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Impact of blue, red, and far-red light treatments on gene expression and steviol glycoside accumulation in *Stevia rebaudiana*

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ABSTRACT

Stevia rebaudiana (Bertoni) Bertoni is a plant that biosynthesizes a group of natural sweeteners that are up to approximately 400 times sweeter than sucrose. The sweetening components of *S. rebaudiana* are steviol glycosides (SGs) that partially share their biosynthesis pathway with gibberellins (GAs). However, the molecular mechanisms through which SGs levels can be improved have not been studied. Therefore, transcription levels of several SG biosynthesis-related genes were analyzed under several light treatments involved in GA biosynthesis. We detected higher transcription of *UGT85C2,* which is one of the UDP-glycosyltransferases (UGTs) involved in catalyzing the sugar-transfer reaction, under red/far-red (R/FR) 1.22 light-emitting diodes (LEDs) and blue LEDs treatment. In this study, it was demonstrated that transcription levels of SG-related genes and the SGs content are affected by light treatments known to affect the GA contents. It is expected that this approach could serve as a practical way to increase SG contents using specific light treatments.

1. Introduction

Stevia rebaudiana (Bertoni) Bertoni is a perennial plant that belongs to the Asteraceae family and is native to Brazil and Paraguay. Its principal sweetening components are steviol glycosides (SGs), which can confer strong sweetness and are mainly extracted from leaves (Singh and Rao, 2005). *S. rebaudiana* is used by diabetic patients as a diet therapy, and its extracts exhibit pharmacological effects, such as anti-insulin resistance, insulin secretion promotion, anti-hypertensive and anti-obesity (Chen et al., 2005, 2006; Dyrskog et al., 2005) properties. SGs are diterpene secondary metabolites and share their biosynthesis pathway with gibberellins (GAs) through the formation of *ent*-kaurenoic acid (Fig. 1 i). Steviol (Fig. 1 ii) serves as a basic skeleton to which β-glucose is added to advance SG biosynthesis (Geuns, 2003). According to Geuns (2003), the contents of SGs are 4–20% of the dry leaf weight, but depend on the cultivar and growing conditions. The largest percentage of SGs are composed of stevioside (Fig. 1 v), whose yield is 0.6–7.9% (w/w), followed by rebaudioside-A (reb-A) (Fig. 1 vi), whose yield is 0.3–6.5% (w/w) (Vouillamoz et al., 2016). The sweetness intensity of stevioside (Fig. 1 v) is approximately 193 times greater than that of 2% sucrose, and the sweetness intensity of reb-A (Fig. 1 vi) is approximately 400 times higher than that (Schiffman et al., 1995). Schiffman et al. (1995) also investigated the relationship between the sweetness and taste of sweeteners. SGs have strong sweetness. However, they can also taste bitter at the same time. The SG reb-A (Fig. 1 vi) (Fig. 1 vi) is sweeter and less bitter than stevioside (Fig. 1 v). In addition, recent investigations have demonstrated that several minor SGs are stronger sweet-tasting compounds than stevioside (Fig. 1 v) and reb-A (Fig. 1 vi) (Hellfritsch et al., 2012; Espinoza et al., 2014). As an example, rebaudioside M, which has more β-glucose bound to steviol (Fig. 1 ii) than stevioside (Fig. 1 v) or reb-A (Fig. 1 vi), is 200–350 times sweeter than sucrose, and its bitter aftertaste is reduced compared to reb-A (Fig. 1 vi) (Prakash et al., 2014).

In previous genetic research on *S. rebaudiana*, Ohlrogge and Benning (2000) and Brandle et al. (2002) analyzed expressed sequence tags (ESTs), and various genes from the SG biosynthetic pathway have been sequenced. In recent years, SGs have even been investigated at the molecular level.

Kaurene oxidase (KO) is an enzyme that is common to the SG and GA biosynthetic pathways. It is an especially important enzyme

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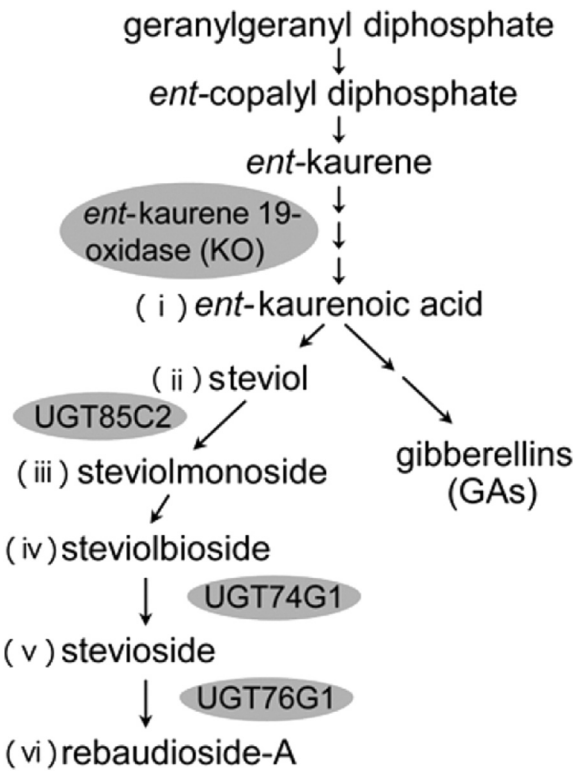

Fig. 1. Metabolic pathway of steviol glycosides (SGs) and gibberellins (GAs). The enzymes are: *ent*-kaurene 19-oxidase (KO), and uridine diphosphate-dependent glycosyltransferase (UGT). This pathway is a simplified schematic pathway referred to by Richman et al. (2005); Mohamed et al. (2011).

in SG and GA biosynthesis (Helliwell et al., 1998, 1999) because it is one of the most highly expressed SG-related genes (Kumar et al., 2012).

