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REVIEW

Optimization Process of Plant Growth Environment for Improving Content Compounds Using Physiological and Genetic Information in a Closed-type Plant Factory

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Abstract

A closed-type plant factory (plant factory) can control growth environments in confined spaces with artificial lights and hydroponic systems. The growth environment of a plant factory can be modulated without having to consider the natural climate, so that high value plants rich in useful compounds or low in toxic compounds can be uniformly and consistently produced. However, the process of optimizing growth condition settings to increase or decrease the target compounds tends to be complicated, given the various control parameters in a plant factory. An efficient optimization process for producing high value plants was shown using physiological and genetic information in the case of *Stevia rebaudiana* (stevia). This case study was conducted to determine the optimal conditions for producing stevia rich in sweet steviol glycosides (SGs) compounds. All environmental condition settings were selected based on previous studies relating to the biosynthesis of such SGs as stevioside (Stev) or rebaudioside-A (Reb-A). A yield evaluation of Stev and Reb-A was conducted based on the transcription levels of SGs-related genes. The process of producing SGs-rich stevia provides an example of an efficient optimization process for producing high value plants with increased or decreased target compounds in a plant factory system.

Discipline: Agricultural Engineering
Additional key words: control light environment, high value plants, stevia, sweetening

Introduction

A closed-type plant factory (plant factory) is a type of closed plant production system that makes use of artificial lights and hydroponics in confined spaces isolated from the external natural environment. There are several differences between plant factory and field or greenhouse cultivation (Kozai et al. 2019). Plant factories are confined spaces separate from the natural environment; therefore, the interior climate of a plant factory can be kept uniform, compared with a field or greenhouse (Fig. 1). Moreover, plant factory systems maximize the use of nutrient solutions and are otherwise agrochemical-free to minimize environmental pollution. Yield and quality of produce tend to improve in plant factory systems, as compared with those of a field or greenhouse in terms of environmental control. In addition, the utilization of aerial space by using vertical farming techniques and shelves increases spatial efficiency. Graamans et al. (2018) reported that under appropriate conditions the cultivation cycle length for lettuce production in plant factories was reduced by half as compared with greenhouse cultivation. They also reported that due to consistent and uniform plant factory production, annual dry weight production was greater in plant factories than in greenhouses. In a comparison between plant factory and field cultivations of stevia, Yoneda (2018) investigated an expression of steviol glycosides (SGs) related gene. UDP-glycosyltransferase *UGT85C2*, which is related to a rate-limiting step in the biosynthesis of SGs with the yield quantity (Mohamed et al. 2011, Guleria et al. 2011). The relative transcription

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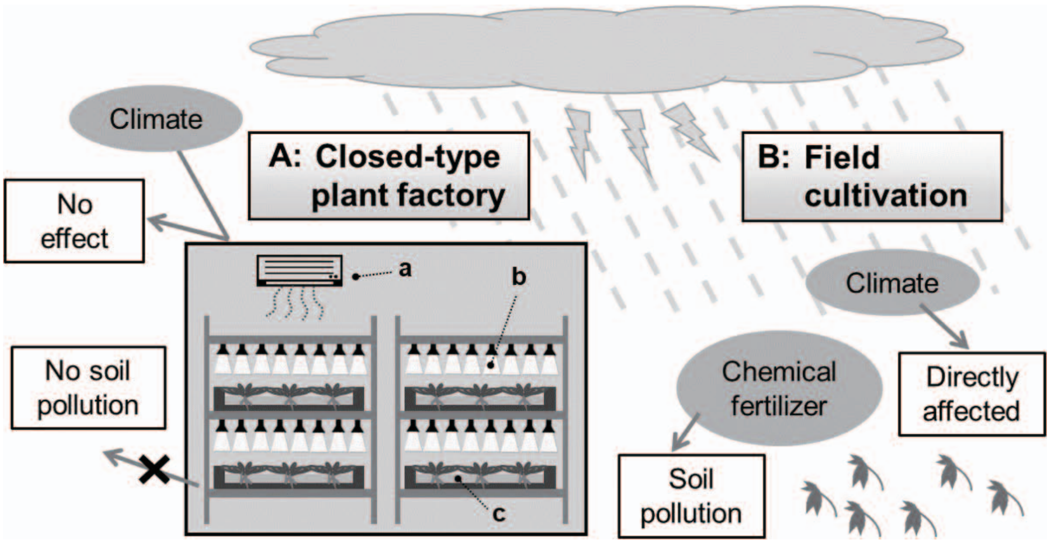

