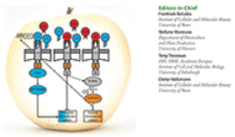
PLANT signaling & behavior



Plant Signaling & Behavior

Taylor & Francis
Taylor & Francis Group

ISSN: (Print) 1559-2324 (Online) Journal homepage: http://www.tandfonline.com/loi/kpsb20

New Insights Into Artemisinin Regulation

Zongyou Lv, Lei Zhang & Kexuan Tang

To cite this article: Zongyou Lv, Lei Zhang & Kexuan Tang (2017): New Insights Into Artemisinin Regulation, Plant Signaling & Behavior, DOI: 10.1080/15592324.2017.1366398

To link to this article: http://dx.doi.org/10.1080/15592324.2017.1366398

Accepted author version posted online: 24 Aug 2017.

Mini-Review

New Insights Into Artemisinin Regulation

Zongyou Lv[1,2], Lei Zhang[2], Kexuan Tang[1]*

1 Joint International Research Laboratory of Metabolic & Developmental Sciences, Key Laboratory of Urban Agriculture (South) Ministry of Agriculture, Plant Biotechnology Research Center, Fudan-SJTU-Nottingham Plant Biotechnology R&D Center, Shanghai Jiao Tong University, Shanghai 200240, China

2 Department of Pharmaceutical Botany, School of Pharmacy, Second Military Medical University, Shanghai 200433, China

Submitted: 27 July 2017

Accepted:

Key Words: *Artemisia annua* L; Artemisinin; hormones; miRNA; transcription factors; transporter

***Correspondence to:**

Kexuan Tang, Email: kxtang@sjtu.edu.cn; Fax, 86-21-34206916.

Abstract

Artemisinin is a sesquiterpene lactone coming from the traditional Chinese herb *Artemisia annua* L. Artemisinin combination therapies (ACTs) are the main recommended treatment for malaria. Transcription factors regulation of artemisinin belong to different families including AP2/ERF, bHLH, MYB and WRKY. Plant hormones jasmonic acid (JA), abscisic acid (ABA), salicylic acid (SA) and gibberellins (GA) have been described as positively affecting artemisinin biosynthesis in *A. annua*. Transporter and miRNA open up new possibilities for the biosynthesis of high value artemisinin. We review recently major developments regarding regulator which play a center role in artemisinin biosynthesis, and provide suggestion for further studies.

TEXT

Artemisinin is a sesquiterpene lactone which comes from glandular trichomes of the traditional Chinese herb *Artemisia annua* L. Artemisinin combination therapies (ACTs) are the main recommended treatments for malaria by WHO. It can be used for the treatment of cancer, febrile diseases, inhibition of *Mycobacterium tuberculosis*, and curing for diabetes also. There are many people living in the areas with high risk of the malaria, unfortunately, and malaria brings about over 430 000 child deaths in Africa every year. The price of artemisinin is too expensive for many

people in South East Asia and Africa, so there is an urgent need to reduce the production costs for the developing-world people. However, the content of artemisinin is low in planta (0.1-0.8%); the increase of artemisinin content is very important for stabilizing supplies and satisfying projected global demand. Many works have been done to increase the artemisinin supply, including transgenic breeding high-yielding *A. annua* strains, yeast and tobacco metabolic. The engineering chemical synthesis of artemisinin is not economically feasible because of its low yield and high cost. The semisynthesis of artemisinin in yeast is expensive, making it uncompetitive with the plant-based production and stable supplies the market.

To increase the content of artemisinin, many experimental works have been done. Even though a lot of efforts toward increasing the artemisinin content have been made, there is still room for improvement artemisinin. To improve the drug production and decrease its price, we included studies of regulating artemisinin biosynthesis in *A. annua* in this mini-review.

