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Trichomes + roots + ROS = artemisinin: regulating artemisinin biosynthesis in *Artemisia annua* L

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Abstract

Artemisinin is a highly effective sesquiterpene lactone therapeutic produced in the plant, *Artemisia annua*. Despite its efficacy against malaria and many other infectious diseases and neoplasms, the drug is in short supply mainly because the plant produces low levels of the compound. This review updates the current understanding of artemisinin biosynthesis with a special focus on the emerging knowledge of how biosynthesis of the compound is regulated *in planta*.

Keywords

Artemisinin; Trichome; ROS; Reactive oxygen species; Jasmonic acid; Salicylic acid; Abscisic acid; Gibberellic acid; Cytokinin

Introduction

In 2008, there were an estimated 253 million cases of malaria resulting in 863,000 deaths (World Health Organization [WHO] 2009). Approximately 85% of the cases and 89% of the deaths occurred in Africa where many countries are undeveloped and poor. While artemisinin is very effective against malaria, it remains too expensive for many people in these countries. Its high cost is due mainly to the high demand for the drug and low production *in planta*; *Artemisia annua* yields are typically less than 1.2% of dry weight (Kindermans *et al.* 2007). Much work is being done to increase the artemisinin supply, including breeding high-yielding *A. annua* strains (Graham *et al.* 2010), producing transgenic *A. annua* and engineering the artemisinin biosynthetic pathway into *Escherichia coli*, yeast, and tobacco (Arsenault *et al.* 2008; Covello 2008; Zhang *et al.* 2010). Artemisinin is not only effective against malaria but other diseases as well, including: different types of cancer, other parasites like schistosomiasis, and some viruses such as

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hepatitis B (Efferth 2009). The drug also appears to be useful in treating some animal diseases (Ferreira and Gonzalez 2008). Even though there is a lot of effort toward increasing the artemisinin supply, the biosynthetic pathway is not entirely clear and only recently have studies begun to elucidate its regulation. In this review, we update recent earlier reviews (Weathers *et al.* 2006; Covello *et al.* 2007) but with a focus on what is known currently about the regulation of artemisinin biosynthesis in *A. annua*.

Biosynthesis of Artemisinin

Artemisinin (AN; Fig. 1) is a sesquiterpene lactone that stems from isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). These 5-carbon building blocks of all terpenes can originate from either the cytosolic mevalonate (MVA) pathway or the methylerythritol phosphate (MEP) pathway in the plastid (Fig. 1). In 2007, Towler and Weathers showed that, through inhibition with mevinolin or fosmidomycin, AN is produced using IPP/DMAPP from both the MVA and MEP pathways, respectively. This result was quantitatively confirmed by Schramek *et al.* (2010) using isotopologue profiling of *A. annua* plants with $^{13}CO_2$; the farnesyl diphosphate which eventually yields AN is composed of one isoprene unit from the MEP pathway and two isoprene units from the MVA pathway.

Although FPP can lead to a number of other compounds in *A. annua* (Weathers *et al.* 2006), the first step toward artemisinin biosynthesis is the cyclization of FPP to amorpha-4,11-diene by amorphadiene synthase (ADS; Chang *et al.* 2000; Mercke *et al.* 2000; Wallaart *et al.* 2001). Amorpha-4,11-diene is then oxidized twice by a cytochrome P450, CYP71AV1 (CYP), to form artemisinic alcohol and then artemisinic aldehyde (Ro *et al.* 2006; Teoh *et al.* 2006). From here, the exact pathway to artemisinin and other end products is not as clearly understood. It seems there are two possible routes from artemisinic aldehyde: one route to arteannuin B (AB) and one to AN. The route to AB occurs via oxidation of artemisinic aldehyde to artemisinic acid (AA) either by CYP or an aldehyde dehydrogenase (Aldh1), apparently culminating in AB production (Teoh *et al.* 2009; Fig. 1). Although Dhingra and Narasu (2001) suggested that AB could be converted to artemisitene (AT) and then to AN in a cell-free system, to our knowledge there has been no other work to validate enzymatic *in planta* conversion of AB to AN via AT. Indeed, Brown and Sy (2007) provide data pointing to a lack of conversion of AA to AN. Thus, it is currently thought that AB is one end product of two branches of the pathway to *seco*-cadinanes (including AN and AB) in *A. annua*.

