

Sugars proportionately affect artemisinin production

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Abstract Little is known about the effect of sugars in controlling secondary metabolism. In this study, sugars alone or in combination with their analogs were used to investigate their role in the production of the antimalarial drug, artemisinin, in *Artemisia annua* L. seedlings. Compared to sucrose, a 200% increase in artemisinin by glucose was observed. Different ratios of fructose to glucose yielded artemisinin levels directly proportional to increases in relative glucose concentration. When the glucose analog, 3-*O*-methylglucose, was added with glucose, artemisinin production was dramatically decreased, but hexokinase activity was significantly increased compared to glucose alone. In contrast, neither mannose nor mannitol had any significant effect on artemisinin yield. In comparison with 30 g/l sucrose, artemisinin levels were significantly reduced by 80% in the presence of 27 g/l sucrose + 3 g/l palatinose, which cannot be transported into cells through the sucrose transporter. Together these results suggest that both monosaccharide and disaccharide sugars are likely acting not only as carbon sources but also as signals to affect the downstream production of artemisinin, and that the mechanism of these effects appears to be complex.

Keywords Sugar signal · Sugar analog · Hexokinase

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Introduction

Sugars have recently been recognized as molecules that can be sensed in plants thereby inducing signals that affect metabolism, development, growth, and gene expression (Jang and Sheen 1994; Rolland et al. 2006). Both monosaccharide and disaccharide sugars can act as signals in plants (Loreti et al. 2000; Rolland et al. 2006).

Relatively little, however, is known about how sugars act to control production of plant secondary metabolites. Larronde et al. (1998) reported that in cell suspension cultures of *Vitis vinifera*, sucrose dramatically stimulated the production of anthocyanins. Stilbene level, however, was only slightly affected. They further showed that mannose, a glucose analog that can be transported into plants and phosphorylated by hexokinase, can mimic the effect of sucrose in the production of anthocyanins, while another glucose analog, 3-*O*-methylglucose (3OMG), which can be taken up into plant cells but only slowly phosphorylated by hexokinase (HXK), can not. When mannoheptulose, a specific inhibitor of HXK, was added, anthocyanin production by sucrose was inhibited. These results suggested that HXK appeared to be involved in a sugar signal transduction pathway related to anthocyanin production (Vitrac et al. 2000). In a recent study by Price et al. (2004) using *Arabidopsis* seedlings, a DNA microarray analysis revealed that gene expression related to secondary metabolism was also regulated by glucose.

Artemisinin (Fig. 1) is an important antimalarial sesquiterpene lactone therapeutic that is produced by *Artemisia annua* L. Although the drug is produced only in the shoots of whole plants at highest levels just at or

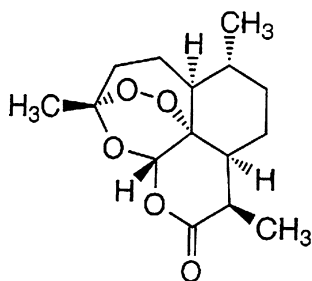


Fig. 1 Structure of artemisinin

before flowering, the roots play an important but as yet undefined role in production; rooted shoot cultures produce significantly more artemisinin than rootless shoot cultures (Ferreira and Janick 1996). Previously we reported that in *A. annua* hairy roots, artemisinin production was stimulated by glucose but inhibited by fructose in comparison to sucrose at the same carbon level (Weathers et al. 2004). Significant differences were observed in artemisinin production between sucrose and sucrose + fructose, but not between sucrose, and sucrose + glucose although the same amount of carbon was supplied for each condition.

This study used *A. annua* whole plants. In contrast to what was observed for hairy roots, whole plants respond quite differently to both monosaccharides and disaccharides, and show a directly proportional increase in artemisinin as the ratio of glucose increased relative to fructose. Growth responses were also different, and together these results provide the first report documenting these observations in whole plants of *A. annua*. Because sugars can act as both carbon sources and signals, different sugar analogs were also used to provide a very preliminary indication of possible regulatory roles of these sugars in artemisinin production.