Fig. 1. Closed-type plant factory and field cultivation
A closed-type plant factory (A) is able to control growth conditions using air conditioning (a), artificial lights (b), and hydroponic environment (c). Field cultivation (B) depends on the natural environment.

levels of *UGT85C2* in stevia under appropriate conditions in a plant factory were about 200 times greater than those under a sunny place in field cultivation (Fig. 2).

From this finding, it is evident that plant factories can consistently produce high value plants when using optimal environmental conditions for increasing or decreasing the target compounds. Goto (2012) demonstrated that the anthocyanin content of lettuce increased by using a higher percentage of blue light-emitting diodes (LEDs) at the same photosynthetic photon flux (PPF) in plant factories. Low-potassium lettuce or Komatsuna can be produced using a hydroponic solution without the application of potassium (Ogawa et al. 2012). However, selecting the optimal conditions for producing high value plants tends to be complicated, given the various environmental control parameters such as lighting, air conditioning, and hydroponic conditions. The optimization process for selecting environmental conditions has not been systematized, and plant factories may sometimes take a round-robin approach when testing various control parameters (Fig. 3-A).

The review below describes a process using physiological and genetic information will shorten the time required for determining optimal conditions for plant factories (Fig. 3-B). The main objective of this review was to demonstrate an efficient optimization process for producing high value plants in plant factories, which can be adapted to other promising plants.

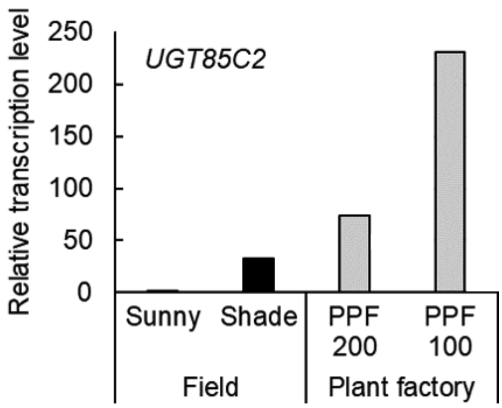

Fig. 2. *UGT85C2* expression levels in stevia between field cultivation and closed-type plant factory (plant factory)
Stevia of field was grown in a sunny place (Sunny) or in a shaded place (Shade). Those plants were grown from overwintering buds and harvested in September. Stevia of plant factory was grown under 200 μmol m^{-2} s^{-1} (PPF 200) or 100 μmol m^{-2} s^{-1} (PPF 100) at 24°C/20°C under 16L/8D for 4 weeks. Those plants were cuttings from a mother plant which was grown under the same condition without light intensity, 120 μmol m^{-2} s^{-1}. Data extracted from Yoneda (2018).

