Plant Physiology and Biochemistry 74 (2014) 70–83

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Effect of prolonged water stress on specialized secondary metabolites, peltate glandular trichomes, and pathway gene expression in *Artemisia annua* L.

Ritesh K. Yadav, Rajender S. Sangwan, Farzana Sabir, Awadesh K. Srivastava, Neelam S. Sangwan*

Metabolic and Structural Biology Department, CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), P.O. CIMAP, Lucknow, UP 226015, India

ARTICLE INFO

Article history:
Received 20 June 2013
Accepted 16 October 2013
Available online 25 October 2013

Keywords:
Artemisia annua
Artemisinin
Artemisinic acid
Dihydroartemisinic acid
1,8-Cineole
Glandular trichome
Water stress

ABSTRACT

Artemisia annua L. accumulates substantial quantities of unique sesquiternoid artemisinin and related phytomolecules and characteristic essential oil in glandular trichomes, present on its leaves and inflorescence. Water stress is a major concern in controlling plant growth and productivity. In this study, our aim was to find out the modulation of artemisinin and essential oil constituents in plants grown under prolonged water stress conditions. *A. annua* CIM-Arogya plants grown in pots were subjected to mild (60% $\pm$ 5) and moderate (40% $\pm$ 5) water stress treatment and continued during entire developmental period. Results revealed that artemisinin, arteannuin-B, artemisinic acid, dihydroartemisinic acid and essential oil content were positively controlled by the growth and development however negatively modulated by water deficit stress. Interestingly, some of minor monoterpenes, all sesquiterpenes and other low molecular weight volatiles of essential oil components were induced by water deficit treatment. Camphor which is the major essential oil constituents did not alter much while 1, 8 cineole was modulated during development of plant as well as under water stress conditions. Water deficit stress induces a decrease in glandular trichome density and size as well. The dynamics of various secondary metabolites is discussed in the light of growth responses, trichomes and pathway gene expression in plants grown under two levels of prolonged water stress conditions.

1. Introduction

Artemisia annua L. (Asteraceae) yields artemisinin, the sesquiterpenoidal compound which is widely used in malaria treatment. Artemisinin is central component of artemisinin combination therapy which is currently the most effective malaria drug and World Health Organization (WHO) recommended it for the treatment of drug-resistant and cerebral malaria [1,2]. The plant derived productivity of artemisinin is still a limitation to fulfil the growing demand of the drug worldwide. The growing shortage of artemisinin has catalysed intense research efforts to understand its dynamics of production by the plant and intended to enhance its production *in planta* or *in vitro* including synthetic biology. This also involves understanding of metabolic modulation of artemisinin biosynthesis by intrinsic and extrinsic factors [3]. The biosynthetic pathway of sesquiterpenoid artemisinin is almost completely elucidated (Fig. 1). The isopentenyl pyrophosphate for artemisinin originates from both cytosolic and plastidic isoprenogenesis [4]. Briefly, farnesyl diphosphate synthase catalyzes the synthesis of farnesyl diphosphate (FPP) from two units of IPP and one unit of dimethyl allyl pyrophosphate (DMAPP), as all sesquiterpenoids and triterpenoids are biosynthesized [5]. Catalytic conversion of FPP into amorpha-4,11-diene through amorpha diene synthase activity constitutes the committed step for biosynthesis of artemisinin and congener metabolites (artemisinic acid and arteannuin B) of the pathway. In metabolic sequence, amorpha-4,11-diene is sequentially oxidized to generate artemisinic acid involving a single cytochrome P_{450} enzyme, CYP71AV1 [6]. Recently, a double bond reductase (DBR2) [7] and an aldehyde dehydrogenase (Aldh1) [8] involved in the biosynthesis were also isolated and characterized. These enzymes are involved in the conversion of artemisinic aldehyde to its dihydro form and then to dihydroartemisinic acid, respectively. Aldh1 also convert artemisinic aldehyde to artemisinic acid, an activity also ascribed to CYP [7]. Dihydro derivative is converted to artemisinin either directly or through mediation of arteannuin B, respectively [9] (Fig. 1).

* Corresponding author.
E-mail addresses: nsangwan5@gmail.com, nss.cimap@gmail.com (N.S. Sangwan).

0981-9428/$ – see front matter © 2013 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.plaphy.2013.10.023