Materials and methods

Culture, growth, and synchronization of *A. annua* seedlings

Artemisia annua L. seeds (YU strain, 2004 WPI crop) were surface sterilized in 10% (v/v) bleach followed by 70% (v/v) ethanol. Then seeds were washed with 10 ml 0.1% (v/v) sterile PPMTM [Plant Preservative Mixture; Plant Cell Technology (PCT Inc.), Inc., Washington, DC, USA], 3 times, 5 min each time. The sterilized seeds were imbibed in 30 ml 0.1% sterile PPM in a 125 ml Erlenmeyer flask in the dark for 3 days in the refrigerator. After that, seeds were further incubated in

30 ml Gamborg's B5 medium (Gamborg et al. 1968) with 3% (w/v) sucrose at pH 5.7 in the dark in the refrigerator for 3 days, then transferred to continuous cool-white fluorescent light ($100 \mu\text{Em}^{-2} \text{s}^{-1}$) at 24°C, and at 140 rpm on a shaker. After 5 days most seeds developed to the two-cotyledon stage. To eliminate exogenous sugar effects, the seedlings were washed with sugar-free B5 medium seven times, after which the seedlings were then maintained in the dark for 1 day. Ten seedlings at the two-cotyledon stage were manually selected and then inoculated into a Petri dish containing 20 ml autoclaved B5 medium with 0.23% (w/v) Phytigel to which filter-sterilized sugars were added. Cultures were maintained at 24°C under continuous cool-white fluorescent light ($100 \mu\text{Em}^{-2} \text{s}^{-1}$) for 14 days. For liquid culture, ten seedlings at the two-cotyledon stage were inoculated into 125 ml Erlenmeyer flasks containing autoclaved 20 ml B5 medium with filter-sterilized sugars.

All sugars and their respective analogs were filter sterilized using separate 0.22 μm sterile syringe filters before addition into B5 medium. Although all sugars and analogs were added to either Petri dishes or Erlenmeyer flasks at a final concentration of carbon equivalent precisely to 3% (w/v; 30 g/l = 100%) sucrose, textual reference is rounded for simplicity. For example, 100% Glc molar equivalent to 100% Suc (30 g/l) is 31.58 g/l, but stated as 30 g/l.

Analysis of growth and development

After 2 weeks growth on Petri dishes or in Erlenmeyer flasks, seedlings were removed, washed with diH₂O and blotted dry with paper towels. The leaves of each seedling were counted. Each seedling was cut at the base of the hypocotyl yielding the shoot and root portions. Individual shoots and roots were dried at 60°C for at least 16 h and the dry mass of each was measured. All dead or contaminated seedlings were discarded.

Extraction and analysis of artemisinin

The dried shoots of all seedlings harvested from the same Petri dish or Erlenmeyer flask were pooled together, weighed, and extracted with 1 ml toluene in a chilled water sonicator for 30 min. The supernatant was decanted and the sonication was repeated twice. The three supernatants from shoots harvested from the same Petri dish or Erlenmeyer flask were pooled and dried under nitrogen at 30°C. Samples were stored in the freezer at -20°C until analysis by HPLC. Artemisinin in each sample was analyzed by HPLC as the

Q260 derivative according to the method of Smith et al. (1997).

Assay of hexokinase activity

Seedlings grown in Petri dishes for 14 days were harvested, and shoots from 2 to 3 plates containing the same medium were pooled together and frozen using liquid nitrogen, and then ground using mortar and pestle. The powder was halved. One half of frozen shoot powder was dried, weighed, extracted and analyzed by HPLC for artemisinin as described above, and the other half was stored in the freezer at -80°C until hexokinase activity was assayed.

About 0.1 g of frozen fresh powdered plant material and liquid nitrogen were added to the mortar and pestle. After the liquid nitrogen evaporated, 10% insoluble polyvinylpyrrolidone was added and further homogenized. Then 1 ml ice-cold extraction buffer containing 50 mM HEPES-KOH (pH 7.5), 5 mM MgCl_2 , 1 mM EDTA (pH 7.0), 15 mM KCl, 2.5 mM dithiothreitol, 0.1% Triton X-100, 10% glycerol and 1 tablet of protease inhibitor per 50 ml extraction buffer (Roche Diagnostics, #1836145) was added and thoroughly ground again. The extract was centrifuged at $17,400\times g$ for 3 min at 4°C and the crude supernatant was directly used for assay of hexokinase activity and total protein.

Hexokinase activity was measured using a coupled assay with glucose-6-phosphate dehydrogenase and NAD reduction. The assay mixture contained 50 mM Bicine-KOH pH 8.5, 15 mM KCl, 5 mM MgCl_2 , 2.5 mM ATP, 1 mM NAD, and 2 units glucose 6-*P* dehydrogenase in 450 μl at room temperature. For one assay, 450 μl of the assay mixture, 400 μl H_2O , and 100 μm plant extract were added in 1 ml cuvette, mixed, and the spectrophotometer (Hitachi U2800) was zeroed at 340 nm. Then, 50 μl of 0.1 M glucose was added to initiate the reaction and the absorbance at 340 nm was monitored for 30 min using the time scan method on the instrument. The protein concentration was determined according to the method of Bradford (1976) using BSA as standard. Unless otherwise stated all chemicals were obtained from Sigma-Aldrich Chemical Co., St. Louis, MO, USA.