In the other branch of the *seco*-cadinane pathway post-CYP, artemisinic aldehyde is converted to dihydroartemisinic aldehyde by the double bond artemisinic aldehyde reductase (DBR2; Zhang *et al.* 2008) and then converted to DHAA by Aldh1 (Teoh *et al.* 2009). DHAA is then converted to AN probably by more than one non-enzymatic, spontaneous photo-oxidation reactions (Wallaart *et al.* 2001; Brown and Sy 2007). Recently, Rydén *et al.* (2010) showed that a new enzyme, dihydroartemisinic aldehyde reductase (Red1), in the pathway appears to be competing for dihydroartemisinic aldehyde, the precursor to DHAA, thereby reducing potential AN production. Once better understood, inhibition of these alternate pathway enzymes offers potential targets for increasing production of AN.

There also exists in *A. annua* two well-defined chemotypes, which account for differences in flux of artemisinic metabolites within the pathway (Wallaart *et al.* 2000). One chemotype produces mainly AN and its precursor DHAA, while the other produces mainly AB and its AA precursor. These variants can apparently be maintained as separate genetic stocks (Delabays *et al.* 2001). It is possible that understanding the regulatory mechanisms will elucidate how such pathway variations occur within these genetic sub-types.

Morphological and Developmental Regulation of Artemisinin

Trichomes

Trichomes are appendages that develop from the epidermal cells of leaves, stems, and flowers and occur in a variety of shapes and sizes: unicellular or multicellular, branched or unbranched, and glandular or nonglandular. Glandular trichomes are important to artemisinin biosynthesis because they store many secondary metabolites including AN (Duke *et al.* 1994; Ferreira and Janick 1996a).

AN is produced and sequestered in the glandular secretory trichomes (GSTs) of *A. annua* which are composed of ten cells (Fig. 2): two basal cells, two stalk cells, four sub-apical cells, two apical cells, and a subcuticular space where AN accumulates (Ferreira and Janick 1996a,b;Olsson *et al.* 2009). GSTs are located mainly on the leaves and flowers of *A. annua* (Ferreira and Janick 1996a). Since trichomes are the sites of synthesis of AN, it should be expected that more trichomes would result in more AN produced, and indeed, there is a strong correlation between AN concentration and trichome density (Kapoor *et al.* 2007;Arsenault *et al.* 2010a;Graham *et al.* 2010). It was also found that younger leaves develop more trichomes and produce more AN than older leaves (Ferreira and Janick 1996a;Arsenault *et al.* 2010a). Interestingly, in mature plants, however, senescent leaves provide nearly half of the total AN in the plant (Lommen *et al.* 2007).

Through microdissection of *A. annua* glandular trichomes and RT-PCR, transcripts of early genes in the AN biosynthesis pathway (*ADS*, *CYP71AV1*, *DBR2*) were found only in the apical cells of the trichome, while transcripts of *DXR* from the plastid-localized MEP pathway were found only in the sub-apical cells (Olsson *et al.* 2009). The apical and basal cells are not green, but the four subapical cells are, so the presence of *DXR* transcripts in these latter cells is consistent with functional chloroplasts. Furthermore, transcripts of *FPS* were found in both the apical and sub-apical cells suggesting that the nonplastid MVA pathway is functional in both cell types. It appears that different cells in the glandular trichomes play different roles in the AN pathway. Although no artemisinin was detected in glandless *A. annua*, it is still unclear if any of the genes in the AN pathway are expressed elsewhere in the leaves (Duke *et al.* 1994).

Lommen *et al.* (2006) suggested that upon maturation, trichomes collapse and release AN, which may inhibit the AN biosynthetic pathway. Arsenault *et al.* (2010a) later tested this hypothesis by spraying the foliage of soil-grown plants during the vegetative stage with either 100 μg/ml AN or AA in 70% ethanol and measuring the mRNA transcripts of *HMGR*, *FPS*, *ADS* and *CYP*. Compared to the control plants sprayed with only 70% ethanol carrier, plants sprayed with AN only showed decreased transcription of *CYP*. In contrast, plants sprayed with AA showed inhibition of both *ADS* and *CYP*. This suggested that the AN biosynthetic pathway is regulated through feedback inhibition and that ruptured trichomes may reduce production.