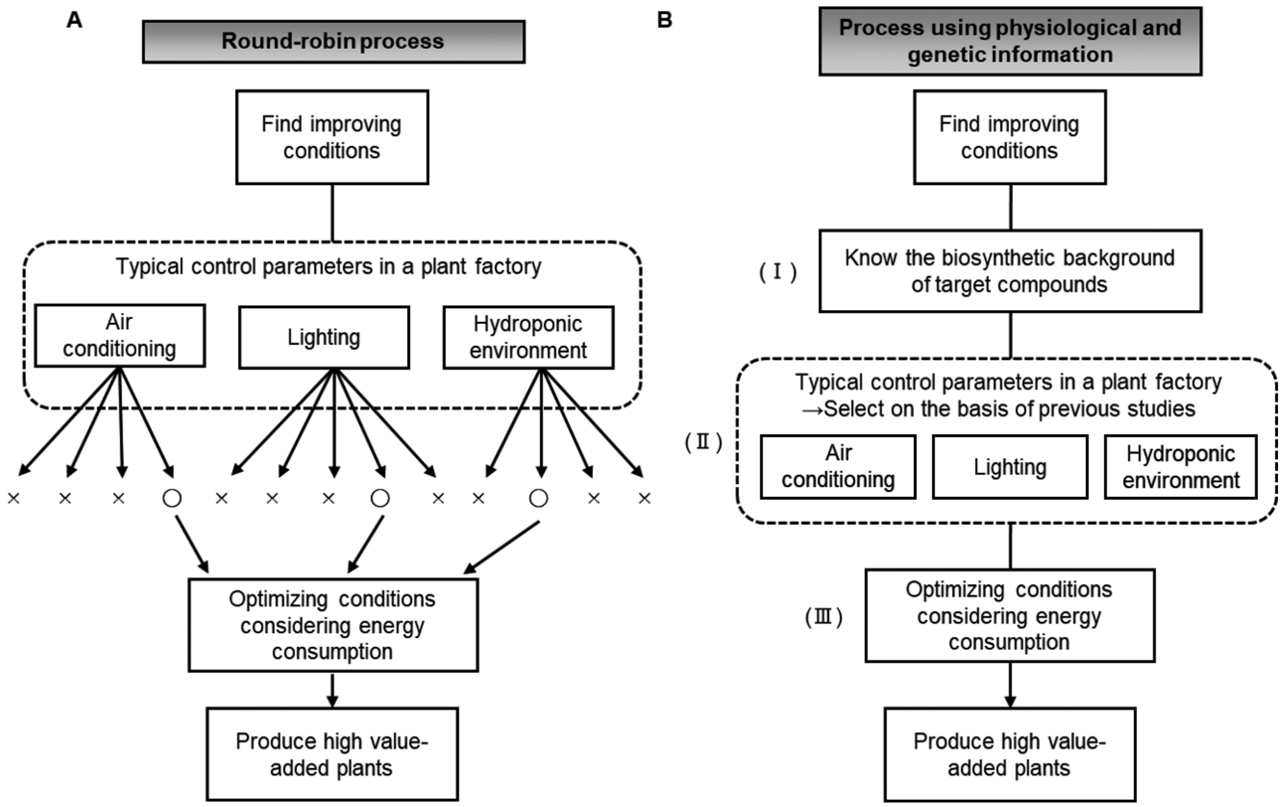

Fig. 3. Models of producing high value-added plants
A round-robin process (A). A process using physiological and genetic information (B).

The process of using physiological and genetic information on stevia

The process of producing SG-rich stevia using physiological and genetic information has been shown as an effective and systematic example of producing high value plants (Fig. 3-B).

Stevia is a natural sweetener, and its main sweet compounds—SGs named stevioside (Stev) and rebaudioside-A (Reb-A) (Fig. 4 (i) and (ii))—are about 200-400 times sweeter than sucrose (Schiffman et al. 1995). These sweet compounds have attracted considerable interest as a potential component of diet therapy for diabetic patients (Chen et al. 2005, Chen et al. 2006, Dyrskog et al. 2005). However, cold weather limits the germination, growth, and yield of stevia (Shock 1982, Singh & Rao 2005). It is valuable and therefore suitable for year-round cultivation in plant factories in order to increase the yield of SGs. In this review, the process of producing SG-rich stevia focuses on two types of information for systematizing the optimization process: (1) physiological and (2) genetic information.