Biosynthesis of Artemisinin

The biosynthetic pathway of artemisinin is clear today along with other pathways of terpene metabolism in *A. annua*. Farnesyl diphosphate (FPP) is the direct substrate of artemisinin biosynthesis. Under the catalysis of a series enzymes, such as amorphadiene synthase (ADS),

cytochrome P450 (CYP71AV1), artemisinic aldehyde Á11(13) reductase (DBR2) and aldehyde dehydrogenase 1 (ALDH1), FPP can change into dihydroartemisinic acid (DHAA)[1-2] (Figure 1).

The overexpression of artemisinin biosynthetic pathway genes is very effective in increase the artemisinin content. However, there are four enzymes (ADS, CYP71AV1, DBR2 and ALDH1) in artemisinin biosynthetic pathway, and the rate-limiting enzyme is unknown. Previous study indicated that the artemisinin content was determined by the chemotype (high-artemisinin production and low-artemisinin production) of CYP71AV1. In fact, the highly active CYP71AV1 is decided by an amino acid residue (Ser479).[3] Feedback inhibition of artemisinin in *A. annua* indicated that the mRNA level of *CYP71AV1* was inhibited by artemisinic acid.[4] In the CYP71AV1 mutation plant, artemisinin was not detected.[5] There is no doubt that CYP71AV1 is the rate-limiting enzyme in the artemisinin biosynthetic pathway. Therefore, *CYP71AV1* is a necessary gene when overexpress the artemisinin biosynthetic pathway genes.

Many genes exhibited a trichome-specific activity in glandular trichomes or T shape trichomes. *ADS* and *DBR2* showed a trichome-specific activity in glandular trichomes, and *CPS* and *ECS* showed a trichome-specific activity in T shape trichomes, *CYP71AV1* and *BFS* exhibited a trichome-specific activity both in glandular trichomes and T shape trichomes (Figure 1).[6-7] *AaWRKY1* driven by *CYP71AV1* promoter improved the transcription of *CYP71AV1* much more

effectively than the 35s promoter.[8] Therefore, trichome-specific promoters may be useful for improving the artemisinin content.

Transcription Factors Regulation of Artemisinin

Since the transcription factors are important positive or negative regulators in the secondary metabolism, it is a powerful tool for the modulation of compound production and the impact of flux contribution through metabolic pathways in plants. AP2/ERF proteins contain an AP2 DNA-binding domain of approximately 60 amino acids and play important roles in the regulation of the secondary metabolism. Previously, an AP2/ERF transcription factor ORAC2 played key roles in the terpenoid indole alkaloid biosynthesis.[9] Later, another AP2/ERF transcription factor ORAC3 increased the expression level of several metabolite biosynthetic genes and enhanced accumulation of terpenoid indole alkaloids in *Catharanthus roseus.*[10] Several AP2/ERF transcription factors in *A. annua* regulation of artemisinin have been found, including *AaERF1*, *AaERF2*, *AaORA* and *AaTAR.*[11-13] The bHLH transcription factors regulation of trichome initiation and anthocyanin accumulation by interacting with MYB family proteins to form a complex (bHLH-Myb-TTG1) have been reported in Arabidopsis. The bHLH transcription factors AaMYC2 and AabHLH[14,15] can regulate the artemisinin in *A. annua*. In addition, a MYB transcription factor AaMYB1 connects GA signaling with the artemisinin biosynthesis in *A. annua.*[16] WRKY transcription factors function as key regulators of disease resistance.[17] There are

two WRKY (AaWRKY1 and AaGSW1) transcription factors playing important roles in modulating artemisinin content.[18,19] A bZIP transcription factor AabZIP1 and a NAC (NAM, ATAF and CUC) transcription factor AaNAC1 have been found to enhance the artemisinin content.[20,21] Recently, a home-domain-leucine zipper (HD-ZIP) IV transcription factor AaHD1 has been found to promote the *A. annua* glandular trichome initiation.[22] It is expected that the transcription factor can be combined with a more rational design of metabolic engineering approaches for improving the artemisinin content.