Maes *et al.* (2010) recently showed that trichome density significantly increased in response to jasmonic acid (JA) and 6-benzylaminopurine (BAP). Seedlings that were typically either high or low AN producers were sprayed and the associated soil was drenched with hormones every 2 d for 5 wk. When they compared trichome size between these two groups of plants, only the low producers showed a significant increase in trichome size. When artemisinic metabolites were measured, however, both the high and the low AN-producing plants showed significant increases in AA, AB, DHAA, and AN in response to JA. Overall, however, the largest percentage increases in AN and AB occurred in the low AN-producing plants. Together these results further substantiate that there may be upper limits to both trichome density as well as AN content.

Roots

In vitro-cultured shoots of *A. annua* that develop roots produce more AN than shoots without roots (Ferreira and Janick 1996b). Similarly, compared to unrooted shoots growing in liquid culture, only rooted shoots of *A. annua* responded to DMSO elicitation of AN production (Mannan *et al.* 2010). Furthermore, when DMSO was applied only to leaves of rooted plants, there was no change in AN production. Together, these results show that despite the fact that the roots produce neither AN nor its precursors, they play a critical role in AN biosynthesis and possibly in the perception of signals that affect its synthesis. How the roots affect AN production is still unclear, since negligible amounts of transcripts of *ADS* (Kim *et al.* 2008), *CYP* (Teoh *et al.* 2006), *DBR2* (Zhang *et al.* 2008), and *Aldh1* (Teoh *et al.* 2009) were found in the roots. Clearly more research is needed to elucidate how roots increase AN in the shoots of *A. annua*.

Shifting from vegetative to reproductive growth

As *A. annua* plants develop from vegetative to reproductive growth, AN levels increase (Ferreira *et al.* 1995; Lommen *et al.* 2006; Ma *et al.* 2008). Recently, Arsenault *et al.* (2010a) measured AN, AB, and their respective precursors, DHAA and AA, as well as transcripts of six genes in the AN biosynthetic pathway (*HMGR*, *FPS*, *DXR*, *DXS*, *ADS*, *CYP*) in two types of leaf tissue, flower buds, and full flowers in soil-grown *A. annua* in different developmental stages: vegetative, floral budding, and full flowering. When comparing the transcript levels of these six genes in similar leaf tissues at vegetative, budding and flowering stages, the highest levels of *HMGR*, *DXR*, and *DXS* were found in leaves at the budding stage and the highest level of *FPS* in leaves at the flowering stage. Although the highest level of *FPS* was coincident with the highest AN level detected, it is unclear how FPS plays a role in AN regulation since overexpression of the FPS gene in *A. annua* has provided conflicting results. While several studies showed a significant increase in AN when FPS was overexpressed (Chen *et al.* 2000; Han *et al.* 2006; Banyai *et al.* 2010), others reported no change from controls (Ma *et al.* 2008) or a decrease in AN yield in some transformants (Banyai *et al.* 2010). On further analysis, Banyai *et al.* (2010) observed some gene silencing when two copies of the gene were inserted. In cases where there was a single gene insertion, position of the *Agrobacterium tumefaciens* delivered transgene was suggested as a possible explanation. Despite these conflicting results using transgenic *A. annua*, untransformed plants increased expression of FPS under various stimuli and this generally correlated with increases in AN production (Arsenault *et al.* 2010a).

As plants transition to flowering, transcript levels of *ADS* and *CYP* showed a very different pattern than the previously mentioned four genes. Instead of the highest transcript levels of *ADS* and *CYP* appearing during budding or flowering, as one might expect, they were highest during vegetative growth and then declined significantly during floral development (Arsenault *et al.* 2010a). Similarly, levels of DHAA were highest in vegetative plants compared to budding and full flower plants. These data suggest that the key metabolic genes responsible for directing terpenoid biosynthesis toward AN may be negatively related to AN production, or that increased AN negatively regulates these genes *in vivo*. At least during the reproductive stage, when it is the highest, AN appears to inhibit the transcription of *ADS* and *CYP*, which are AN-specific biosynthetic genes. Indeed, this has been confirmed through exogenous application of AN (Arsenault *et al.* 2010a).

Artemisinin levels are highest either just before or after full flower, suggesting that the flowering process may be involved in regulation of AN biosynthesis (Ferreira *et al.* 1995; Ma *et al.* 2008; Arsenault *et al.* 2010a). This now appears unlikely as two studies that induced premature flowering using the early flowering gene CONSTANS (Wang *et al.* 2004) or the flowering promoting factor1 (Wang *et al.* 2007) showed no significant increase

in AN levels compared to wild type controls. This suggested that the high level of AN found before or after the flowering stage is not directly linked to the act of flowering.

