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Review

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Artemisia annua glandular secretory trichomes: the biofactory of antimalarial agent artemisinin

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Abstract Artemisinin, the key ingredient of first-line antimalarial drugs, has large demand every year. The native plant, which produces small quantities of artemisinin, remains as its main source and thus results in a short supply of artemisinin. Intensified efforts have been carried out to elevate artemisinin production. However, the routine metabolic engineering strategy, via overexpressing or down-regulating genes in artemisinin biosynthesis branch pathways, was not very effective as desired. Glandular secretory trichomes, sites of artemisinin biosynthesis on the surface of *Artemisia annua* L. (*A. annua*), are the new target for increasing artemisinin yield. In general, the population and morphology of glandular secretory trichomes in *A. annua* (*Aa*GSTs) are often positively correlated with artemisinin content. Improved understanding of *Aa*GSTs will shed light on the opportunities for increasing plant-derived artemisinin. This review article will refresh classification of trichomes in *A. annua* and provide an overview of the recent achievements regarding *Aa*GSTs and artemisinin. To have a full understanding of *Aa*GSTs, factors that are associated with trichome morphology and density will have to be further investigated, such as genes, microRNAs and phytohormones. The purpose of this review was to (1) update the knowledge of the relation between *Aa*GSTs and artemisinin, and (2) propose new avenues to increase artemisinin yield by harnessing the potential biofactories, *Aa*GSTs.

Keywords *Artemisia annua* L. · Artemisinin biosynthesis · Glandular secretory trichomes · Engineering *Aa*GSTs

1 Introduction

Malaria is a global health problem. Nowadays, there are still estimated 198 million cases of malaria and 584,000 deaths worldwide [1]. Artemisinin, a kind of sesquiterpene lactone endoperoxide, was extracted from traditional Chinese herb *Artemisia annua* (*A. annua*, sweet wormwood). It has been used for many years in the treatment of malaria. Besides, artemisinin and its derivatives have attracted additional interests because of their potential activities in fighting against viruses, cancers and autoimmune diseases [2–4]. With so many applications of artemisinin, the demand for this chemical compound has been constantly going up. However, the supply of which could not meet the needs after 2009 [5]. It is highly necessary to enhance artemisinin production so as to satisfy future demand and therefore cut the price [6].

Generally speaking, there are two supply chains in artemisinin market, which are the traditional chemical extraction (or natural artemisinin, NA) and semisynthetic artemisinin (SSA) [7]. NA had long been used as the only source of artemisinin before 2013. With the development of biosynthetic technology, SSA through yeast engineering shows potentials to increase artemisinin production [8]. It is essential that future production of both natural and

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semisynthetic artemisinin be coordinated, to ensure long-term, sustainable production, at a price which is fair to both the producers and buyers [7]. In 2014, the estimated NA was 120–140 MT and the price was around US$ 270–300/kg; the estimated SSA was 35–60 MT and the price was around US$ 400/kg [7]. Naturally occurring artemisinin, isolated from *A. annua*, is still the main source of artemisinin with a price advantage. However, the productivity of artemisinin in wide-type *A. annua* is very low, only 0.01 %–1.09 % of dry weight [9]. In the production of low-value-added products, the yield of plants is an important design variable to optimize.

Metabolic engineering is a powerful approach to gain accesses of bioactive compounds, which accumulate only at low quantities in their naturally producing plant. By breaking the rate-limiting steps or blocking competitive pathways, scopolamine content in *Hyoscyamus* [10], water-soluble phenolic acids [11] and fat-soluble diterpenes [12] in *Salvia miltiorrhiza* hairy root have been successfully increased. However, none of the metabolic engineering methods for producing artemisinin has a sufficiently high production level to allow for commercialization of the process [13]. It is difficult for the routine metabolic engineering strategy to enhance the plant-derived artemisinin since there is more than one bottleneck involved in artemisinin biosynthetic pathway and the whole pathway is still not completely clear [14, 15]. Via overexpressing genes in artemisinin biosynthesis or down-regulating genes in branch pathway, artemisinin production was just slightly increased [16].