Statistical analysis

Each sugar experiment was repeated 2–6 times and the results pooled and averaged. Data were analyzed using SPSS 14.0 for MS Windows (SPSS Inc), using ANOVA and the Mann-Whitney *U* test, or the Kruskal-Wallis test depending on the constraints of the data pool

followed by Dunn's post hoc test, when the sample sizes were different (Glantz 2001).

Results and discussion

Sucrose (Suc), glucose (Glc), and fructose (Fru) are the most common sugars existing in all plants. They can be easily transported into plant cells, readily converted to each other, and normally metabolized through glycolysis. Thus, it was necessary to first understand the individual effects of these three common sugars on growth and artemisinin production in *A. annua* seedlings.

Effects of single, common sugar metabolites on growth and artemisinin production

The effects of Glc or Fru were compared with Suc because Suc is the common carbon source used in axenic seedling cultures. Equimolar carbon (equivalent to the amount of carbon in 3% w/v Suc; 30 g/l) of Suc, Glc, or Fru was added into B5 medium and seedlings were cultured in Petri dishes for 14 days. When seedlings were grown in Glc, the number of true leaves was significantly increased, but decreased when Fru was provided; Glc also inhibited root growth (Fig. 2a). This resulted in a higher ratio of shoot to root biomass when seedlings were grown in Glc compared to Suc. Importantly, seedlings grown in medium with 30 g/l Glc produced about twice the artemisinin of those grown in Suc while artemisinin production from Fru was about half of that in Suc (Fig. 2b). These results showed that compared to Suc, Glc stimulated artemisinin production, while Fru inhibited it in *A. annua* whole plants. Although hairy roots also showed maximum production of artemisinin when grown in glucose, the level produced in fructose was twice that in sucrose (Weathers et al. 2004). In general the effects in whole plants varied considerably from those observed for hairy roots.

Effects of mixtures of sugars on growth and artemisinin production

Because Suc can be hydrolyzed rather rapidly into Glc and Fru via external and internal invertases (Sturm and Tang 1999), addition of extracellular Glc or Fru in the presence of Suc will inevitably change the ratio of these three sugars. For example, when a small amount of Glc is added with Suc, the relative Glc concentration is increased relative to cultures provided only Suc. Similarly, when a little Fru is added to Suc and provided exogenously, the relative Fru concentration is increased. A mixture of sugars should, thus, alter

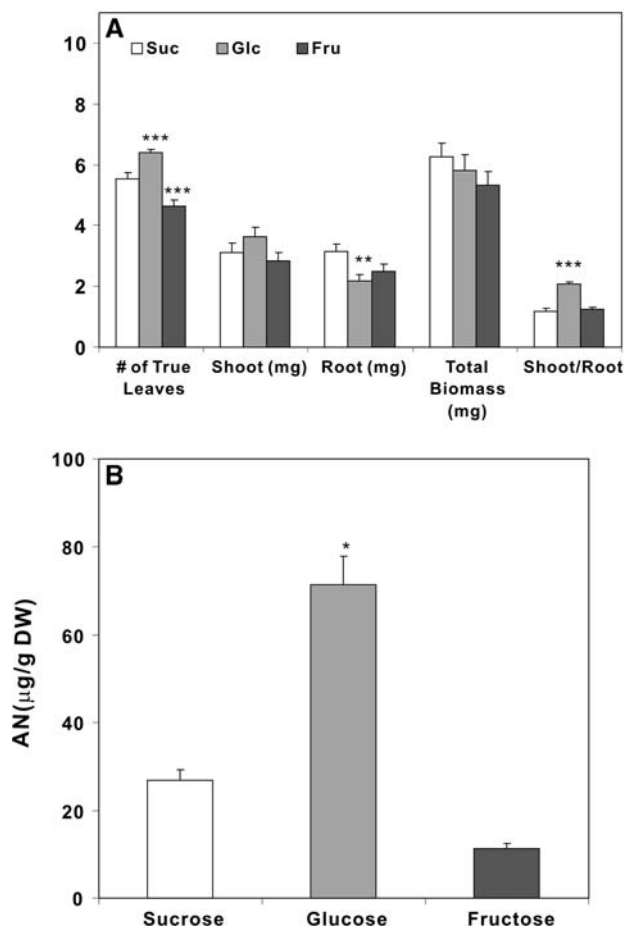


Fig. 2 Effects of sucrose, glucose, and fructose on growth and artemisinin production. **a** Growth responses to all three sugars, **b** effect on artemisinin production; 100% of any sugar = the amount of carbon in 30 g/l sucrose; AN, artemisinin. Data are mean of total replicates + SE. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