Environmental Regulation of Artemisinin

Abiotic

Plants can respond to their environment by altering shape, pigment content, and their secondary metabolites. Not surprisingly, some environmental factors also alter AN production. Lulu *et al.* (2008) showed that chilling can affect both AN content and transcript levels of *ADS* and *CYP* in *in vitro* cultures. Using qPCR and HPLC analysis of AN levels, they observed after 24 h 11- and 7-fold increases in *ADS* and *CYP*, respectively, in *A. annua* plant cultures chilled to 4°C for 30 min compared to non-chilled cultures. Although chilling also doubled AN levels, higher temperatures (42°C) did not seem to affect AN biosynthesis or transcript levels of either *ADS* or *CYP*. Yang *et al.* (2010) also showed marked increases in *ADS*, *CYP*, and *DXS* in response to chilling. In contrast to Lulu *et al.* (2008), the Yang group also showed a significant increase in both *ADS* and *CYP* in response to a 1-h dose of UV light.

Drought also affects AN levels in plants (Marchese *et al.* 2010). Pot-grown plants deprived of water for either 38 or 62 h showed increased AN levels in leaves. Only the shorter duration treatment was effective as it did not reduce leaf biomass. Yang *et al.* (2010) substantiated this response by showing *ADS* increased in shoots when plant roots were allowed to dry for 6 h. In contrast, water logging decreased *ADS*, but increased *CYP* transcription; *DBR2* also increased. These results may be important for selecting optimal harvest time as there are two perceived benefits: increased AN content overall and reduced water content of plants, thereby reducing drying time.

Various minerals also affect AN yields. For example, boron at limited application levels can increase AN significantly with little or no effect on biomass (Aftab *et al.* 2010). Levels of H_2O_2 also increased with boron application, so the increase in AN may be explained by a ROS stress response. Phosphate was also shown to increase AN levels, but the mechanism of the stimulus is not known (Kapoor *et al.* 2007).

Biotic

Although pathogens and pathogen-related compounds like chitosan are known to be elicitors of secondary metabolites in many plants, only a few reports seem to focus on studies with whole *A. annua* plants. Chitosan is an elicitor of AN production and appears to function by inducing transcription of the *AaWRKY1* transcription factor that seems to regulate the *ADS* promoter (Ma *et al.* 2009). In another example, Kapoor *et al.* (2007) showed that addition of mycorrhizae to roots of potted plants increased AN levels in leaves by as much as 300%, depending on the fungal species, with *Glomus fasciculatum* performing best. This gain also correlated closely with increased trichome density, a response observed in other species of mycorhizzal plants (Kapoor *et al.* 2007). In comparison to uninoculated plants, addition of phosphate to plants did not alter AN levels. This study shows that certain biotic factors can play a significant role in altering the morphology of the *A. annua* leaves by increasing trichome numbers and that the perception of a stimulating signal again occurs via the roots. Overall, there are clearly a number of different environmental effects which are beginning to be elucidated to show how they may alter regulation of the AN biosynthetic pathway.

Phytohormones and Other Signaling Molecules

GA, ABA, and BAP effects

Gibberellic acid (GA_3), abscisic acid (ABA), and cytokinins are phytohormones known to affect AN production. GA_3 and BAP are also known to stimulate trichome development (Maes *et al.* 2008). When the *A. tumefaciens* cytokinin biosynthetic gene, isopentenyl transferase was transferred and constitutively expressed in *A. annua*, the *in vivo* content of cytokinins increased along with chlorophyll and AN (Sa *et al.* 2001) suggesting that biosynthesis of AN is regulated by cytokinins. This is not surprising since trichome development is known to be stimulated by BAP. Although the level of AN was increased in these transgenic plants, root mass decreased by about 50%, indicating that either the source (bacterial gene) or amount of cytokinin produced were problematic.

In a later study, Maes *et al.* (2010) sprayed and soil-drenched two *A. annua* cultivars with either water, 50 μM BAP, or 100 μM GA_3 and saw that both phytohormones stimulated filamentous trichome development, but only BAP stimulated glandular trichome development in both cultivars. Although the two cultivars showed an increase in glandular trichomes, the low-artemisinin-producing cultivar showed a greater increase than the high-artemisinin-producing cultivar (Maes *et al.* 2010). Also, compared to the control, only GA_3 increased the trichome size of the low-artemisinin-producing but not the high-artemisinin-producing cultivar. This suggested that trichomes in the high-artemisinin cultivar were already replete with AN.

