Daminozide enhances the vigor and steviol glycoside yield of stevia (Stevia rebaudiana Bert.) propagated in temporary immersion bioreactors

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Received: 2 January 2022 / Accepted: 7 March 2022 / Published online: 23 March 2022
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Abstract
Stevia (Stevia rebaudiana Bertoni) produces steviol glycosides (SG), a group of diterpenoid secondary metabolites widely used as natural sweeteners. SGs share the same precursor with gibberellins (GAs), ent-kaurenoic acid. In this study, we attempted to increase the SG content in stevia propagated in Temporary Immersion Bioreactors (TIBs) by blocking ent-kaurenoic acid entry to the GA synthesis pathway using Daminozide (0, 10, 20 ppm), a known GA inhibitor. The maximum increase in biomass weight and SG content (up to 40% for stevioside and rebaudioside A) was observed following 10 ppm Daminozide treatment. The treatment also increased transcript accumulation for genes involved in SG biosynthesis, such as SrKA13H, SrUGT85C2, and SrUGT76G1, indicating that the SG pathway became more active due to GA pathway inhibition. Furthermore, GA inhibition following inhibitor exposure was also indicated by the upregulated expression of a GA biosynthesis gene (GA3ox) due to feedback regulation, and the downregulated expression of a GA catabolism gene (GA2ox2) as a result of feed-forward regulation.

Key message
This study reports the impact of late-stage GA biosynthesis inhibition by chemical treatment to the modulation of SG yield from stevia in vitro culture; SG and GA pathways are related.

Keywords Daminozide · Gibberellin · Stevia · Steviol glycosides · Temporary immersion bioreactors

Introduction
Diabetes is a serious threat to global health. Approximately 463 million people in the world are living with diabetes and by 2045, the case numbers are estimated to increase by 51% world-wide and 74% in Southeast Asia (IDF 2019). This has led to a demand for sugar substitutes with a lower calorie count and glycemic index. Stevia (Stevia rebaudiana), also known as honey leaf, has been consumed as a natural sweetener due to its zero calorie and zero glycemic index properties. Stevia is now widely used for various food, beverage, and pharmaceutical products after being approved in the USA (2008) and Europe (2011) (Yadav and Guleria 2012). The global stevia market is expected to grow by 8.2% throughout 2019 to 2025 (Industry Research 2019).

Due to its high demand, efficient methods for the mass propagation of stevia are pertinent. This may be achieved through in vitro propagation in a bioreactor system. Stevia can be easily propagated through in vitro shoot cuttings, with a single cut of node growing to 6–14 nodes in 4 weeks in a medium without growth regulators (Singh and Dwivedi 2014). Moreover, bioreactors provide more space for plant growth, which is ideal for scaling up. However, hyperhydricity may sometimes occur in liquid culture-based systems,
causing growth disorders such as distorted leaf anatomy, chlorophyll deficiency, and other vitrification symptoms (Debnath 2011). This is because liquid culture-based systems may limit gas exchange in plants. Therefore, Temporary Immersion Bioreactors (TIBs) can be used to overcome such a problem as they allow for balanced plant exposure to liquid media and air. These system features an automatic control device to allow for periodic contact of liquid medium with the plant (Steingroewer et al. 2013) thereby preventing hyperhydricity. Several types of TIBs have been developed and used for industrial plant micropropagation, including the RITA® bioreactor system (https://cirad.fr, CIRAD, France). This system consists of autoclavable polypropylene containers with two compartments separated by a table and supported by a grid and a central pipe. The upper containers are for the plants, while the lower containers are for the liquid medium. The container caps are connected to a time-controlled air supply secured with Millipore filters (Georgiev et al. 2014). The RITA® bioreactor system has been successfully used for the mass production of stevia (Ramírez-Mosqueda et al. 2016; Vives et al. 2017; Bayraktar 2019).