artemisinin production. Considering that normal plant cells would always have dynamic sugar concentrations, experiments were done using 27 g/l Suc supplemented with 3 g/l of either Glc or Fru, keeping total carbon constant (equivalent to 3% Suc). Although addition of 3 g/l Glc or 3 g/l Fru to 27 g/l Suc did not affect seedling growth significantly compared to 30 g/l Suc (Fig. 3a), seedlings showed a rather large decrease in artemisinin levels (Fig. 3b). This surprising decrease in artemisinin was in contrast to that in 30 g/l Glc (Fig. 2b) and to our work with hairy roots (Weathers et al. 2004). Taken together these data further suggested that mixtures of sugars have profound and complex effects on artemisinin production.

The previous experiments suggested that Suc, Glc, and Fru may be interacting with one another to affect the production of artemisinin in *A. annua* seedlings. To compare how Fru and Glc might work coordinately in

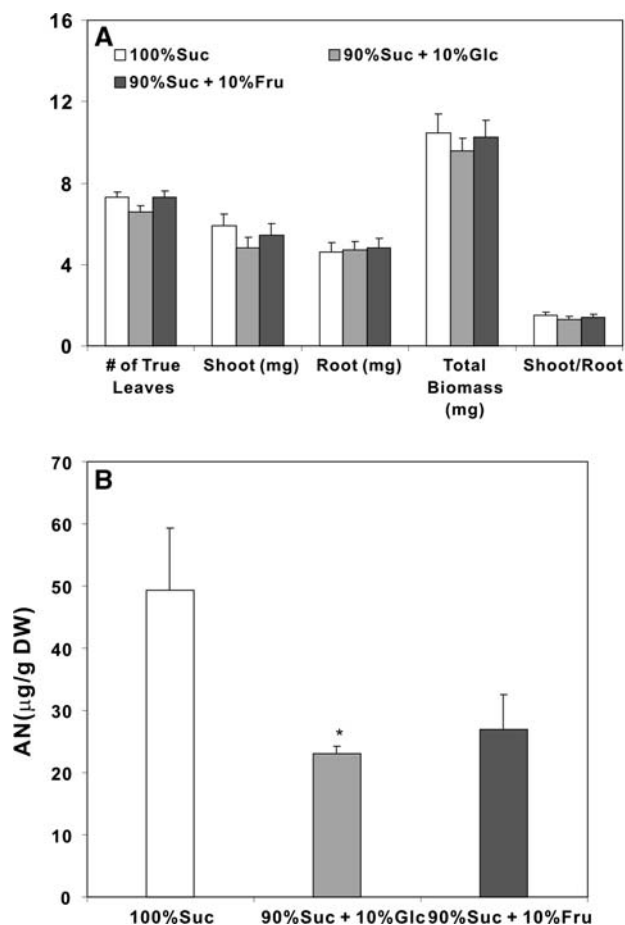


Fig. 3 Effect on growth and artemisinin production of sucrose combined with small amounts of either glucose or fructose. **a** Growth responses, **b** artemisinin production in 100% (30 g/l) Suc vs 90% (27 g/l) Suc + 10% (3 g/l) Glc, or vs 90% (27 g/l) Suc + 10% (3 g/l) Fru. Data are mean of total replicates + SE. * $P < 0.05$

altering growth and especially artemisinin production, it was important to eliminate Suc from the culture media because its presence confounded our ability to interpret responses from monosaccharides. Five different ratios of these two sugars were, therefore, tested on *A. annua* seedlings. As the concentration of Glc increased relative to Fru, overall biomass steadily decreased (Fig. 4a). While the number of true leaves increased, root mass decreased with increasing Glc level (Fig. 4a). This resulted in a slightly increasing shoot/root biomass ratio as the amount of Glc increased relative to Fru (Fig. 4a). Although there appears to be some correlation between decreasing biomass and increasing artemisinin concentrations, variations are not similarly proportionate.