ABA plays an important role in a plant's response to different biotic and abiotic stresses; also, it is elevated often in response to drought. When soil-grown *A. annua* plants were sprayed with 1–100 μM ABA, there was a significant increase in AN content compared to controls (Jing *et al.* 2009). There also was an increase in *HMGR*, *FPS*, and *CYP* transcripts in ABA-treated plants after 4, 12, and 8 h, respectively. This is consistent with the observations of Yang *et al.* (2010) who reported increased *ADS* expression in plants when roots were subjected to drought.

JA and SA effects

Like many secondary metabolites, AN is thought to play a part in plant defense, including against oxidative stress. JA is a secondary messenger known to activate a plant's defense system against oxidative stress and to induce production of secondary metabolites (van der Fits and Memelink 2000). Wang *et al.* (2009) sprayed soil-grown plants with 300 μM methyl jasmonate and 6 d after the treatment saw a significant increase in AN compared to controls. Because trichomes are the site of AN biosynthesis and sequestration, Liu *et al.* (2009) also measured trichome density 14 d after JA treatment. Both trichome density and AN levels increased after JA treatment compared to controls. Later Maes *et al.* (2010) sprayed and soil-drenched two *A. annua* cultivars with 100 μM JA or water and observed a response similar to that of GA_3 treatment: enhanced trichome density, larger trichomes, and more AN. JA stimulated AN and DHAA production concurrent with increases in *FPS* and *DBR2* expression in a high-artemisinin cultivar. JA also stimulated AB and AA production concurrent with increases in *ALDH1* in the low-artemisinin cultivar (Maes *et al.* 2010). In addition, JA increased expression of *ADS*, *CYP*, and *CPR* in both cultivars.

Salicylic acid (SA) is another plant signal involved in defense (Durrant and Dong 2004). When SA was sprayed onto *A. annua* plants, AN, AA, and DHAA all increased (Pu *et al.* 2009). Only *ADS* and *HMGR* showed a measurable increase in transcripts. The ROS molecules, H_2O_2 and O_2^- both showed significant increases about 4 h after treatment, and this timing coincided with a significant increase in AN. This study was later confirmed by Guo *et al.* (2010).

Other Regulation of Artemisinin

Competing pathways

Inhibition of squalene synthase (SQS) in other plants has been shown to be coordinately regulated with sesquiterpene cyclases. For example, Vögeli and Chappell (1988) showed that in tobacco when sterols were inhibited, sesquiterpene production increased; conversely, inhibition of sesquiterpene synthesis resulted in enhanced sterol synthesis. In *A. annua*, when SQS was inhibited with miconazole, AN yield increased (Kudakasseril *et al.* 1987; Towler and Weathers 2007). Moreover, studies using either an antisense or interference strategy with *A. annua* (Yang *et al.* 2008; Feng *et al.* 2009), or promoter replacement in *Saccharomyces cerevisiae* (Paradise *et al.* 2008), showed that when SQS was inhibited, AN metabolites and transcripts of genes in the AN biosynthetic pathway both increased. Feng *et al.* (2009) further showed that downregulation of *SQS* results in preferential upregulation of *ADS* instead of the other competing sesquiterpene cyclases.

Transcription factors

As mentioned previously, a transcription factor, AaWRKY1, was found in *A. annua* and affects AN biosynthesis. AaWRKY1 binds to the W-box cis-acting elements of the ADS promoter and likely regulates ADS gene expression (Ma *et al.* 2009). WRKY transcription factors are plant specific transcription factors that bind to the W-box of the promoter of defense-related genes (Ülker and Somssich 2004). These transcription factors are known to contain a conserved amino acid sequence (WRKYGQK) and a zinc finger-like motif. Using the plant cis-acting regulatory DNA element database, Ma *et al.* (2009) found that the *ADS* promoter contains two reverse-oriented TTGACC W-boxes. Subsequently, AaWRkY1 was isolated by constructing a cDNA library using mRNA isolated from GSTs and shown to contain the consensus WRKY sequence and a zinc finger-like motif. Ma *et al.* (2009) showed through an electrophoretic mobility shift assay that AaWRKY1 was able to bind to the ADS promoter but unable to bind to a mutated ADS promoter. They also showed that an increase in *AaWRKY1* resulted in an increase in *ADS* in transformed *A. annua*. JA also upregulates *AaWRKY1*, which correlates well with the *ADS* results observed by Maes *et al.* (2010).