First, it is important to have knowledge about the physiological background such as the biosynthetic and inactivation pathways of the target compounds, in order to select effective conditions. In the case of stevia, the biosynthetic pathway of Stev and Reb-A is partially shared with a biosynthetic pathway of gibberellin (GA) hormones, to *ent*-kaurenoic acid as the common substrate (Fig. 4). There are two branch pathways from *ent*-kaurenoic acid to Reb-A (SG pathway) and from *ent*-kaurenoic acid to GAs (GA pathway). In comparing the number of studies made on the relationship between environmental stimulation and GAs, little has been reported about the environmental effects on SG biosynthesis. Conversely, several studies have reported on the relationship between GA biosynthesis and environmental conditions. Given that there is a correlation between environment stimulations for the yield of GAs and SGs due to the relationship between the biosynthesis pathways of GAs and SGs, the previously reported environmental condition settings of GAs would be applicable for the control of SGs production.

Second, a genetic characterization based on an analysis of transcription levels that correlate with the yield of target compounds offers advantages in terms of sampling size and cultivation cycle. The sampling size required for genetic analysis is generally smaller than a

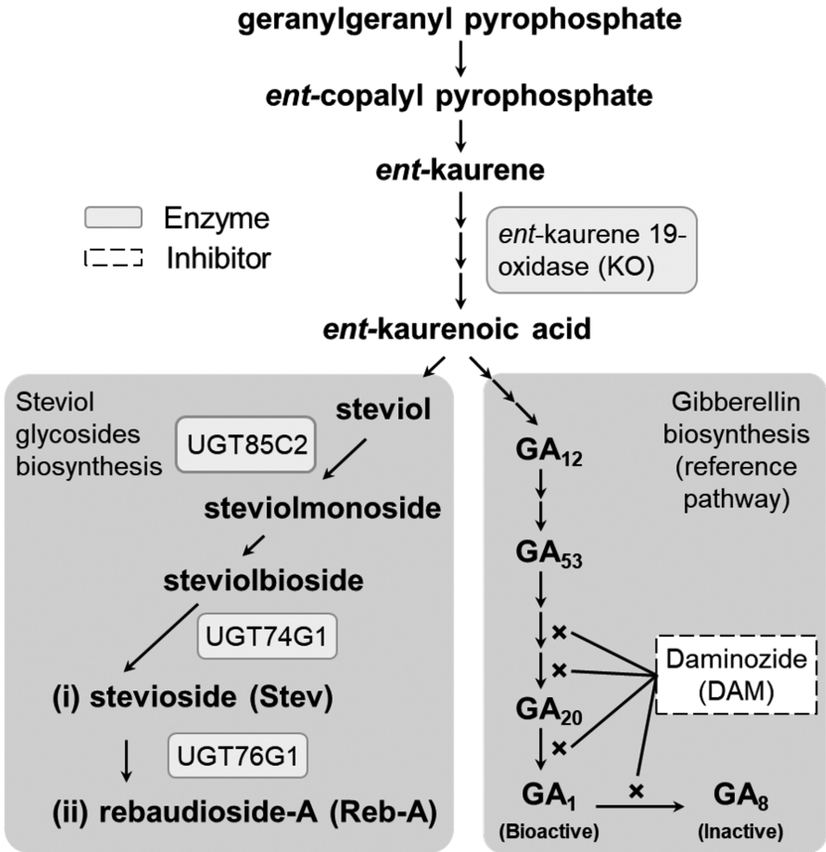

Fig. 4. Biosyntheses of stevia sweet components and gibberellins
Referred from Rademacher (2000), Yoneda et al. (2017a), Yoneda et al. (2018), and Yoneda (2018).

piece of leaf, and this scale is lower than that required for content yield analysis. Therefore, the cultivation cycle would be shorter than that of conventional cultivation for determining the target compound content. In the case of stevia, several studies have reported a positive correlation between transcription levels, especially for *UGT85C2*. Consequently, the genetic analysis of stevia in a plant factory was completed within approximately one-fourth of the period required for field cultivation (Yoneda 2018).

The target compounds were Stev and Reb-A—the most abundant and second most abundant sweet compounds in stevia, respectively (Crammer & Ikan 1986, Chatsudthipong & Muanprasat 2009). There are three steps for increasing these compounds in plant factory systems (Fig. 3-B, (I)-(III)).