Another issue in the manufacture of stevia is how to enhance its sweetness, which emerges from the sweet compounds called steviol glycosides (SGs). SGs are steviol aglycons that are attached to sugar molecules(s) at the C13-hydroxyl and C19-carboxylic acid sites (Hellfritsch et al. 2012). SGs differ in the type and number of sugars attached to the diterpenoid steviol structure. Most attached sugars are glucose (Purkayastha et al. 2016), while some others are xylose or rhamnose (Shafii et al. 2012). The number of sugar molecules attached determines the sweetness level of SGs (Hellfritsch et al. 2012). For example, rebaudioside A, which carries four additional glucose units, is sweeter and has a less bitter aftertaste than stevioside, which has only three additional glucose units (Prakash et al. 2014b). Stevioside and rebaudioside A are the most abundant SGs in stevia, which account for 5.8% and 1.8% of leaf dry weight (Gardana et al. 2010). These compounds are 350 times sweeter than sucrose (Mogra and Dashora 2009). In addition, at least 35 other SGs have been identified in stevia. One of the sweetest compounds is rebaudioside M, also known as rebaudioside X, which carries six additional glucose units but is present at very low concentrations in stevia plant cells (Prakash et al. 2014b). Recently, rebaudioside M, along with rebaudioside D, rebaudioside I, and rebaudioside IX, have been developed as the next-generation premium stevia sweeteners (Prakash et al. 2014b, a, 2017; Olsson et al. 2016).

SGs are synthesized from ent-kaurenoic acid via the methylerythritol 4-phosphate (MEP) pathway (Xu et al. 2021) in plant chloroplasts and endoplasmic reticulum. ent-Kaurenoic acid is then converted into steviol by kaurenoic acid 13-hydroxylase (KAH) in the cytoplasm (Yoneda et al. 2018). However, in the cytoplasm, ent-kaurenoic acid is also metabolized into the plant hormones gibberellins (GA) through the activity of ent-kaurenic acid oxidase (KAO), followed by the 2-oxoglutaric acid-dependent (2ODD) group of enzymes such as GA 20-oxidase (GA20ox), GA 3-oxidase (GA3ox), and GA 2-oxidase (GA2ox) (Olszewski et al. 2002). This leads to the idea that these pathways might be manipulated to increase SG synthesis.

Many studies have attempted to increase SG biosynthesis by inhibiting certain stages of GA biosynthesis using retardants in ex vitro cultivation of stevia. Hajihashemi and Geuns (2017) used Paclobutrazol to block the conversion of ent-kaurene to ent-kaurenoic acid, resulting in the decrement of both GA and SG. In contrast, Yoneda et al. (2018) applied Daminiozide to block the conversion of GA12-aldehyde into bioactive GAs and their derivatives, resulting in an enhancement of SG level and related biosynthesis gene expression. Daminiozide has a similar chemical structure to 2-oxoglutaric acid (2OG), and thus both compounds may competitively bind to the 2ODD group of enzymes involved in late-stage GA biosynthesis (Rademacher 2006). Interestingly, the use of retardants is also correlated to the increment of plant yield (Karimi et al. 2014; Saptari et al. 2020). Accordingly, this study aimed to provide protocols for in vitro propagation of stevia to yield high biomass and SG content by chemically manipulating the SG and GA pathways using Daminiozide treatments in TIB. In vitro propagation is a solution to cope with land, climate, and geographical challenges in stevia cultivation.

### Methods

#### Explant preparation

In vitro stevia shoots were provided by the Laboratory of Cell Culture and Micropropagation, Indonesian Research Institute for Biotechnology and Bioindustry (IRIBB). The shoots were cut into each nodal segment and conditioned in a petri dish (16 cm in diameter) containing 10 mL of sterile liquid medium. The medium consisted of Murashige and Skoog (MS) basal minerals, 30 g/L sucrose, and 0.5 g/L active charcoal, and was adjusted to a pH of 5.7. Shoot conditioning was conducted for four days in a culture room equipped with LED lighting at 20 μmol/m²/s (12 h light/12 h dark photoperiod) and at 25 °C.

#### TIB culture

TIB culture was prepared using the same media composition as described above, except that the active charcoal was omitted. Daminiozide was added to the medium at three concentrations: 0 ppm (control), 10 ppm, and 20 ppm. The media were then sterilized in an autoclave at 121 °C and
1 kg/cm² pressure for 15 min, and were left overnight before use. Afterwards, 175 mL of sterile media were aseptically poured into each TIB vessel in the Laminar Air Flow (LAF). Subsequently, 40 conditioned stevia shoots were inoculated into each TIB. TIBs were installed in the system for the automation of the immersion period set at 30 min every 6 h. The TIB system was incubated in a culture room equipped with LED lighting at 20 μmol/m²s (12 h light/12 h dark photoperiod) and at 25 °C.

**Growth measurements**

Growth parameters were observed after 40 days incubation in TIBs, including shoot height, number and length of nodes, size of leaves, and fresh and dry weight of shoot biomass. Shoot height was measured from the base to the highest leaf tip. Leaf size was measured by the length and width of the leaves. Subsequently, biomass weight was measured based on the leaves and total shoot biomass. All data were subjected to the analysis of variance (ANOVA) followed by Tukey’s post hoc test at a 0.05 significance level.