Sugars

Sugars are known to stimulate production of many secondary metabolites, including AN (Wang and Weathers 2007). When *A. annua* seedlings were inoculated into Gamborg's B5 medium containing 3% (*w/v*) sucrose, glucose, or fructose for 14 d, glucose stimulated AN production while fructose inhibited production. Increasing the ratio of glucose to fructose showed a direct correlation with increasing AN level (Wang and Weathers 2007).

In a subsequent study, Arsenault *et al.* (2010b) reported that expression of the genes *HMGR*, *FPS*, *DXS*, *DXR*, *ADS*, and *CYP* in the AN biosynthetic pathway as well as production of AN metabolites, AN, AB, DHAA, and AA were also affected. AN was the highest 2 d after inoculation of seedlings into B5 medium containing glucose, and as AN increased from day 1 to day 2, DHAA began to decrease (Arsenault *et al.* 2010b). In medium containing fructose, both AN and DHAA decreased, further supporting the earlier observation by Wang and Weathers (2007) that fructose inhibits AN biosynthesis (Arsenault *et al.* 2010b). On the other hand, AA and AB continued to decline after inoculation into medium containing glucose. In medium containing fructose, however, the AA concentration was always higher than AB. It appeared that AA does not show the same precursor kinetic patterns as was seen with DHAA and AN. Although after 1 d glucose- and fructose-fed plants increased *HMGR* transcripts, 3.5- and 2.5-fold, respectively, compared to seedlings fed sucrose, *FPS* increased 8-fold but only in the glucose-fed seedling (Arsenault *et al.* 2010b). Glucose also increased

DXS and *DXR* expression in seedlings 1 d after inoculation. After a week's incubation, and compared to fructose-fed seedlings, glucose-fed seedlings showed a 3-fold increase in *ADS* expression relative to sucrose-fed seedlings. *CYP* expression, on the other hand, showed a bimodal increase 2 and 7 d after inoculation of seedlings into glucose. Only glucose induced expression of all six genes involved in AN biosynthesis. While not practical from the standpoint of production, these studies show that *in planta* regulation of AN production, at least in young seedlings, is subject to fluctuations in native sugars that are in constant flux in plants.

DMSO

Although AN is known to be phytotoxic and is thought to be involved in plant defense, it has also been suggested that DHAA eliminates reactive oxygen species (ROS) by reacting with ROS to form AN as a by-product (Mannan *et al.* 2010). Staining with 3,3-diaminobenzidine (DAB) for hydrogen peroxide, a common ROS in plants, Mannan *et al.* (2010) reported an increase in hydrogen peroxide in leaves of 14-d rooted shoots that were exposed to DMSO. The highest amounts of AN were detected in rooted shoots exposed to 0.25% (*v/v*)and 2% (*v/v*) DMSO after 7 d. It was also found that AN levels of rooted shoots exposed to 0.25% (*v/v*) DMSO produced the highest AN level, which continued to increase after 7 d. Interestingly, analysis of DHAA and AA, the precursors of AN and AB, respectively, in the same experiment described above showed that DMSO was able to increase production of DHAA but not AA. The highest level of *CYP* transcripts were detected in cultures exposed to 0.5% (*v/v*) DMSO after 24 h, but at 0.5% (*v/v*) DMSO, AN level was the lowest compared to cultures exposed to 0.1%, 0.25%, 1%, and 2% (*v/v*) DMSO. There were no significant changes to *ADS* transcripts level in any of the DMSO concentrations.

Ascorbic acid (vitamin C, AsA) is a ROS scavenger, and Mannan *et al.* (2010) hypothesized that the hydrogen peroxide induced by DMSO should decrease in the presence of AsA. When DMSO-treated plants were incubated with either 10 or 20 mM AsA, levels of peroxide decreased and production of AN significantly decreased by about 80%. This study showed that although DMSO is not part of the natural biochemistry of the plant, it can be a useful tool in helping to elucidate the role of ROS in AN biosynthesis. Indeed, ROS, possibly as hydrogen peroxide, is playing a role in AN biosynthesis and DHAA is likely acting as a ROS sink with concomitant production of AN.