Transporter Regulation of Artemisinin

Plants produce a lot of secondary metabolites and these secondary compounds can be accumulated in a temporal and spatial manner in the plants special organs, such as in trichomes. The ATP-binding cassette (ABC) transporters are involved in the transport of secondary metabolites, such as alkaloids, phenol, terpenoids, and wax.[23] Previously, a transporter TolC played an important role in amorphadiene efflux in *E. coli*. Therefore, the overexpression of tolC+ macAB (ABC family transporters) or emrAB or emrKY (MFS family transporters), the concentration of amorphadiene was found to be enhanced by more than threefolds.[24] Recently, an ABC transporter AaPDR2 from *A. annua* was found to enhance the accumulation of DHAA and AA in the apoplast of *N. benthamiana* leaves.[25] The transporters open up new possibilities for the biosynthesis of high value terpenes in plants or in heterologous expression systems.

miRNA

Plant microRNAs (miRNAs) are usually 21-24 nucleotide (nt) noncoding RNAs in length, which play important regulatory roles in development, secondary metabolism, flowering, seed size, biomass, and involve in modulating the juvenile-to-adult phase transition and so on. MiR156 is an important miRNA in plants. Previous studies have shown that miR156 negatively regulates the anthocyanin accumulation by directly preventing the expression level of SPL9, which can bind to the promoter region of dihydroflavonol reductase DFR.[26] Recent study indicated that the miR156-SPL module can modulate the (E)-β-caryophyllene concentration by regulating the mRNA level of the sesquiterpene synthase gene *TPS21*. In addition, the miR156-SPL module can regulate the sesquiterpenes patchoulol by increasing patchoulol synthase (PatPTS) gene expression.[27] Therefore, the model of miRNA-TFs are highly conserved in plants and it can be used in enhancing the sesquiterpene artemisinin production.

Phytohormones and Other Signaling Molecules

Plant hormones jasmonic acid (JA), abscisic acid (ABA), salicylic acid (SA) and gibberellins (GA) have been described to positively affect artemisinin biosynthesis in *A. annua*. JA can activate some transcription factors, which modulate the artemisinin content by directly bound to the promoter of the artemisinin biosynthetic pathway genes.[16] Also, JA can increase the trichome density and

decrease the feedback inhibition of artemisinin in *A. annua*.[16] Another important hormone ABA which can activate transcription factor AaBZIP1, directly regulate the accumulation of artemisinin.[21] SA is an important plant signal involved in defense. It enhances the expression level of *ADS* and increases the AN, AA, and DHAA content.[28] However, the mechanism of SA modulation of artemisinin content is unknown and needs to be studied. GA causes the increase of the transcript levels of *FDS*, *ADS* and *CYP71AV1* and positively affects the trichome proliferation, elevating the accumulation of artemisinin in *A. annua.*

Different plant hormones play the same roles in the regulation of the secondary metabolism. Therefore, there are hormones crosstalk in modulating the same phenomenon. Understanding the mechanism of hormones or crosstalk between hormones regulation artemisinin may lead to innovative strategies being devised for molecular genetic modification to improve artemisinin to a great extent. Not only plant hormones, other exogenous factors, such as cold, drought, sugars, salinity, ultraviolet and active oxygen species (AOS)[29] also play positive roles in the artemisinin biosynthesis.

Concluding Remarks

Although the transcription factors, key enzymes, plant hormones, miRNA, transporter, plant hormones and exogenous factors (Figure 2) can positively or negatively regulate the expression of

artemisinin biosynthetic pathway genes and effect on artemisinin content, further studies are needed to increase knowledge on networks of these regulators that modulating artemisinin.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Acknowledgment

This work was funded by China Transgenic Research Program (grant no. 2016ZX08002001-006) and Shanghai Leading Academic Discipline Project (Horticulture).