**Stevioside and rebaudioside A accumulation**

The accumulation of stevioside and rebaudioside A was analyzed using high-performance liquid chromatography (HPLC) based on a method reported by Martono et al. (2016). Samples were prepared by drying stevia leaves in the oven at 105 °C for five hours. The dry biomass was then mashed into powder and extracted using an ultrasonicator and a 60% ethanol solvent. Afterwards, the extract was injected into the HPLC system for analysis. The HPLC used Eurosphere C-18 (30 °C) for the stationary phase, and for the mobile phase it used a mixture of water–methanol (90:10 v/v, pH = 3.0), acetonitrile, and trifluoroacetic acid (TFA) (65:35:0.01 v/v/v), at a 0.6 mL/min flow rate. A UV detector (UV Smartline, Knauer, GmBH) at 210 nm wavelength was used for the analysis. Stevioside and rebaudioside A with 99% purity (Wako, Japan) were used as standards in the range of 20–120 μg/mL. Standard curves were made by plotting standard concentrations (μg/mL) vs. peak area (mAU.min). The linearity for the stevioside and rebaudioside A curves was $R^2 = 0.9994$ and $R^2 = 0.9998$, respectively.

**SG and GA metabolic gene transcription analysis**

The expression of genes related to SG and GA metabolism was analyzed using quantitative real time PCR (qPCR). Samples for the analysis were stevia leaves from the 2nd–3rd nodes of the shoot collected after 40 days of culture incubation. Total RNAs (50 mg fresh weight) were extracted from the samples using GeneAll® Ribospin™ Plant according to the manufacturer’s instructions. RNA purity and concentration were determined using a NanoDrop spectrophotometer, while RNA quality and integrity were assessed on 1.0% agarose gel. RNA extracts with good quality, purity, and concentration were reverse transcribed into cDNAs using AccuPower® RT PreMix (Bioneer) according to the manufacturer’s instructions. qPCR was performed using SensiFAST™ SYBR® Hi-ROX One-Step (Bioline) with the following thermal cycling: 95 °C for 25 s of denaturation, 60 °C for 15 s of annealing, and 72 °C for 20 s of amplification, repeated for 45 cycles.

The SG metabolism genes analyzed in this study were SrUGT76G1 (AY345974.1), SrUGT74G1 (AY345982.1), SrUGT85C2 (AY345978.1), SrKA13H (DQ398871.3), while the GA metabolism genes were KAO, GA2ox1, GA2ox2, GA3ox, and ATH1. Transcript accumulation of those genes was compared to the SrActin (AF548026) internal standard. Primers for the amplification of GA3ox were obtained from Singh et al. (2017), while those for the rest of the genes analyzed in this study were designed using Geneious Prime software.

**Results and discussion**

**Stevia growth on TIBs**

The morphological growth of stevia in TIBs was affected by the Daminozide treatment. Plantlets treated with Daminozide were stunted and had shorter internodes (Fig. 1b) compared to the control group. Daminozide concentrations are inversely related to plantlet height and internode length (Figs. 1a and 2). The control group has an average plantlet height of 14.6 ± 2.1 cm and average internode length of 1.9 ± 0.1 cm, while the shortest plantlets were derived from 20 ppm Daminozide treatment with 6.5 ± 1.0 cm of average height and 1.0 ± 0.1 cm of average internode length (Fig. 1a and b). The 20 ppm Daminozide treatment exhibited the strongest effect (55.5%) in shortening plantlet stems, and consequently, the plantlets in this treatment had fewer nodes (6.5 ± 1.1) compared to other treatment groups (Fig. 1c).

These findings suggest that low GA activity or concentrations interfere with GA function in stem development. In the cells, GA is recognized by the GIBBERELLIN INSENSITIVE DWARF1 (GID1) receptor, promoting structural changes in the receptor (Ueguchi-Tanaka et al. 2007). This allows GID1 to interact with DELLA proteins, causing DELLAs to be inactivated and degraded by the proteasome ScGID2/SLY1 (Sun 2011). DELLAs function as repressors that, among other molecules, block PHYTOCHROME INTERACTING FACTOR 4 (PIF4), a transcription factor for genes involved in cell and hypocotyl elongation (de Lucas et al. 2008). Meanwhile, the application of Daminozide may decrease the GA level in cells, restraining DELLAs
from degradation. GA-DELLA interactions in hypocotyl and stem elongation have been studied in several plants, such as Arabidopsis thaliana, Oryza sativa, and Pisum sativum (Ueguchi-Tanaka et al. 2007; de Lucas et al. 2008; Weston et al. 2008).