Validation of the relationship between GAs and SGs

In the first stage of the model (Fig. 3-B (I)), it is important to know the biosynthetic background of the target compounds. It is presumed that GA-related glycosides are a kind of storage form of GAs in order to inactivate GAs (Schneider et al. 1992, Schneider & Schliemann 1994). In the case of stevia, SGs are diterpene glycosides, and the biosynthesis of SGs is partially shared with the GA pathway. In order to validate the relationship between GAs and SGs, exogenous GA and GA inhibitor treatments were conducted (Yoneda et al. 2018, Yoneda 2018, Fig. 5). One of the GA inhibitors, daminozide (DAM), inhibits a downstream GA pathway that is separate from the biosynthesis of SGs (Fig. 4). As a result, both groups of 6-week-old stevia plants subjected to exogenous GA and DAM treatments once a week after two weeks of the rooting period showed increased transcription levels of the SG-related genes and an increased percentage yield of SGs than the control (Fig. 5). These results suggest that there was an interaction between GA treatments and SGs production. However, weekly exogenous GA and DAM treatments would take time and effort.

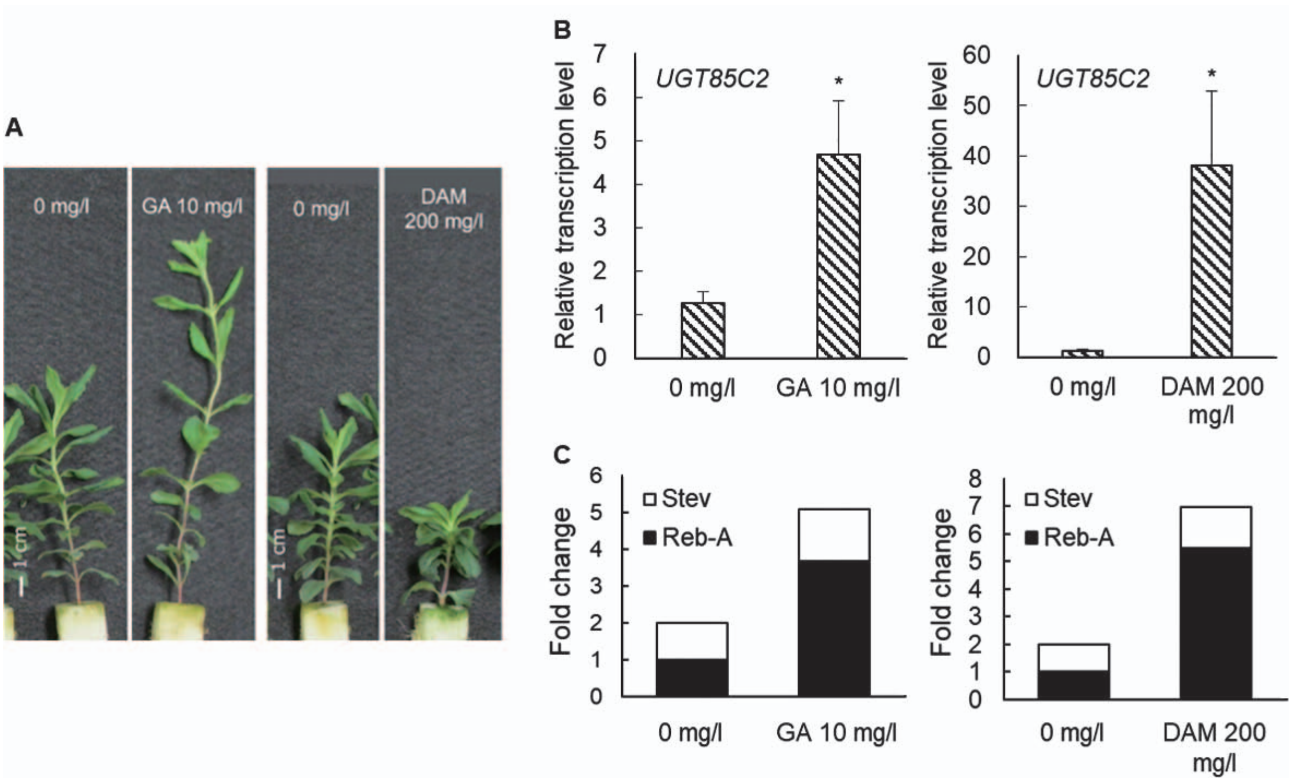

Fig. 5. Effect of gibberellin (GA) and inhibitor treatments for stevia

Morphology of 6-week-old stevia after cutting subjected to 0 mg/l as control, GA 10 mg/l, and daminozide (DAM) 200 mg/l treatments (A). Each sample was subjected to treatment solution with a surface-active agent once a week after two weeks of the rooting period. Relative transcription levels of *UGT85C2* under 0 mg/l, GA 10 mg/l, and DAM 200 mg/l treatments (B). Fold change of stevia sweet components including stevioside (Stev) and rebaudioside-A (Reb-A) subjected to 0 mg/l, GA 10 mg/l, and DAM 200 mg/l treatments (C). The asterisks indicate a significant difference (*t*-test, $^*P < 0.05$). Adapted from Yoneda et al. (2018) and Yoneda (2018).