References

1. Zhang Y, Teoh KH, Reed DW, Maes L, Goossens A, Olson DJH, Ross ARS, Covello PS. The molecular cloning of artemisinic aldehyde Δ11 (13) reductase and its role in glandular trichome-dependent biosynthesis of artemisinin in *Artemisia annua*. J Biol Chem 2008; 283:21501-8; PMID:18495659; http://dx.doi.org/ 10.1074/jbc.M803090200
2. Olofsson L, Engström A, Lundgren A, Brodelius PE. Relative expression of genes of terpene metabolism in different tissues of *Artemisia annua* L. BMC Plant Biol 2011; 11:45; PMID: 21388533; http://dx.doi.org/ 10.1186/1471-2229-11-45

3. Komori A, Suzuki M, Seki H, Nishizawa T, Meyer JJM, Shimizu H, Yokoyama S, Muranaka T. Comparative functional analysis of CYP71AV1 natural variants reveals an important residue for the successive oxidation of amorpha-4, 11-diene. FEBS Lett 2013; 587:278-84; PMID: 23246612; http://dx.doi.org/ 10.1016/j.febslet.2012.11.031

4. Arsenault PR, Vail D, Wobbe KK, Erickson K, Weathers PJ. Reproductive development modulates gene expression and metabolite levels with possible feedback inhibition of artemisinin in *Artemisia annua*. Plant Physiol 2010; 154:958-68; PMID: 20724645; http://dx.doi.org/ 10.1104/pp.110.162552

5. Czechowski T, Larson TR, Catania TM, Harvey D, Brown GD, Graham IA. *Artemisia annua* mutant impaired in artemisinin synthesis demonstrates importance of nonenzymatic conversion in terpenoid metabolism. Proc Natl Acad Sci USA 2016; 113:15150-55; PMID: 27930305; http://dx.doi.org/ 10.1073/pnas.1611567113

6. Wang H, Olofsson L, Lundgren A, Brodelius PE. Trichome-specific expression of amorpha-4, 11-Diene synthase, a key enzyme of artemisinin biosynthesis in *Artemisia annua* L., as reported by a promoter-GUS fusion. Am J Plant Sci 2011; 2:619-28; http://dx.doi.org/10.4236/ajps.2011.24073

7. Jiang W, Lu X, Qiu B, Zhang F, Shen Q, Lv Z, Fu X, Yan T, Gao E, Zhu M. Molecular cloning and characterization of a trichome-specific promoter of artemisinic aldehyde Δ11 (13)

reductase (DBR2) in *Artemisia annua*. Plant Mol Biol Rep 2014; 32:82-91; http://dx.doi.org/ 10.1007/s11105-013-0603-2

8. Han J, Wang H, Lundgren A, Brodelius PE. Effects of overexpression of *AaWRKY1* on artemisinin biosynthesis in transgenic *Artemisia annua* plants. Phytochemistry 2014; 102:89-96; PMID: 24629804; http://dx.doi.org/ 10.1016/j.phytochem.2014.02.011
9. Menke FL, Champion A, Kijne JW, Memelink J. A novel jasmonate- and elicitor-responsive element in the periwinkle secondary metabolite biosynthetic gene Str interacts with a jasmonate- and elicitor-inducible AP2-domain transcription factor, ORCA2. Embo J 1999;18:4455-63; PMID: 10449411; http://dx.doi.org/ 10.1093/emboj/18.16.4455
10. Van der Fits L, Memelink J. ORCA3, a jasmonate-responsive transcriptional regulator of plant primary and secondary metabolism. Science 2000; 289:295-7; PMID: 10894776
11. Lu X, Zhang L, Zhang F, Jiang W, Shen Q, Zhang L, Lv Z, Wang G, Tang K. AaORA, a trichome-specific AP2/ERF transcription factor of *Artemisia annua*, is a positive regulator in the artemisinin biosynthetic pathway and in disease resistance to *Botrytis cinerea*. New Phytol 2013; 198:1191-202; PMID: 23448426; http://dx.doi.org/ 10.1111/nph.12207
12. Tan H, Xiao L, Gao S, Li Q, Chen J, Xiao Y, Ji Q, Chen R, Chen W, Zhang L. TRICHOME and ARTEMISININ REGULATOR 1 is required for trichome development and artemisinin biosynthesis in *Artemisia annua*. Mol Plant 2015; 8:1396-411; PMID: 25870149; http://dx.doi.org/ 10.1016/j.molp.2015.04.002