Many studies have investigated the enzymes in SG biosynthesis that are shared by the GA biosynthesis pathway, but are unique to these pathways. UGT85C2, UGT74G1, and UGT76G1 are UDP-glycosyltransferases (UGT) that catalyze sugar-transferring reactions to add glucose to the steviol (Fig. 1 ii) basic skeleton (Richman et al., 2005). UGT85C2 catalyzes the synthesis of steviolmonoside (Fig. 1 iii) from steviol (Fig. 1 ii). UGT74G1 catalyzes the synthesis of several SGs, including stevioside (Fig. 1 v), from steviolbioside (Fig. 1 iv). UGT76G1 also catalyzes several SGs and can add glucose to stevioside (Fig. 1 v) to form reb-A (Fig. 1 vi).

The relationship between these enzymes and the SGs content has been previously researched. For example, Guleria et al. (201 1) reported that transcription levels of *KO*, *UGT85C2*, and *UGT76G1* were upregulated after treatment with 5% sucrose compared to their transcription levels after treatment with 3% sucrose. Mohamed et al. (2011) suggested that the glycosylation involved in *UGT85C2* is a rate-limiting step in SG biosynthesis. Based on these studies, transcription analysis of these enzymatic genes can serve as an indirect analysis of the levels of SGs.

Some studies have demonstrated a relationship between SGs and GA. Because these compounds share the same biosynthesis pathway up to *ent*-kaurenoic acid (Fig. 1 i), an effect on the accumulation of SGs can be expected. Kumar et al. (2012) reported that *SrUGT74G1* was upregulated by GA treatment. Similarly, Hajihashemi et al. (2013) reported that transcription of *KO* was significantly upregulated following GA treatment compared to transcription in the control. Recent investigations have further demonstrated that SG biosynthesis is closely related to GA biosynthesis.

Numerous studies have investigated genes encoding GA biosynthesis enzymes. The transcription level of these genes is controlled by light (Ait-Ali et al., 1999; Zhao et al., 2007). It is well known that a red-light photoreceptor, called phytochrome, is involved in GA regulation (Toyomasu et al., 1998; Smith, 2000). There are two phytochrome conformations: Pfr (active form) and Pr (inactive form). Pfr is converted into Pr under 730 nm far-red light. Pr is converted into Pfr under 660 nm red light. In several species, such as cowpea, *Arabidopsis thaliana,* and cucumber, GA biosynthesis is promoted by far-red light (Kamiya and García-Martínez, 1999; García-Martinez and Gil, 2001). In studies examining the effects of light quality on *S. rebaudiana*, the levels of SGs were higher when *S. rebaudiana* was grown under short-day conditions with night-interruption by red light-emitting diodes (LEDs) compared to the control conditions (Ceunen et al., 2012).

Another property of light has also been implicated in GA regulation. It has been reported that cryptochrome, which is a blue light photoreceptor, contributes to the inactivation of GA_1, which is a bioactive GA under blue light treatment (Zhao et al., 2007).

Given that the SGs content can be easily modulated by changing supplemental light, it can be expected to be able to increase the yield of SGs using particular light qualities. However, the relationship between SGs and the light qualities that affect GA biosynthesis has not been investigated thoroughly. The details about these light conditions are still unclear. Therefore, *S. rebaudiana* we investigated under various light conditions that are involved in GA biosynthesis to increase the volume of SGs. To confirm that SGs-rich

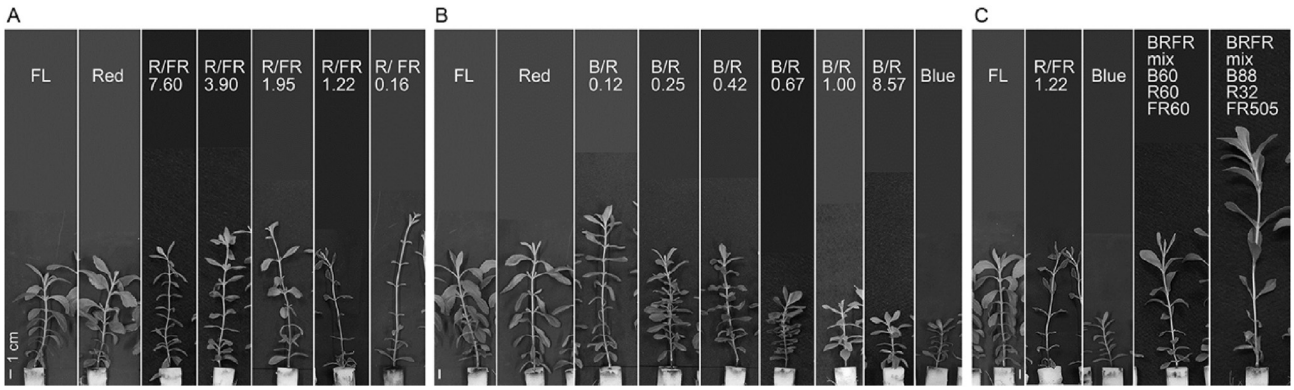

Fig. 2. Effect of various light quality treatments. Various red and far-red LEDs light conditions (A). Various blue and red LEDs light conditions (B). Various blue, red and far-red LEDs light conditions (C). Fluorescent lamp (FL), red LEDs (Red), red and far-red LEDs ratio (R/FR), blue and red LEDs ratio (B/R), blue LEDs (Blue), blue, red and far-red LEDs mix (BRFR mix). Scale bar indicates 1 cm. All of samples were collected 6 weeks after cutting.

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