Artemisinin production steadily increased as the Glc to Fru ratio increased (Fig. 4b). These results were consistent with the results using only Fru or only Glc (Fig. 2) and showed that increasing the concentration

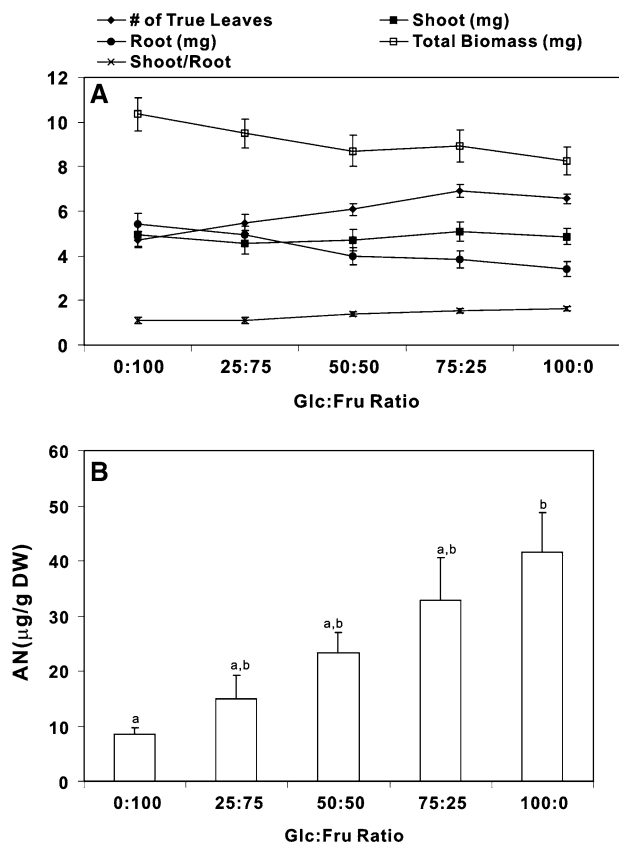


Fig. 4 Effects of variations in the Glc/Fru ratio on growth and artemisinin production. **a** Effect on growth, **b** effect on artemisinin production. There were 5 replicates for each ratio condition except 75/25, which contained 4; 100% of any sugar = 30 g/l. Data are mean of total replicates \pm SE. Groups that are statistically similar ($P > 0.05$) are labeled with the same letter

of Glc relative to Fru appears to stimulate more shoot than root growth and clearly stimulates artemisinin production. Both Fru and Glc can be phosphorylated by HXK but substrate specificity appears to vary with individual HXKs (Gonzali et al. 2002). For example, in the gametophyte of the moss, *Physcomitrella patens*, the *hxx1* knockout mutant showed much greater HXK activity with fructose than with glucose as substrate while the inverse was observed for wild type plants (Olsson et al. 2003).

Effects of Glc and its analogs on growth and artemisinin production

In preliminary studies using eight sugar analogues on *A. annua* hairy roots, we observed that in 30 g/l of five mono and three disaccharide analogues, there was no root growth (unpublished data). In all but one case, use of 10% (3 g/l) of the analogues +90% (27 g/l) sugar roots grew normally. Galactose was always toxic.

Considering that growth was not substantially different between 30 g/l sugar and 27 g/l sugar +3 g/l of its analogue, subsequent experiments using *A. annua* seedlings followed a similar design.

Three Glc analogs, 3-*O*-methylglucose (3OMG), mannose (Man), and mannitol (Mtl) were used to provide some preliminary insight into how *A. annua* growth and artemisinin production may be affected by Glc. Because there is considerable variability in uptake and transport of sugar analogues among plant species, the results here must be viewed with caution (Gibson 2000) and not be interpreted as confirmation of a sugar signaling mechanism. Rather, the data serve as indicating the direction of future study.

Although the Glc analog, 3OMG, can be effectively transported into the cytoplasm of many plant cells via plasma membrane monosaccharide transporters (Lalonde et al. 1999; Gibson 2000; Ho et al. 2001; Loreti et al. 2001) and then phosphorylated to 3-*O*-methylglucose-6-phosphate via HXK, this occurs about five orders of magnitude slower than for glucose or Man (Cortès et al. 2003). Since 3OMG appears to have no influence on HXK activity (Gonzali et al. 2002), it has been used to investigate whether hexokinase is a sensor in sugar signaling. Inhibition by 3OMG of a metabolic process therefore suggests that HXK is involved in regulation of that process. Man is not particularly common in plants; however, it can be transported into the cell via a monosaccharide transporter and phosphorylated by HXK to mannose-6-phosphate. Thereafter it is very slowly metabolized (Pego et al. 1999; Baskin et al. 2001). Alteration of a metabolic process by Man would suggest that some step downstream of HXK might be involved as a regulator. Mtl, on the other hand, is not metabolized by most plants, and cannot enter plant cells (Gibson 2000). It is commonly used as an agent to alter osmotic pressure and is not usually used in sugar analog studies. If Mtl affected some metabolic response, this would suggest either an osmotic effect, or the participation of a monosaccharide transporter in the process.