13. Yu ZX, Li JX, Yang CQ, Hu WL, Wang LJ, Chen XY. The Jasmonate-Responsive AP2/ERF transcription factors AaERF1 and AaERF2 positively regulate artemisinin biosynthesis in *Artemisia annua* L. Mol Plant 2011:1-13; PMID: 22104293; http://dx.doi.org/ 10.1093/mp/ssr087

14. Ishida T, Kurata T, Okada K, Wada T. A genetic regulatory network in the development of trichomes and root hairs. Annu Rev Plant Biol 2008;59:365-86; PMID: 18257710; http://dx.doi.org/ 10.1146/annurev.arplant.59.032607.092949

15. Ji Y, Xiao J, Shen Y, Ma D, Li Z, Pu G, Li X, Huang L, Liu B, Ye H. Cloning and characterization of AabHLH1, a bHLH transcription factor that positively regulates artemisinin biosynthesis in *Artemisia annua*. Plant Cell Physiol 2014;55:1592-604;PMID:24969234; http://dx.doi.org/10.1093/pcp/pcu090

16. Shen Q, Lu X, Yan T, Fu X, Lv Z, Zhang F, Pan Q, Wang G, Sun X, Tang K. The jasmonate-responsive AaMYC2 transcription factor positively regulates artemisinin biosynthesis in *Artemisia annua*. New Phytol 2016; 210:1269-81; PMID: 26864531; http://dx.doi.org/10.1111/nph.13874

17. Eulgem T, Somssich IE. Networks of WRKY transcription factors in defense signaling. Curr Opin Plant Biol 2007;10:366-71; PMID: 17644023; http://dx.doi.org/10.1016/j.pbi.2007.04.020

18. Ruan JX, Li JX, Fang X, Wang LJ, Hu WL, Chen XY, Yang CQ. Isolation and characterization of three new monoterpene synthases from *Artemisia annua*. Front Plant Sci 2016;7:638; PMID: PMC4861830; http://dx.doi.org/10.3389/fpls.2016.00638

19. Ma D, Pu G, Lei C, Ma L, Wang H, Guo Y, Chen J, Du Z, Wang H, Li G. Isolation and characterization of AaWRKY1, an *Artemisia annua* transcription factor that regulates the amorpha-4, 11-diene synthase gene, a key gene of artemisinin biosynthesis. Plant Cell Physiol 2009;50:2146-61;PMID: 19880398; http://dx.doi.org/10.1093/pcp/pcp149

20. Lv Z, Wang S, Zhang F, Chen L, Hao X, Pan Q, Fu X, Li L, Sun X, Tang K. Overexpression of a novel NAC domain-containing transcription factor (AaNAC1) enhances the content of artemisinin and increases tolerance to drought and *Botrytis cinerea* in *Artemisia annua*. Plant Cell Physiol 2016;57:1961-71; PMID: 27388340; http://dx.doi.org/ 10.1093/pcp/pcw118

21. Zhang F, Fu X, Lv Z, Lu X, Shen Q, Zhang L, Zhu M, Wang G, Sun X, Liao Z, et al. A basic leucine zipper transcription factor, AabZIP1, connects abscisic acid signaling with artemisinin biosynthesis in *Artemisia annua*. Mol Plant 2015; 8:163-75; PMID: 25578280; http://dx.doi.org/ 10.1016/j.molp.2014.12.004