Scientists have found that artemisinin is synthesized and stored in a structure called glandular secretory trichomes, locating on the surface of overground *A. annua* [17, 18]. Engineering *Aa*GSTs (density and morphology) might be an alternative way to elevate artemisinin yield [19, 20]. It has already been proved that different types of glandular trichomes (GLTs) have different metabolite compounds in *Tomato* [21] and *Solanum* [22].

This review endeavors to show the current research progress of key enzymes in artemisinin biosynthesis and reveal the correlation between artemisinin production and *Aa*GSTs. It also covers the latest advances in the studies of genes, microRNAs (miRNAs) and phytohormones, which may be related to the morphology and density of *Aa*GSTs.

2 The classification and function analysis of trichome

Trichomes are specialized tissues on the surface of many plants. The trichomes protrude from epidermis and have a great diversity in shape, size and density [23]. Their diversity is almost as great as the number of species on which they are encountered or even within species and individual plants (Fig. 1). Trichomes can be divided into glandular (mostly multicellular) and non-glandular (mostly unicellular) according to their morphology. The *Lamiaceae* (menthol) [24], *Solanaceae* (tomato) [21], *Asteraceae* (*A. annua*) [23] and *Cannabaceae* (*Cannabis sativa*) [25] are particularly rich in glandular trichomes (GLTs). The *Malvaceae* (cotton) [26] and *Cruciferae* (*Arabidopsis*) [27] are rich in non-glandular trichomes (NGTs).

In *A. annua*, there are two kinds of trichomes named glandular secretory trichomes (*Aa*GSTs) and T-shaped non-glandular trichomes (*Aa*TNGs), which all originate from aerial epidermis cells (Fig. 1a–d) [17, 28]. *Aa*GSTs are a 10-celled biseriate translucent structure of two stalk cells, two basal cells, four subapical cells and two apical cells (Fig. 1a, b). *Aa*TNGs are filamentous 5-celled T-trichomes presenting in the basal bracts and pedicel of the capitulum (Fig. 1d). The top of *Aa*TNGs is formed by a greatly elongated cell [29].

Trichomes are the first protective barriers of plant. They can protect plant from external stress by chemical or physical tools. NGTs function as physical barriers of herbivores, fungi, bacteria, pathogens, salts, metals, freezing coldness and UV from environment [30]. GLTs are valuable to protect plant from external stress, such as herbivore [21, 31] and drought [32], through large amounts of secondary metabolites that are poisonous to natural enemies [33, 34].

*Aa*GSTs are a kind of GLTs with the capacity to synthesize, store and secrete plenty of metabolites, such as terpenoid, alkaloid, catechol tannins, polysaccharide and calcium oxalate [35]. Artemisinin is produced in both apical and subapical cells and accumulated in subcuticular space of *Aa*GSTs [18, 36]. In early times, the apical cells rather than subapical cells were considered to produce sesquiterpenoid artemisinin [36]. However, laser microdissection and qPCR results of *Aa*GSTs indicated that artemisinin biosynthesis took place in both apical and subapical cells [37]. The *A. annua* transcriptome research of trichomes further proved this assumption [38]. *Aa*TNGs are a kind of NGTs that keep external threats away from *A. annua*. Moreover, transcriptome study of trichomes from *A. annua* showed that *Aa*TNGs might have the same ability as *Aa*GSTs since they both have specific gene expressions in sesquiterpenoid and triterpenoid biosynthetic pathways.

For these reasons, trichomes (including glandular and non-glandular) are promising targets to produce high-value plant products in many plants. In *A. annua*, *Aa*GSTs are the chemical factories of artemisinin. Moreover, the connection between *Aa*TNGs and artemisinin needs to be further investigated.