In the presence of 3 g/l 3OMG (+27 g/l Glc), the numbers of true leaves, shoot mass, root mass and total biomass decreased between 12 and 37% compared to seedlings grown in 30 g/l Glc (Fig. 5a). Shoot mass, root mass, and total biomass of seedlings were also remarkably inhibited by the addition of 3 g/l Mtl (Fig. 5a). No significant differences in growth were observed, however, between addition of 3 g/l (+27 g/l Glc) Man and the 30 g/l Glc control (Fig. 5a).

Artemisinin production, on the other hand, decreased by about 95% compared to the 30 g/l Glc control, when 3 g/l 3OMG (+27 g/l Glc) was added to

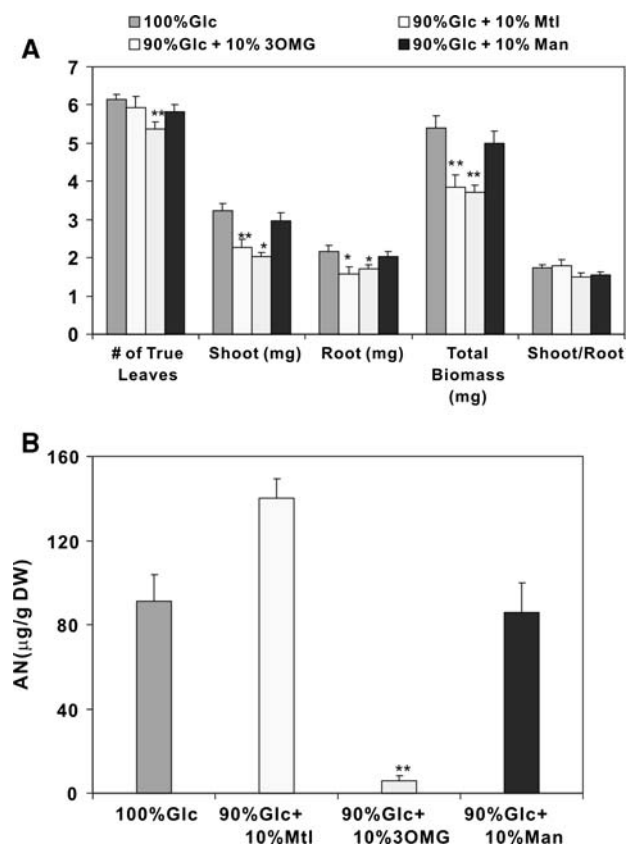


Fig. 5 Effects of glucose and its analogs on growth and artemisinin production. **a** Growth, **b** artemisinin production. Data are mean of total replicates + SE. There were 8 replicates for each condition except for 27 g/l Glc + 3 g/l Mtl, which only contained 3 replicates. ** $P < 0.01$

the medium (Fig. 5b), while neither Man nor Mtl had any significant effect on artemisinin production (Fig. 5b). The 3OMG analog cannot be phosphorylated effectively by HXK (Cortès et al. 2003), and artemisinin production was significantly decreased compared to the 30 g/l Glc control when only 3 g/l 3OMG (+27 g/l Glc) was added (Fig. 5b). Considering also that the Glc analog, Man, an effective substrate of HXK, produced a level of artemisinin equivalent to the 30 g/l Glc control, these results together suggested one of two things: either Glc is acting as a signal in the control of artemisinin production and 3OMG is disrupting that signal, or 3OMG is an inhibitor of artemisinin production.

HXK activity was also measured in seedlings grown on 30 g/l Glc and on 3 g/l 3OMG (+27 g/l Glc). Compared to the 30 g/l Glc control, HXK activity was significantly increased when 3OMG was added (Fig. 6). Taken together, these results suggested that Glc may be affecting a downstream control on artemisinin production.