Validation of the relationship between GA-related production conditions and SGs

In the second stage of the model (Fig. 3-B (II)), the control parameters to optimize treatment procedures for plant factories and increase or decrease the target compounds were selected based on the previous studies. It would be possible to find a minimum procedure for producing high value plants when referring to past research as compared with unplanned experimental design. For simplicity and efficiency, it is advantageous to select such well-established treatments as air conditioning, lighting, or the hydroponic environment in a plant factory for producing high value plants. Many studies have reported on the relationship between GA content and lighting. When the balance of red/far-red (R/FR) light was altered to favor a low R/FR ratio, the amount of GAs produced increased in *Arabidopsis thaliana* and cucumber (Kamiya & García-Martínez 1999). In contrast, blue light treatment, typically employed to inhibit stem elongation, contributes to the inactivation of bioactive GAs in *Arabidopsis thaliana* (Zhao et al. 2007). Consequently, both treatments of R/FR and blue LEDs were found to increase the transcription levels of SG-related genes and the percentage yield of SGs, as compared with the control (Yoneda et al. 2017a, Yoneda 2018, Fig. 6). According to exogenous GA and DAM treatments, the promoting action or inhibitory action of GAs affected the SGs yield. These results suggest that light treatment for increasing GAs content may also affect SGs production.