22. Yan T, Chen M, Shen Q, Li L, Fu X, Pan Q, Tang Y, Shi P, Lv Z, Jiang W, et al. HOMEODOMAIN PROTEIN 1 is required for jasmonate-mediated glandular trichome initiation in *Artemisia annua*. New Phytol 2016; 213:1145-55;PMID: 27659595; http://dx.doi.org/ 10.1111/nph.14205

23. Yazaki K. ABC transporters involved in the transport of plant secondary metabolites. Febs Lett 2006;580:1183-1191; PMID: 16364309; http://dx.doi.org/10.1016/j.febslet.2005.12.009

24. Zhang C, Chen X, Stephanopoulos G, Too HP. Efflux transporter engineering markedly improves amorphadiene production in *Escherichia coli*. Biotechnol Bioeng 2016;113:1755-63; PMID: 26804325; http://dx.doi.org/10.1002/bit.25943

25. Wang B, Kashkooli AB, Sallets A, Ting HM, de Ruijter NC, Olofsson L, Brodelius P, Pottier M, Boutry M, Bouwmeester H, et al. Transient production of artemisinin in *Nicotiana benthamiana* is boosted by a specific lipid transfer protein from *A. annua*. Metab Eng 2016;38:159-69; PMID: 27421621; http://dx.doi.org/10.1016/j.ymben.2016.07.004

26. Gou JY, Felippes FF, Liu CJ, Weigel D, Wang JW. Negative regulation of anthocyanin biosynthesis in Arabidopsis by a miR156-targeted SPL transcription factor. Plant Cell 2011;23:1512-22; PMID: 21487097; http://dx.doi.org/10.1105/tpc.111.084525

27. Yu Z, Wang L, Zhao B, Shan C, Zhang Y, Chen D, Chen X. Progressive regulation of sesquiterpene biosynthesis in arabidopsis and patchouli *(Pogostemon cablin*) by the miR156-targeted SPL transcription factors. Mol Plant 2015; 8:98-110; PMID: 25578275; http://dx.doi.org/10.1016/j.molp.2014.11.002

28. Pu G, Ma D, Chen J, Ma L, Wang H, Li G, Ye H, Liu B. Salicylic acid activates artemisinin biosynthesis in *Artemisia annua* L. Plant Cell Rep 2009;28:1127-35; PMID: 19521701; http://dx.doi.org/10.1007/s00299-009-0713-3

29. Pulice G, Pelaz S, Matias-Hernandez L. Molecular farming in *Artemisia annua*, a promising approach to improve anti-malarial drug production. Front Plant Sci 2016;7:329; PMID: 27047510; http://dx.doi.org/10.3389/fpls.2016.00329

Figure Legends

Figure 1

Artemisinin biosynthetic pathway in *A. annua*. Enzymes in red have been cloned from *A. annua*.

There are two kinds of trichomes in *A.* annua, containing glandular trichomes and T-shape trichomes. Artemisinin biosynthetic pathway genes are mainly expressed in the glandular trichomes. Artemisinin branch pathway genes are mainly expressed in the T-shape trichomes. *GAS* has the same expression patterns with artemisinin biosynthetic pathway genes may expressed at the glandular trichomes. FPP is the intermediate product at the branch point of terpenoid metabolism and then is transformed into amorpha-4,11-diene and artemisinin.