The production of artemisinin in the presence of 3 g/l Mtl + 27 g/l Glc is statistically equivalent to the 30 g/l Glc control (Fig. 5b) suggesting that a monosaccharide transporter is probably not involved in the sensing process because Mtl does not enter the cell (Gibson 2000). These results are consistent with the study of Jang and Sheen (1994). In their study, neither L-Glc, which cannot be efficiently transported into plant cells, nor 3OMG, which can be transported into plant cells but cannot be effectively phosphorylated by HXK, repressed the expression of photosynthetic genes compared to Glc. Further, in the presence of Man, which is known to be phosphorylated by HXK, but not metabolized further, artemisinin yields were not significantly affected, results also similar to the studies by Jang and Sheen (1994). Taken together these results using the 3 Glc analogs, 3OMG, Man, Mtl, are consistent with a possible role for HXK in regulating artemisinin biosynthesis.

When the activity of HXK was measured in seedlings grown in 3 g/l 3OMG, HXK specific activity increased compared to that of seedlings grown in 30 g/l Glc (Fig. 6). These results have several possible interpretations. First, HXK, a known Glc signal sensor, may affect artemisinin production through its catalytic activity. The addition of 3 g/l 3OMG actually decreased by 3 g/l the total carbon that was metabolically available and since HXK activity increased concomitantly by 3 g/l (Fig. 6), it is possible that the increase in HXK activity may be in response to the total carbon available to the seedlings after 14 days in culture. Several studies, however, have indicated that Glc signaling is uncoupled from Glc metabolism (Jang and Sheen 1994; Jang et al. 1997). Further, HXK1 mutants

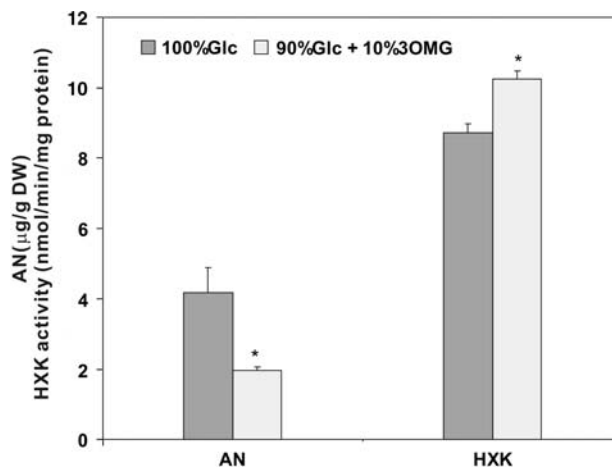


Fig. 6 Effects of 30 g/l glucose or 27 g/l glucose + 3 g/l 3OMG on artemisinin production and hexokinase activity. Data are the mean of three replicates + SE. * $P = 0.05$

lacking catalytic activity still showed various signaling functions (Moore et al. 2003). All of the signaling functions previously studied, however, have been related to gene expression or related to plant development, not secondary metabolism, so separation of Glc signaling from Glc metabolism cannot necessarily be assumed with respect to artemisinin production. Second, some unknown metabolite(s) downstream of Glc phosphorylation may be involved in artemisinin production with or without Glc signaling and this metabolite might be what is being sensed by HXK (Xiao et al. 2000). Third, increased HXK activity could also be due to some stress produced by the presence of 3OMG. For example, it was reported by Fox et al. (1998) that HXK activity is stimulated in shoots of *Echinochloa phyllopogon* by anaerobic stress. Further, 3OMG also can act as a competitive inhibitor of the Glc transporter, therefore inhibiting Glc entrance to plant cells which could subsequently induce an increase in HXK activity (Gogarten and Bentrup 1983; Getz et al. 1987). Clearly additional studies of HXK activity in seedlings grown in Suc, Fru, and Glc in combination with its other analogs and in the presence of an HXK inhibitor like *N*-acetyl glucosamine should be undertaken. Coupled with measurement of the mRNA expression of terpenoid genes active in artemisinin biosynthesis, such results would definitively demonstrate the sugar regulation of artemisinin biosynthesis.

Effects of sucrose and a disaccharide analog, palatinose, on growth and artemisinin production

Although monosaccharides are the main metabolic actors in plant cells, disaccharides also play crucial roles. The effect on growth and artemisinin production of Suc and one of its structural analogs, palatinose (Pal; Glc- α -[1 \rightarrow 6]- α -Fru) was also measured.

Pal, an analog of Suc, is not biosynthesized in higher plants, and cannot be recognized and transported by Suc transporters, so it is used to discern signals that might be perceived by a Suc transporter (Bouteau et al. 1999; Fernie et al. 2001; Börnke et al. 2002; Sinha et al. 2002) or at least by some signal external to the plasmalemma. For example, Pal can also induce extracellular invertase (Sinha et al. 2002). In preliminary experiments using *A. annua* hairy roots, growth in 30 g/l Pal was lethal. When grown in 3 g/l Pal (+27 g/l sucrose), however, roots grew equivalent to those in 30 g/l sucrose, but artemisinin was inhibited by 98% (data not shown).

