Molecular mechanisms underlying environmental and epigenetic fruit ripening control in highbush blueberry

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Introduction

Highbush blueberry (*Vaccinium corymbosum*) is grown worldwide and known as a "superfruit" with high economic and nutritional value. However, the short harvest season and non-uniform sequential ripening of individual fruit within a cluster lead to high labor costs. To address these problems, efficient artificial methods for control of blueberry fruit ripening should be developed based on a deeper understanding of the fruit ripening mechanism in blueberry. Fruit ripening involves significant alteration of the color, aroma, flavor, texture, and other metabolites, which is coordinately regulated by intrinsic developmental cues and various environmental factors, including light quality. The blueberry fruit is classified as a typical non-climacteric type in which abscisic acid (ABA) primarily regulates the ripening process. In this study, the effects of ultraviolet-B (UV-B), epigenetic modification, and ABA on highbush blueberry fruit ripening were studied with the aim of clarifying the underlying molecular mechanisms.

Chapter 1 Promotion of fruit ripening and stage-specific modification of anthocyanin metabolism by preharvest long-term exposure to UV-B radiation

Ultraviolet-B light (280–315 nm) is an important environmental signal that regulates plant development and secondary metabolism. However, the effect of long-term preharvest UV-B irradiation on fruit growth and coloration remains unclear. The effect of long-term UV-B treatment involving an environmentally relevant dose on highbush blueberry fruit was tested in this chapter. The preharvest UV-B treatment quickly promoted fruit growth and sugar accumulation. This promotional effect of UV-B on fruit growth and sugar accumulation is not commonly observed in other fruit species. Exposure to UV-B also accelerated ripening and coloration of blueberry fruit, which has been previously reported in certain other species. A dual-luciferase assay revealed that expression of *VcUFGT*, a crucial anthocyanin

biosynthesis-related gene, was positively and negatively regulated by *VcMYBA1* and *VcMYBC2*, respectively, in blueberry. Throughout fruit development, UV-B treatment upregulated *VcMYBPA1* expression, leading to increased *VcUFGT* expression. At the green fruit stage, UV-B treatment enhanced transcription of *HY5*, which encodes a UV-receptor. HY5 was shown to play a role in the upregulation of *VcMYBPA1* and downregulation of *VcMYBC2*, and thereby anthocyanin accumulation was promoted by upregulation of HY5 transcription by UV-B treatment. In mature fruit, UV-B inhibited excessive anthocyanin synthesis by enhanced *VcMYBC2* transcription through a HY5-independent pathway. In conclusion, modified expression of a MYB activator and repressor of *VcUFGT* by UV-B may coordinately balance the accumulation of anthocyanins in fruit in a stage-specific manner.

Chapter 2 Spatiotemporal genome-wide DNA methylation changes during fruit ripening and DNA demethylation-induced cluster ripening

Involvement of epigenetic control, such as DNA methylation, in the regulation of fruit ripening has been recently proposed in some species; however, the detailed mechanism remains unclear owing to the lack of systematic integrative analyses. Whole-genome methylation sequencing of blueberry fruit peel and flesh at different developmental stages (small green, green, pink, and mature stages; SG, G, P, and M, respectively) showed that the status of CHG- and CHH-type methylated cytosines (mCs) changed differently in the peel and flesh from the G to the M stages. mRNA-sequencing analyses indicated that RNA-dependent DNA methylation (RdDM) pathway-related genes (typically *VcAGO4*) were downregulated from the G to the P stages in the peel and flesh. Thereafter, RdDM-related genes were up- and downregulated in the flesh and peel, respectively, at the M stage, which induced hypermethylation in the flesh but hypomethylation in the peel. In blueberry, the increase in ethylene production occurs in parallel with ABA accumulation, which led us to hypothesize that ethylene may induce methylation changes. As expected, ethylene treatment negatively regulated *VcAGO4*, suggesting that the transient ethylene rise in blueberry may trigger DNA methylation reconfiguration and can be memorized in chromatin. Artificial demethylation treatment with 5-azacitidine (5-AzaC) at the G stage advanced fruit coloration and increased anthocyanin contents, resulting in enhanced consistency in fruit ripening in a

cluster. However, this effect was not observed with 5-AzaC treatment at the SG stage. Transcriptome profiles suggested that ABA biosynthesis was activated in the G stage but not in the SG stage, raising the possibility that ABA may be necessary for 5-AzaC-induced cluster ripening. Accordingly, combined treatment of ABA and 5-AzaC at the SG stage induced anthocyanin biosynthesis in the peel through activating the crosstalk between ABA and demethylation, especially of *VcMYBA1*. In addition, combined treatment promoted sugar accumulation in the flesh, accompanied by ABA- and demethylation-induced higher expression of *VcSWEET*s, sugar transporter genes. Taken together, these results suggest that DNA (de)methylation may be a hub regulator for gene expression reprogramming for the onset of ripening by integrating the ethylene cue into ABA-induced fruit ripening.

Chapter 3 Effects of ABA, DNA demethylation, and combined ABA and DNA demethylation treatment on ABA biosynthesis and signaling homeostasis

In this chapter, the impact of combined ABA and DNA demethylation treatment on blueberry fruit ripening was further explored. Genome-wide transcriptome and methylome analyses revealed that ABA treatment promoted ABA biosynthesis and suppressed ABA-activated ABA signaling, whereas 5-AzaC treatment promoted the ABA signaling pathway by inducing demethylation of CHG- and CHH-type mCs. Combined ABA and 5-AzaC treatment inhibited ABA biosynthesis through downregulation of ABA synthesis and transport pathways, and upregulation of ABA catabolic pathways. These results collectively suggested that, in the combined treatment, 5-AzaC-induced hypomethylation may reduce the inhibitory effect of ABA on ABA-activated signals, thereby promoting global expression of ABA-responsive genes. A novel ABA and chromatin crosstalk was observed through the modified expression of *VcWRKY24*, an ABA-induced negative regulator of ABA signaling. ABA and hypomethylation in *VcWRKY24* may antagonistically affect ABA-activated signaling pathways, which may fine-tune ABA transduction. Collectively, combined ABA and DNA demethylation treatment might promote fruit ripening by not only regulating core regulatory genes, but also modifying ABA biosynthesis and signaling homeostasis.

Conclusion

This study showed that blueberry fruit ripening can be modified and manipulated by environmental and epigenetic modifications. Modification of fruit ripening was achieved by activation of crucial ripening-related regulators and modification of the ABA signaling pathway through changes in the DNA (de)methylation status of the genome. On the practical side, this study demonstrated the potential of these modifications for future establishment of artificial treatments for cluster harvesting in blueberry.