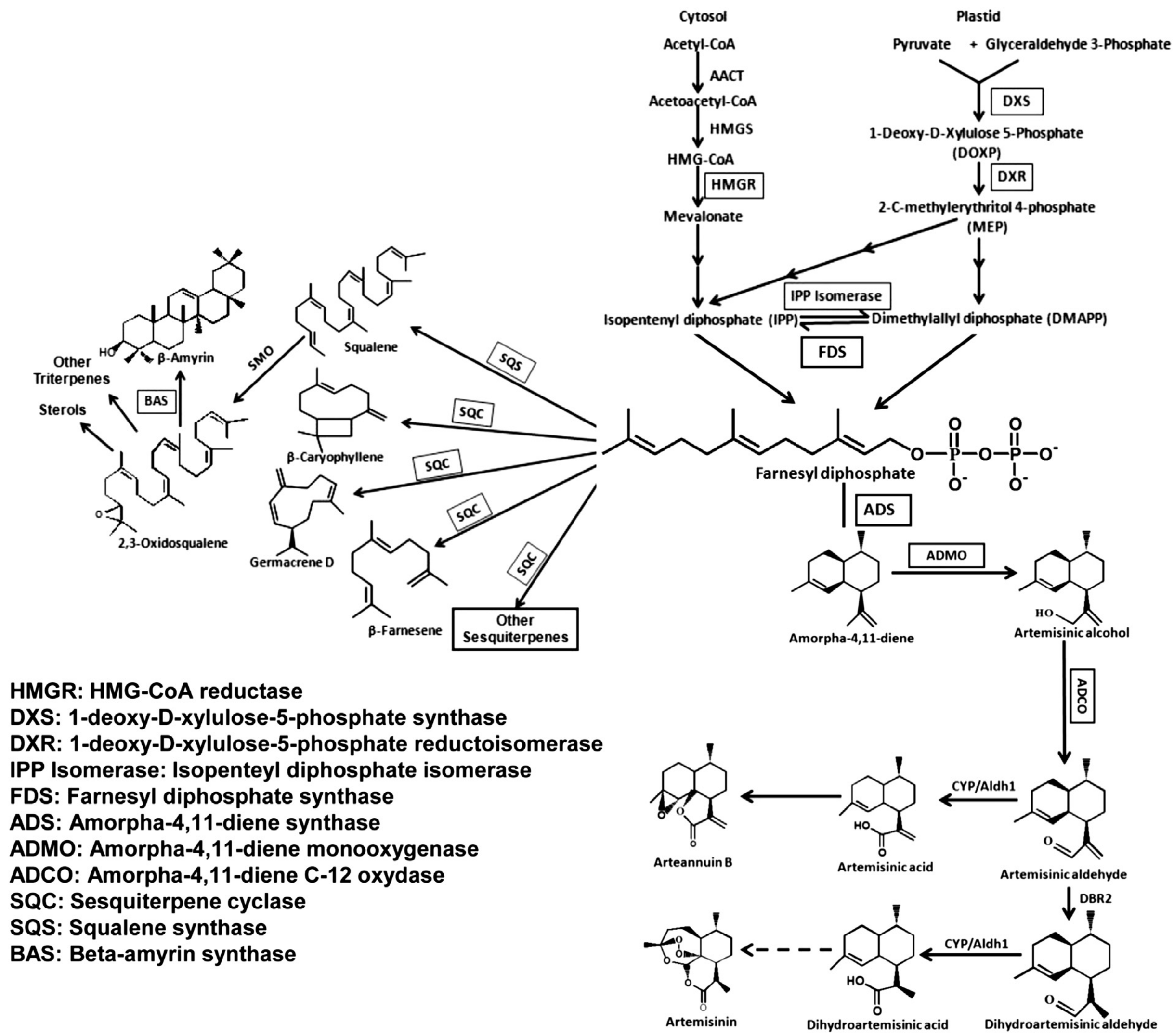

Fig. 1. Schematic presentation of artemisinin and other terpenoid biosynthetic pathway.

Artemisinin and a number of other terpenoids are produced in glandular trichomes (GT) present on aerial surfaces of the plant. The *A. annua* peltate glandular trichomes are 10-celled structures with three pairs of secretory cells. Mono and sesquiterpenes are the major components of the essential oil from *A. annua* and are also produced in trichomes and many secondary metabolism including artemisinin biosynthesis pathway genes are highly expressed in glandular trichomes [7,8,10]. Several studies have shown that artemisinin content can vary widely among cultivars or ecotypes of *A. annua*. It has also been reported that artemisinin content is responsive to the time of harvest, light intensity, and developmental stage. During plant development, artemisinin levels are reported to reach their peak levels either just before flowering or at full bloom [11,12]. Effect of hormonal treatments on artemisinin content has also been reported [10,13–15]. It has been demonstrated that plant growth, behaviour and production of artemisinin are also affected by environmental factors like, irradiation [16], salinity stress [17], chilling stress [18] and elicitation [19]. The effect of water stress imposed for short duration on artemisinin content is reported and reveals that artemisinin content of the plant is affected by short duration water stress [20]. As the water availability is becoming the major challenge, therefore, it is very important to understand the effect of environmental factors specially water stress on the dynamics of biomass and productivity of artemisinin and other secondary metabolites. Though, reports are available in literature exhibiting the effects of water stress on artemisinin in *A. annua* [20]. However, the present study constitutes the most comprehensive analysis in terms of addressing the objectives (i) effects of water deficit stress on artemisinin and other artemisinin related metabolites throughout the development process of plant (ii) effects of water deficit stress on essential oil content and constituents (iii) trichome size (length, width, area) modulation under water deficit stress conditions. Therefore, for understanding the water stress mediated modulation of artemisinin and other terpenoids, the present study deals with the analysis of glandular trichomes, artemisinin and related sesquiterpenoids levels, essential oil accumulation and its constituents in response to two levels (mild and moderate) of water stress for prolonged duration. The study also evaluates the impact of water stress on expression of several secondary metabolic pathway genes and regulatory genetic elements of different biosynthetic pathways viz. mevalonate pathway, MEP/DOXP pathway, artemisinin biosynthetic pathway, and other sesquiterpenoid pathway, triterpenoid pathway,