In contrast to plantlet height, Daminozide treatment had a positive effect on leaf development. Plantlets treated with 10 and 20 ppm Daminozide had larger leaf areas than the control group (Figs. 1d and 2). The largest leaf size (3.02 ± 0.28 cm²) was observed following 20 ppm Daminozide treatment. This result is in agreement with many studies reporting the use of GA inhibitors to increase plant vigor, resulting in dwarf plants with sturdier stems and bigger leaves (Tadesse et al. 2000; Kepenek and Karoglu 2011; Karimi et al. 2015; Sumaryono and Sinta 2011). Tadesse et al. (2000) reported that Daminozide increased S. tuberosum plantlet leaf size in vitro.

There were reports on the antagonistic interaction between GA and cytokinins (CK), the key hormones responsible for leaf morphogenesis (Jasinski et al. 2005; Fleishon et al. 2011; Shwartz et al. 2016). GA inhibits CK signaling (Fleishon et al. 2011). Therefore, GA inhibition might allow for optimal CK function on leaf morphogenesis, as reported for tomato in vitro cultures, in which exogenous GA supplementation resulted in tall plantlets with small and less complex leaves. Meanwhile, reducing GA level or activity resulted in dwarf plantlets with bigger and more complex leaves (Fleishon et al. 2011). Similar results were also observed in this study, where GA inhibition through Daminozide treatment increased stevia plantlet leaf size. The enhancement of leaf production, both in numbers and size, positively affects plant productivity, especially for plants that are harvested for their leaves, such as stevia. Moreover, leaves are the main organs that produce SG.
Leaf size is related to leaf biomass weight. Daminozide increased the fresh biomass weight of the leaves (Fig. 3a), as a consequence to the leaf size increase. However, Daminozide did not affect the leaf dry biomass weight (Fig. 3). Further measurement showed that Daminozide treatment also increased the ratio between leaf biomass and stem-root biomass (Fig. 3). In the control group, plantlet biomass consisted of more stem and root (4:6), while those treated with 10 and 20 ppm Daminozide consecutively yielded more leaf biomass (5:5 and 6:4) (Fig. 3). This is in line with stem growth inhibition by Daminozide (Fig. 1a, b), which also promoted leaf growth (Figs. 1d and 2). Similarly, Karimi et al. (2019) reported that Daminozide application to stevia cultivated in the greenhouse shifted photosynthate allocation to the leaves, as demonstrated by the increase in leaf dry weight. In this study, Daminozide only increased fresh biomass weight, while the dry biomass weight was unaffected, indicating that in in vitro conditions, Daminozide might increase cell water absorption and storage capacity, especially in the leaf cells. The plant’s capacity to absorb and store water could maintain plant vigor (Aharon et al. 2003), which is often attempted by the application of GA inhibitors such as Paclobutrazol (Sumaryono and Sinta 2011; Flores et al. 2018). Daminozide exhibited a similar effect to Paclobutrazol in maintaining plant vigor. In addition, by blocking a different stage in the GA biosynthesis pathway compared to Paclobutrazol, Daminozide may potentially increase SG yield in stevia (Yoneda et al. 2018; Saptari et al. 2020).

**SG yield following daminozide treatment**

Stevia treated with 10 ppm Daminozide had the highest stevioside (8.1 ± 0.4 mg/g of dry weight) and rebaudioside A content (14.6 ± 1.2 mg/g of dry weight) of any
treatment group, while those treated with 20 ppm Daminozide had no significant difference with the control. At 10 ppm, Daminozide significantly increased 42.6% of stevioside and 40.67% of rebaudioside A content compared to the control group (Fig. 4). Stevioside and rebaudioside A yield were calculated by multiplying stevioside and rebaudioside A content by leaf dry biomass weight. The maximum stevioside (4.09 ± 1.05 mg) and rebaudioside A (7.45 ± 1.39 mg) yields were obtained from stevia TIB cultures treated with 10 ppm Daminozide, about two-folds higher than the control group (Table 1). Meanwhile, those treated with 20 ppm Daminozide only generated a 50.4% higher rebaudioside A yield than the control group.

