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Research article

Tissue-specific gene-expression patterns of genes associated with thymol/carvacrol biosynthesis in thyme (*Thymus vulgaris* L.) and their differential changes upon treatment with abiotic elicitors

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A B S T R A C T

Thyme (*Thymus vulgaris* L.) is known to produce a variety of phenolic monoterpenes such as thymol and carvacrol. Thymol and carvacrol are health-promoting, biocide and antitoxin compounds and have been considered as the main constituents of essential oils in *T. vulgaris*. To improve our understanding of the regulation of monoterpene biosynthesis in thyme, the expression of genes related to thymol and carvacrol biosynthesis in different tissues and in response to abiotic elicitors was analyzed. Methyl jasmonate (MeJA), salicylic acid (SA), trans-cinnamic acid (tCA) and UV-C irradiation were applied to *T. vulgare* leaves and transcript levels of early (*DXR*) and late (*TvTPS1*, *CYP71D178* and *CYP71D180*) biosynthetic genes of thymol and carvacrol were measured. The results showed that early step and late step genes in thymol/carvacrol biosynthesis are differentially regulated. *DXR* was not found to be exclusively expressed in glandular trichomes; in contrast, biosynthetic genes including γ-terpinene synthase (*TvTPS1*) and two cytochrome P450s, *CYP71D178* and *CYP71D180,* were preferentially expressed in glandular secretory trichomes. The high expression of late biosynthetic genes in glandular trichomes, which also contain the highest concentration of thymol and carvacrol, suggests that glandular trichomes are the structure in which thymol/carvacrol biosynthesis and accumulation occur. Our results indicate that in addition to abiotic elicitors, developmental and spatial factors also play a key role in the biosynthesis of thymol and carvacrol, most likely relating to glandular trichome density and/or activity. Hence optimization of these factors could be considered as a useful strategy to achieve high yield of valuable compounds in *T. vulgare* or other closely related plant species.

1. Introduction

Thyme (*Thymus vulgaris* L.), (2n = 2x = 30) is a medicinal plant belonging to the Lamiaceae. Its aerial parts have long been used as a traditional remedy for several diseases such as bedwetting, diarrhea, stomach ache, arthritis, colic, sore throat, cough, bronchitis, flatulence, chest congestion and as a diuretic (Fachini-Queiroz et al., 2012). Thyme is native to the western Mediterranean region of Europe (Zarzuelo and Crespo, 2002). The essential oils of thyme have important pharmaceutical uses and are routinely used as flavoring agents in food and cosmetics industry. *Thymus vulgaris* essential oil mainly consists of a mixture of monoterpenes and the primary constituents of the essential oil are often thymol (16.4–42.6%) and its isomer carvacrol (7.6–52.3% %; Amiri, 2012; Nickavar et al., 2005). Thymol and carvacrol are important medicinally due to their antioxidative, antimicrobial, antitussive, expectorant, antispasmodic, and antibacterial properties (Höferl et al., 2009; Youdim et al., 1999; Barnes et al., 2007; Dorman and Deans, 2000; Rodríguez et al., 2013). Of the known compounds in

Abbreviations: CYP, Cytochrome P450; DMADP, dimethylallyl diphosphate; DXP, 1-deoxy-D-xylulose-5-phosphate; DXR, 1-deoxy-D-xylulose-5-phosphate reductoisomerase; GDP, Geranyl diphosphate; *GDS*, geranyl diphosphate synthase; IDP, isopentenyl diphosphate; MEP, methyl erythritol phosphate; MeJA, methyl jasmonate; MVA, mevalonic acid; RGE, relative gene expression; tCA, *trans*-cynnamic acid; PAL, phenylalanine ammonia lyase; SA, salicylic acid; UV, ultraviolet.

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thyme, thymol has attracted considerable attention due to its application in medicine based on its ability to fight tooth decay, infection, gum disease or bad breath. Thymol and carvacrol are found in aerial parts of thyme and it has been suggested that thymol is biosynthesized in plastids of *T. vulgaris* glandular secretory trichomes found on surfaces of aerial tissues surface and stored in the subcuticular space of trichomes (Gershenzon et al., 1989; Crocoll, 2011). Glandular trichomes are specialized external secretory organs which are able to produce, store and/or secrete various natural compounds. Glandular trichomes play a key role in plant growth and development as they are the biosynthetically active place of several important natural compounds in different plant species, *e.g.*, thymol in *Origanum vulgare* (Crocoll et al., 2010), artemisinin in *Artemisia annua* (Olsson et al., 2009), pyrethrins in *Chrysanthemum cinerariaefolium* (Ramirez et al., 2012) and parthenolide in *Tanacetum parthenium* (Majdi et al., 2011). In recent years, there has been increasing interest in the glandular trichomes of various plants with the aim of understanding the details and localization of the biosynthetic pathways of beneficial plant natural products. The structure and function of these secretory structures is well documented in the Lamiaceae and they have been considered as the active site of essential oil biosynthesis, secretion and accumulation (Croteau, 1986; Gershenzon et al., 1989; Yamaura et al., 1992; Huang et al., 2008; Turner et al., 2000a,b; Guo et al., 2013). Although several studies have been conducted on glandular trichomes of plant species belonging to the Lamiaceae, the expression patterns of biosynthetic genes of thymol and carvacrol have not yet been explored in thyme.