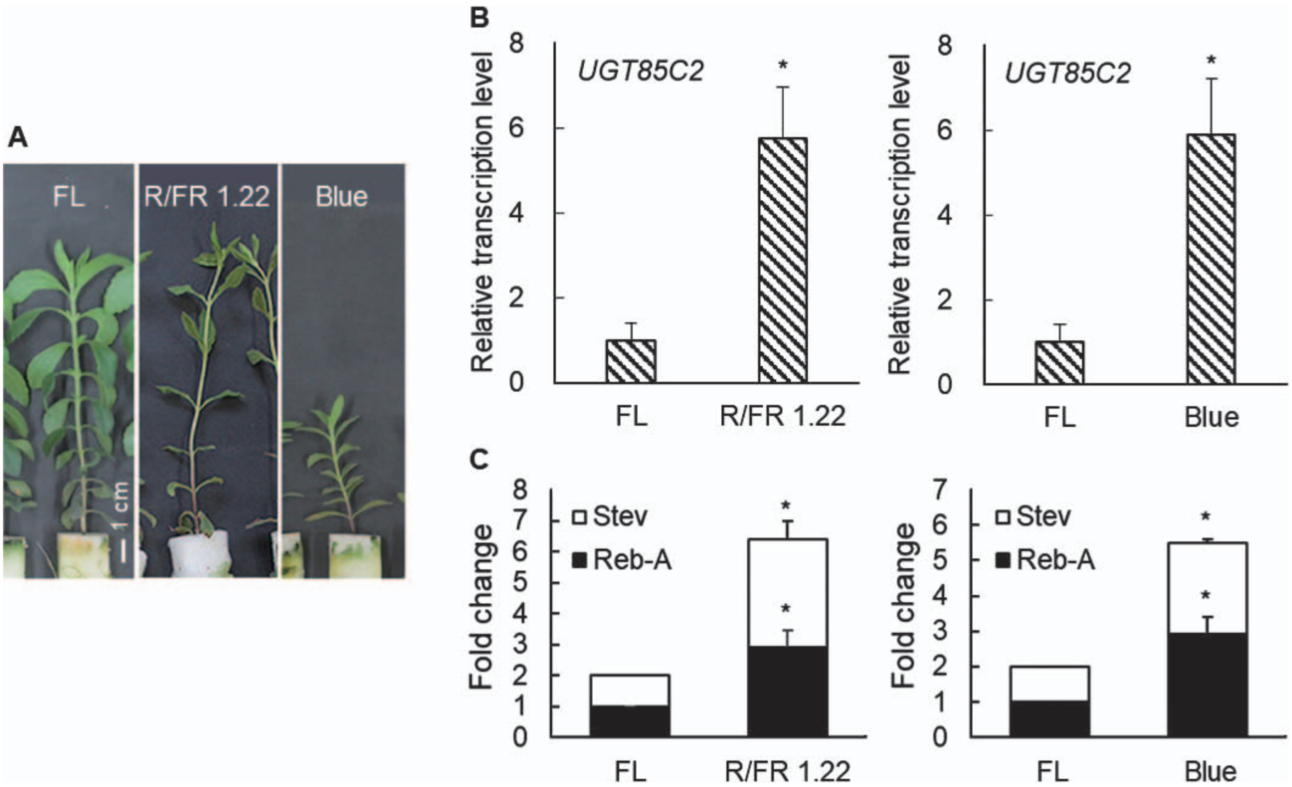

Fig. 6. Effect of light qualities for stevia

Morphology of 6-week-old stevia after cutting under fluorescent lamp (FL) as control, red/far-red (R/FR) 1.22, and blue treatments (A). All cuttings were grown under the same environment for two weeks of rooting period. R/FR 1.22 and blue treatments were used LEDs. Relative transcription levels of *UGT85C2* under FL, R/FR 1.22, and blue treatments (B). Fold change of stevia sweet components including stevioside (Stev) and rebaudioside-A (Reb-A) subjected to FL, R/FR 1.22, and blue treatments (C). The asterisks indicate a significant difference (*t*-test, $*P < 0.05$). Adapted from Yoneda et al. (2017a) and Yoneda (2018).

Searching for optimal conditions by considering energy consumption

In the third stage of the model (Fig. 3-B (III)), considering energy consumption when optimizing conditions is important to reduce running costs. Although plant factories have many advantages such as safety and sustainability when compared with field or greenhouse cultivation, the energy costs of plant production require careful consideration. Plant factories depend on lighting from artificial lights, whereas fields or greenhouses can obtain light from solar energy (Kozai et al. 2019).

In the case of stevia, the optimal light quality was determined in the aforementioned experiments. The optimal day length must be investigated with consideration given to light energy consumption and SGs production. Mohamed et al. (2011) reported that the percentage yield of SGs increased more under short-day photoperiod (12 h) than under long-day photoperiod (16 h) conditions. In order to find the optimal conditions for plant factories, the effects of photoperiod length were investigated (Yoneda et al. 2017b, Yoneda 2018, Fig. 7). The transcription levels of SG-related genes and the percentage yield of SGs under an 8 h photoperiod were higher than those under a 12 h photoperiod. According to Brandle (1998), stevia is an obligate short-day plant. Short-day plants are induced to flower by short days, and the flowering response is attributed to GA-related or phytochrome-related activities (Metivier & Viana 1979, Ceunen & Geuns 2013). These findings suggest that a short-day photoperiod (8 h) could help save light energy and increase the percentage yield of SGs. While leaf biomass under the 8 h condition was lower than that under the 12 h condition, the total leaf area per plant × *UGT85C2* under the 8 h condition was about 1.7-fold larger than that under the 12 h condition (calculated from Yoneda 2018). In terms of plant factory cultivation, a small form may be appropriate for increasing space utilization efficiency, provided that the target component is rich.