![Fig. 3 Stevia biomass after 6 weeks of incubation in TIBs with Daminozide treatments. Different letters indicate significant differences in leaf biomass according to Tukey’s post hoc test at α = 0.05, n = 5](image1)

![Fig. 4 Stevioside (a) and rebaudioside A (b) content in stevia after 6 weeks of incubation in TIBs with Daminozide treatments. Different letters indicate significant differences according to Tukey’s post hoc test at α = 0.05, n = 5](image2)

**Table 1** Total stevioside and rebaudioside A yield from stevia after 6 weeks of incubation in TIBs with Daminozide treatments

| Daminozide concentration (mg/L) | Stevioside yield (mg) | Rebaudioside A yield (mg) |
|---------------------------------|-----------------------|---------------------------|
| 0                               | 2.12 ± 0.20b          | 3.79 ± 0.57c              |
| 10                              | 4.09 ± 1.05a          | 7.45 ± 1.39a              |
| 20                              | 2.67 ± 0.71b          | 5.71 ± 0.90b              |

Different letters indicate significant differences according to Tukey’s post hoc test at α = 0.05, n = 5
These results were slightly different from those reported by Saptari et al. (2020) for the application of Daminozide in thin layer liquid culture, in which there were no differences in stevioside and rebaudioside A yield due to 10 ppm and 20 ppm Daminozide treatment. It indicates that specific optimal conditions for Daminozide treatment are required for each culture type. Results from these in vitro studies complemented the ex-vitro study reported by Yoneda et al. (2018). The optimum concentration for ex-vitro application of Daminozide in stevia to modulate SG yield was 200 ppm compared to the control, whereas for in vitro application it required about 10 times lower concentration.

**SG and GA metabolic gene transcription profiles**

SG-related genes, SrKAI3H, SrUGT85C2, and SrUGT76G1, were significantly upregulated by 10 ppm Daminozide, showing 0.5, 4 and 8 folds of transcript accumulation compared to the control group, respectively (Fig. 5). Moreover, neither of the Daminozide concentrations had an effect on the transcription level of SrUGT74C1, the gene encoding an enzyme for the conversion of steviolbioside to steviol (Fig. 5). These results were similar to those reported by Yoneda et al. (2018), in which Daminozide treatments increased the SG yield per plant and the transcription level of SG-related genes, such as UGT85C2 and UGT76G1. Yoneda et al. (2018) and Karimi et al. (2015) also suggested that UGT85C2 might be a key gene for evaluating SGs in stevia.

In contrast, 20 ppm Daminozide did not affect the transcription level of tested SG-related genes (Fig. 5). Yoneda et al. (2018) also showed similar trends, in which the UGT85C2 and UGT76G1 transcripts were most abundant following 200–500 ppm Daminozide treatment, and higher concentrations of Daminozide led to lower transcription levels of the genes. This is consistent with the SG yield data generated in this experiment, in which SG yield increased significantly following 10 ppm Daminozide treatment, while the 20 ppm treatment resulted in a similar yield to the control (Fig. 4). It suggested that feedback regulation in the SG pathway might occur. However, the mechanism is still unclear and further study is needed to verify such a hypothesis.

Our data regarding SG yield and the transcription profile of SG-related genes may support the hypothesis that Daminozide, which interferes with the downstream stages of the GA biosynthesis pathway, might divert the ent-kaurenoic acid precursor flux towards the SG pathway. The interference in the GA biosynthesis pathway by Daminozide treatment was demonstrated by the transcription profile of GA-related genes. The transcription level of KAO was constant among all treatments (Fig. 6). In contrast, Daminozide modulated the genes for the 2ODD group of enzymes. GA3ox transcripts increased threefold in the treatment groups compared to the control, whereas GA2ox2 was downregulated (Fig. 6). GA2ox2 expression was lower following 10 ppm and 20 ppm Daminozide treatments by 10- and fivefold compared to the control group, respectively.

These results were consistent with previous studies stating that GA homeostasis in higher plants is mainly maintained by negative feedback regulation of GA anabolism and feedforward regulation of GA catabolism, both of which are restricted to the dioxygenase genes downstream of the biosynthesis pathway and do not occur in the earlier pathway (Hedden and Phillips 2000; Honi et al. 2020). Moreover, Hellwell et al. (1998) also reported that genes in early GA biosynthesis of the model plant A. thaliana were not affected by the feedback mechanism. The KAO gene, which encodes for a cytochrome P450 monooxygenase, is not a target of Daminozide and feedback regulation, hence its transcription was not affected in this study. In contrast, increased GA3ox transcript accumulation suggests that negative feedback regulation occurred due to the lack of active GA. The enzyme encoded by this gene catalyzes the conversion of inactive GA intermediates such as GA20 and GA5, to bioactive GA, such as GA1 and GA3. When GA3ox was inhibited by Daminozide, the bioactive GA level might become low, and this triggered the upregulation of genes encoding the enzyme, as also suggested by Hedden and Phillips (2000).