The biosynthetic pathway of thymol and carvacrol has been elucidated recently (Crocoll, 2011). Thymol and carvacrol (phenolic monoterpenes) are biosynthesized from isopentenyl diphosphate (IDP) and dimethylallyl diphosphate (DMADP) which are derived from the MEP (methyl erythritol phosphate) pathway located in plastids (Fig. 1; Rohmer et al., 1993; Dudareva et al., 2005). In the MEP pathway, which is involved in the biosynthesis of thymol and carvacrol, 1-deoxy-D-xylulose-5-phosphate (DXP) is irreversibly converted into 2-C-methyl-D-erythritol-4-phosphate (MEP) by a 1-deoxy-D-xylulose-5-phosphate reductoisomerase enzyme (DXR). This step has been described as the first committed step in the MEP pathway (Takahashi et al., 1998). Geranyl diphosphate synthase (GDS) is a key enzyme in this biosynthetic pathway which catalyze the head-to-tail condensation of IDP and DMADP to geranyl diphosphate (GDP) as the universal precursor of monoterpenes (Lichtenthaler, 1999; Burke et al., 1999). Subsequently, γ-terpinene synthase which is a member of the monoterpene synthase family produces γ-terpinene through cyclization of GDP. Furthermore, enzymes such as CYP71D178, CYP71D180 and CYP71D181 belonging to the cytochrome P450 (CYP) monooxygenases are also involved in further modifications of γ-terpinene backbone to yield thymol and carvacrol (Crocoll et al., 2010; Crocoll, 2011). Thymol is exclusively produced via CYP71D178 while carvacrol is biosynthesized via CYP71D178, CYP71D 180 and CYP71D181 (Fig. 1).

It has been well documented that the essential oil content and consequently thymol and carvacrol biosynthesis and their accumulation varies in different tissues or in response to different environmental factors and stimuli in plant species of Lamiaceae such as *T. vulgaris* and *Origanum vulgare* L. (Verma et al., 2011; Shiyab et al., 2012; Patricelli et al., 2015). In recent years, numerous studies have been conducted to survey the effects of both biotic and abiotic elicitors on secondary metabolite biosynthesis such as ultraviolet (UV) irradiation (Hectors et al., 2014), trans-cynnamic acid (tCA; Hojati et al., 2016), methyl jasmonate (MeJA), and salicylic acid (SA; Majdi et al., 2015; Elyasi et al., 2016). These factors usually change the chemical composition of essential oils in plant species. It has been reported that MeJA and SA treatments trigger secondary metabolism in different plant species, hence the production of volatile compounds is likely regulated by these plant hormones which are involve in plant defense and interaction with environment (Ramakrishna and Ravishankar, 2011; Mirzajani et al., 2015; Patricelli et al., 2015; Elyasi et al., 2016; Xu et al., 2016).

Chemical compounds might have dissimilar effects on different biosynthetic pathways; for example, in *T. parthenium trans*-cinnamic acid has been used as a phenylalanine ammonia lyase (PAL)

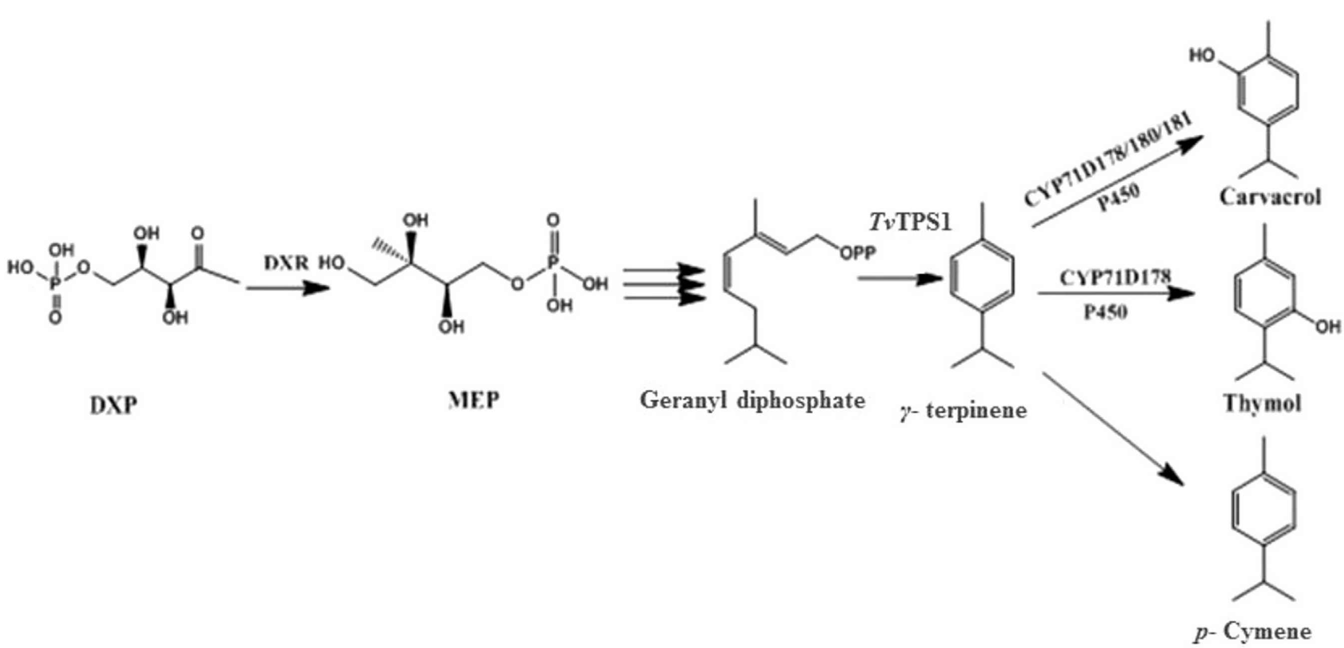

Fig. 1. The biosynthetic pathway of thymol/carvacrol in *T. vulgaris* L. DXP (1-deoxy-D-xylulose-5-phosphate), *DXR* (1-deoxy-D-xylulose-5-phosphate reductoisomerase), MEP (methyl erythritol phosphate), *TvTPS1* (γ-terpinene synthase).