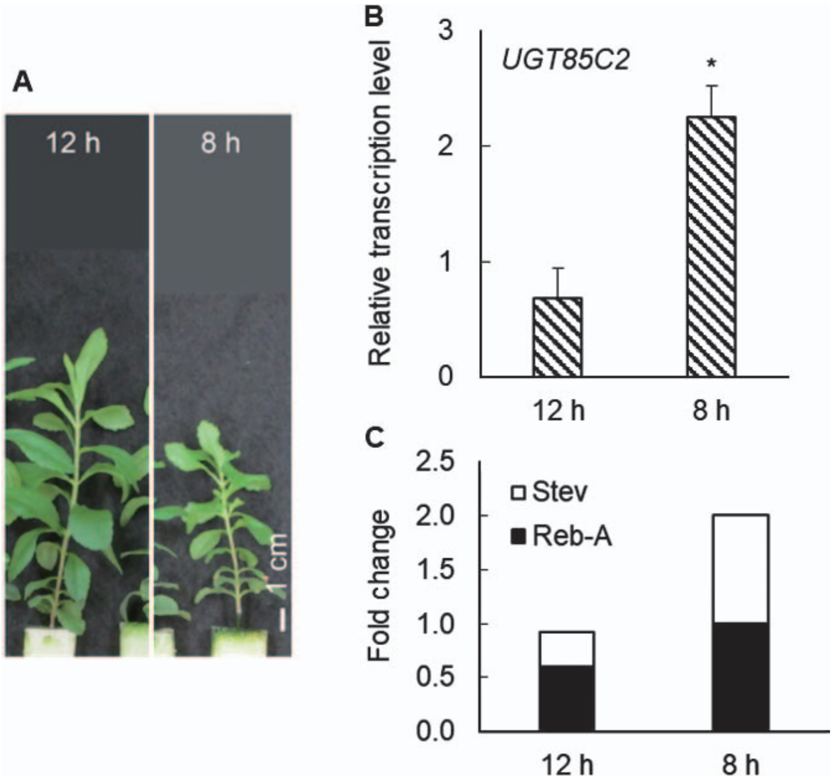

Fig. 7. Effect of day lengths for stevia
Morphology of 6-week-old stevia after cutting exposed to 12 h and 8 h light per day using fluorescent lamps (A). All cuttings were grown under same environment for two weeks of rooting period. Relative transcription levels of *UGT85C2* under 12 h and 8 h treatments (B). Fold change of stevia sweet components including stevioside (Stev) and rebaudioside-A (Reb-A) under 12 h and 8 h treatments (C). The asterisks indicate a significant difference (*t*-test, $^*P < 0.05$). Adapted from Yoneda et al. (2017b) and Yoneda (2018).

Conclusion

A plant factory can precisely modulate various environmental conditions, that is, a large number of environmental combinations. Standard experimental schemes are necessary to improve the productivity of high value plants. In the case of stevia, a relationship exists between the biosynthesis of GAs and the target components (SGs). This physiological information can provide a criterion for selecting environmental conditions. In addition, the gene expression profile of SGs is well-known, and there is a positive correlation between the expression levels of SG-related genes and SGs content. This genetic information can cut analysis time as compared with the analysis of SGs content. According to an analysis of SGs using physiological and genetic information, SG-rich stevia may be produced under short-day photoperiod conditions in plant factory systems, with blue LEDs used as an energy-saving measure. If stevia treated with short-stem blue light also proves to be rich in SGs, it may be suitable for growth in shelf system plant factories.

This systematic process for producing high value plants in plant factories will reduce the labor and time involved in the optimization process as compared with conventional processes. This optimization process is not only suitable for stevia but also for other promising plants.

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