety of heterogeneous, sparse and multimodal omics data; the development of single-cell and spatial multi-omics atlases for staple fruits and vegetable crops; integration of AI-assisted variant-to-function prediction with GWAS and pangenome resources to bridge the gap from statistical association to validated causal genes; and closer linkage of GRN-discovery pipelines with CRISPR/Cas-based functional validation and precision breeding, such that candidate hub genes from multi-omics integration will more rapidly transition into elite breeding lines.

12. CONCLUSION

The use of multi-omics integration has transformed the investigation of anthocyanin and nutrient accumulation in fruits and vegetables from a gene-by-gene approach into a systems-level science that can elucidate the complex interplay between the core MYB-bHLH-WD40 (MBW) complex and its downstream targets, the chromatin and epigenetic states that regulate their activity, and the environmental and hormonal signals that modulate GRN output across development and stress. The comparative case studies presented here from apple, grape, citrus, strawberry, blueberry, tomato, eggplant, potato, sweet potato, lettuce and other crops reveal both a high degree of conservation of the core MBW architecture and significant species- and tissue-specific diversification, driven by the recruitment of accessory transcription factors, transposable-element-mediated promoter evolution, and epigenetic silencing. The same multi-omics and network-inference frameworks are now beginning to produce similarly high-resolution pictures of the transporter and chelator networks involved in iron, zinc, and provitamin A accumulation in the context of ionomics and biofortification. Future investment in integrative, causally validated, and experimentally testable GRN models complemented by CRISPR/Cas-based functional validation and precision breeding shows real potential for systematic improvement of colour, flavour and nutritional quality in horticultural crops on a global scale.

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