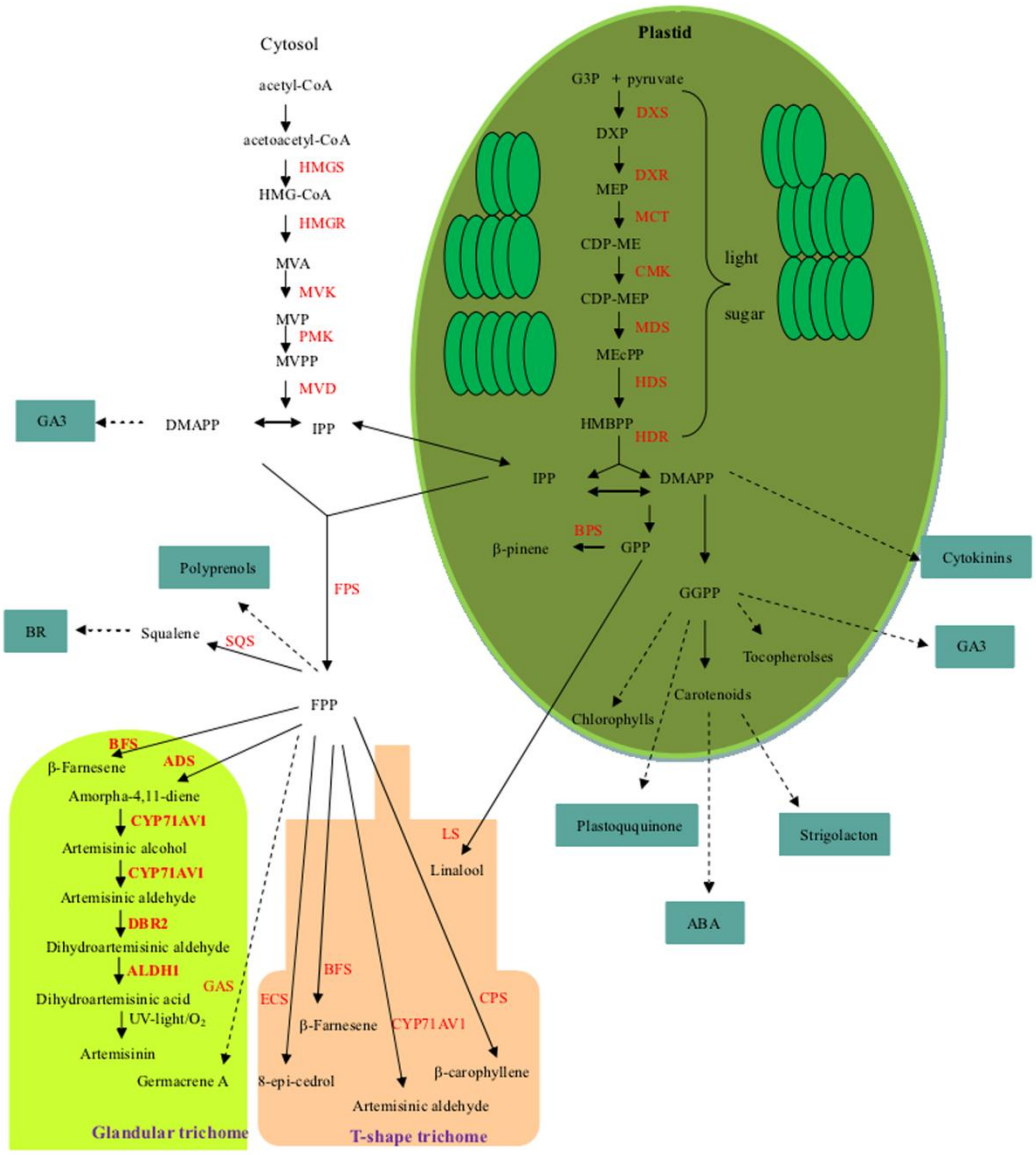


Figure 2

Signaling of network of plant hormones, transcription factors, transporter and miRNAs that modulate artemisinin biosynthesis in *A. annua*.

SCF, Skip/Cullin/F-box; NPR, non-expresser of PR genes; GID1, GA-insensitive dwarf mutant 1; PP2C, protein phosphatase 2C; PYR/PYL/RCAR: pyrabactin resistant/pyrabactin resistant-like/regulatory component of ABA receptor.