Conclusions

Overall, a number of different factors are now known to affect the production of AN in *A. annua*, and these are summarized in Table 1. Until recently, the one broad rule had been: trichomes + roots + ROS = artemisinin. However, it is now clear that, not surprisingly, phytohormones, in particular jasmonic acid, are playing an important role in regulating production of this terpene. As finer points of control become elucidated, our understanding of terpene production in plants will hopefully enable us to better harness the plant for production of this important therapeutic.

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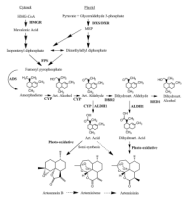

Figure 1.

Artemisinin biosynthetic pathway. *Art acid* artemisinic acid, *ADS* amorpha-4, 11-diene synthase, *Aldh1* aldehyde dehydrogenase 1, *CYP* cytochrome P 450 CYP71AV1, *DBR2* double bond reductase 2, *DXR* 1-deoxyxylulouse 5-phosphate reductoisomerase, *DXS* 1-deoxyxylulose 5- phosphate synthase, *FPS* farnesyl diphosphate synthase, *HMGR* 3-hydroxy-3-methylglutaryl-CoA reductase, *HMG-CoA* 3-hydroxy-3-methylglutaryl-CoA, *MEP* nonmevalonate pathway (plastid pathway), *RED*1 dihydroartemisinic aldehyde reductase 1.

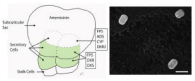

Figure 2.
Schematic (*left*) and photo (*right*)of *Artemisia annua* trichomes. The trichomes on the right are visualized using UV under a confocal microscope; bar = 50 μm. *ADS* amorphadiene 4,11 synthase, *CYP* cytochrome P 450 CYP71AV1, *DBR2* double bond reductase 2, *DXR* 1-deoxyxylulouse 5-phosphate reductoisomerase, *DXS* 1-deoxyxylulose 5-phosphate synthase, *FPS* farnesyl diphosphate synthase.

Table 1

Summary of known regulating factors affecting artemisinin production in *A. annua*

	JA	SA	GA_3	BAP	ABA	DMSO	Glucose	Fructose	AA	AN	Chilling	Drought	Water logging[a]	AM
GENES														
HMGR	nm	+	nm	nm	+	nm	+	+	ns	ns	+	ns	ns	nm
DXS	nm	nm	nm	nm	nm	nm	+	ns	nm	nm	+	+	ns	nm
DXR	nm	nm	nm	nm	nm	nm	+	ns	nm	nm	+	ns	ns	nm
FPS	+	ns	nm	nm	+	nm	+	ns	ns	ns	+	+	ns	nm
ADS	+	+	nm	nm	ns	ns	+	ns	−	ns	+	+	ns	nm
CYP	+	ns	nm	nm	+	+	+	ns	−	−	+	+	ns	nm
DBR2	+	nm	nm	nm	nm	nm	nm	nm	nm	nm	ns	ns	+	nm
ALDH1	+	nm	nm	nm	nm	nm	nm	nm	nm	nm	nm	nm	nm	nm
METABOLITES														
AA	+	+	ns	ns	nm	ns	−	−	nm	nm	nm	nm	nm	nm
AB	+	nm	ns	ns	nm	ns	−	−	nm	nm	nm	nm	nm	nm
DHAA	+	+	ns	ns	nm	+	−	−	nm	nm	nm	nm	nm	nm
AN	+	+	ns	ns	+	+	+	−	nm	−	+	+	nm	+
Glandular trichome #	+	nm	ns	+	nm	nm	nm	nm	nm	nm	nm	nm	nm	nm
Shoot response to root-perceived signal?	Yes	nm	Yes	Yes	nm	Yes	Yes	Yes	nm	nm	nm	nm	Yes	Yes

[a]Yang et al. (2010) suggested that water logging induced hypoxia. +

nm not measured, *ns* not significant, + upregulated or increased; − downregulated or decreased, *AA* artemisinic acid; *AB* arteannuin B; *ABA* abscisic acid; *AM* arbuscular mycorrhizae; *AN* artemisinin; *DMSO* dimethyl sulfoxide; *DHAA* dihydroartemisinic acid; *JA* jasmonic acid; *SA* salicylic acid; *GA*3 gibberellin 3; *BAP* benzylaminopurine