Similar experiments using the same mixture of Pal and sucrose added to seedlings growing in shake flasks resulted in significantly more total biomass produced

compared to seedlings grown in 30 g/l Suc (Fig. 7a). Artemisinin levels were significantly reduced (by 80%) in the presence of Pal (Fig. 7b), similar to results observed for hairy roots (unpublished data). These results suggested that Suc also may be providing some control over artemisinin production, but at a point external to the plasmalemma because Pal can not be transported into cells but can rapidly induce extracellular invertase and, thus, Suc degradation (Börnke et al. 2002; Sinha et al. 2002). Loreti et al. (2000) had previously shown that similar to our results, disaccharides containing a Fru moiety had an inhibitory effect on α -amylase in barley embryos. Even the non metabolizable Fru moiety-containing-disaccharides, Pal, turanose, and lactulose repressed the enzyme indicating that the Fru moiety was necessary for sensing the disaccharide and that it was independent of the Glc sensing system (Loreti et al. 2000).

Contrary to expectations, addition of 3 g/l Glc to 27 g/l Suc did not increase artemisinin yield, but

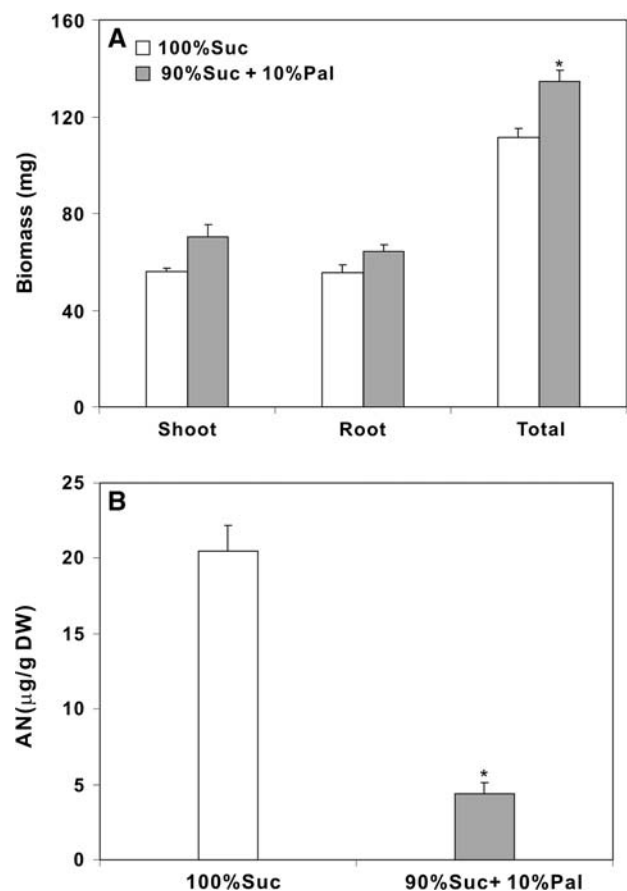


Fig. 7 Effects of sucrose and the palatinose analog on growth and artemisinin production. **a** Effect on growth, **b** effect on artemisinin production; 100% of any sugar = 30 g/l. Data are the mean of three replicates + SE. * $P < 0.05$

substantially reduced it relative to Suc alone (Fig. 3b) or Glc alone (Fig. 2b). This suggests that Suc control of terpenoid metabolism may override the stimulatory effect of Glc on artemisinin production. Furthermore, there is a clear, but markedly different response, when the ratios of only Glc to Fru are altered (Fig. 4b). Taken together these results further suggest a complex crosstalk between disaccharide and monosaccharide signaling in terpenoid biosynthesis in *A. annua*.

Conclusions

This is the first report showing that in *A. annua* whole plants artemisinin production is clearly stimulated by Glc, inhibited by Fru, and sensitive to the relative concentration of these two monosaccharides. To our knowledge this is also the first report showing that production of a secondary metabolite is directly controlled by the relative concentrations of Glc and Fru. By feeding small amounts of different sugar analogs to seedlings, results further suggested that there may also be both monosaccharide and disaccharide sugar sensing mechanisms that are involved in controlling artemisinin production in *A. annua*. Further studies are needed to provide a clear demonstration of an effect of these three sugars on the regulation of terpenoid genes, for example, at the level of mRNA expression, in order to confirm a regulatory role for sugars in artemisinin production.

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