Furthermore, decreased GA2ox2 transcript accumulation also indicated feedforward regulation of genes for GA catabolism. Feedforward regulation of GA2ox due to the low level of GAs was also reported in A. thaliana (Dill and Sun 2001), S. lycopersicum (Ding et al. 2013), and Corchorus sp. (Honi et al. 2020). However, in this study, GA2ox1 transcription was unaffected by the treatments, suggesting that redundant GA oxidase genes have different spatial and temporal expressions. As for GA2ox, at least 7 copies of the gene have been identified in A. thaliana, 11 copies in O. sativa, and 8 copies in Corchorus sp., all of which have different transcription profiles in different organs (Honi et al. 2020).

GA homeostasis regulation is not restricted to genes for GA biosynthesis, but also for genes related to GA signaling. In this study, transcript accumulation of a GA signaling gene, ATH1, increased twofold with 10 ppm Daminozide but was constant with 20 ppm Daminozide treatment (Fig. 6). This indicates that Daminozide might also influence the GA-signaling pathway in a concentration-dependent manner. ATH1 encodes for A. thaliana Homeobox (ATH1), a transcription repressor that works together with the Ovate Family Proteins (OFP1). In A. thaliana, the ATH1-OFP1 complex was reported to inhibit cell elongation, including stem elongation, through the regulation of GA dioxygenase gene expression (García-Martínez and Gil 2001; Wang et al. 2007; Zhang et al. 2018). Similar transcription profile of ATH1 and GA2ox1 was observed following Daminozide
treatments in this study, indicating that \textit{GA2ox1} might be the regulation target of \textit{ATH1} in stevia, or that they might have similar actions in regulating GA homeostasis.

The transcription profile of GA-related genes in this study suggested that GA biosynthesis inhibition and morphological changes might occur in stevia due to Daminozide treatments.

![Steviol glycoside metabolism gene transcript accumulation in stevia after 6 weeks of incubation in TIBs with Daminozide treatments. Different letters indicate significant differences according to Tukey’s post hoc test at $\alpha = 0.05$, $n = 3$.](image)
treatment. GA inhibition was intended to divert more ent-kaurenoic acid into the SG biosynthesis pathway, thereby resulting in more SGs as demonstrated by the increase of stevioside and rebaudioside A contents (40%), especially with 10 ppm Daminozide treatment (Fig. 7). This is also supported by the upregulation of genes encoding SG biosynthesis enzymes, such as SrKA13H, SrUGT85C2, and SrUGT76G1.

Altogether, regarding the results of this study, it should be mentioned that the SG and GA pathways are related and might affect each other, especially from the ent-kaurenoic acid branch. It is also suggested that glycosylation by UDP-dependent glycosyltransferases (UGTs) is a key regulatory step in SG biosynthesis (Xu et al. 2021; Zhou et al. 2021). UGTs are substrate-specific enzymes (Madhav et al. 2013; Wu et al. 2020). Structural analysis showed a variety in the glc2 pocket residues of the acceptor-binding site of these enzymes, which determined their substrate specificity (Lee et al. 2019). Therefore, the availability of substrates for the SG pathway might affect enzyme activity for SG production. Accordingly, chemical treatment using Daminozide to inhibit the GA biosynthesis pathway from ent-kaurenoic acid could be a potential strategy to enhance SG production in stevia. The overall summary of the influence of 10 ppm Daminozide on SG content, SG-related gene transcription, and GA-related gene transcription was illustrated in Fig. 7.
Acknowledgements  This work was financially supported by the Indonesian Educational Funding program from the Indonesian Endowment Fund for Education (LPDP-RI), awarded to R.T. Saptari.

Authors contributions  RTS conducted the experiments, carried out the data analysis, and prepared the manuscript. RRE conceptualized the research design for in vitro propagation and metabolite analysis and evaluated the manuscript. RAP conceptualized the research design for molecular analysis, evaluated and edited the manuscript. All authors have read and approved the manuscript.

Funding  The funded was provided by Indonesian Endowment Fund for Education (LPDP-RI), Grant No (BPI-2016).

Declarations

Conflict of interest  The authors declare no conflict of interest.

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