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The effect of drought stress on the expression of key genes involved in the biosynthesis of phenylpropanoids and essential oil components in basil (*Ocimum basilicum* L.)

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ABSTRACT

Basil (*Ocimum basilicum* L.), a medicinal plant of the Lamiaceae family, is used in traditional medicine; its essential oil is a rich source of phenylpropanoids. Methylchavicol and methyleugenol are the most important constituents of basil essential oil. Drought stress is proposed to enhance the essential oil composition and expression levels of the genes involved in its biosynthesis. In the current investigation, an experiment based on a completely randomized design (CRD) with three replications was conducted in the greenhouse to study the effect of drought stress on the expression level of four genes involved in the phenylpropanoid biosynthesis pathway in *O. basilicum* c.v. Keshkeni luvelou. The genes studied were chavicol *O*-methyl transferase (*CVOMT*), eugenol *O*-methyl transferase (*EOMT*), cinnamate 4-hydroxylase (*C4H*), 4-coumarate coA ligase (*4CL*), and cinnamyl alcohol dehydrogenase (*CAD*). The effect of drought stress on the essential oil compounds and their relationship with the expression levels of the studied genes were also investigated. Plants were subjected to levels of 100%, 75%, and 50% of field capacity (FC) at the 6–8 leaf stage. Essential oil compounds were identified by gas chromatography/mass spectrometry (GC-MS) at flowering stage and the levels of gene expression were determind by real time PCR in plant leaves at the same stage. Results showed that drought stress increased the amount of methylchavicol, methyleugenol, β-Myrcene and α-bergamotene. The maximum amount of these compounds was observed at 50% FC. Real-time PCR analysis revealed that severe drought stress (50% FC) increased the expression level of *CVOMT* and *EOMT* by about 6.46 and 46.33 times, respectively, whereas those of *CAD* relatively remained unchanged. The expression level of *4CL* and *C4H* reduced under drought stress conditions. Our results also demonstrated that changes in the expression levels of *CVOMT* and *EOMT* are significantly correlated with methylchavicol ($r = 0.94$, $P \leq 0.05$) and methyleugenol ($r = 0.98$, $P \leq 0.05$) content. Thus, drought stress probably increases the methylchavicol and methyleugenol content, in part, through increasing the expression levels of *CVOMT* and *EOMT*.

1. Introduction

Plant essential oils, including general terpenoids and phenylpropanoids as major constituents, are important sources of aromatic and flavoring in food, industrial, and pharmaceutical products (Charles and Simon, 1990). Phenylpropanoids are a group of small phenolic molecules, which are key elements in many important herbs and species, including cloves, cinnamon, basil and tarragon (Tahsili et al., 2012). *Ocimum basilicum*, belonging to family Lamiaceae, is one of the best known genera for its medicinal properties and economically important aromatic oils (Rastogi et al., 2014). The *Ocimum* genus comprises annual and perennial herbs and shrubs native to the tropical and subtropical regions of Asia, Africa, and Central South America and includes 50–150 species and subspecies (Javanmardi et al., 2002; Labra et al., 2004). The glands of *O. basilicum* ($2n = 48$) are rich in phenylpropenes as well as monoterpenes and sesquiterpenes (Gang et al., 2001; Iijima et al., 2004; Werker et al., 1993). It is indigenous to Persia and India (Bilal et al., 2012) and is widely cultivated for the production of essential oils and containing high proportions of phenylpropanoid derivatives, such as eugenol, methyleugenol, chavicol,

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metheylchavicol, often combined with various proportions of linalool, a monoterpen, and sesquiterpenes (Tahsili et al., 2012). These compounds are synthesized and stored in peltate glands found on the surface of leaves, stems, and flowers (Iijima et al., 2004). Among the plants known for medicinal value, the plants of genus *Ocimum* are rich in phenolic compounds and are very useful for their therapeutic potentials (Ramesh and Satakopan, 2010). Studies indicate that *O. basilicum* possesses analgesic, anti-inflammatory, antimicrobial, and cardiac stimulant properties (Bilal et al., 2012). Major aroma compounds found in volatile extracts of basil exhibited varying amounts of anti-oxidative activity. Furthermore, ingestion of these aroma compounds may help to prevent *in vivo* oxidative damage, such as lipid peroxidation, which is associated with cancer, premature aging, atherosclerosis, and diabetes (Lee et al., 2005). Despite the wide uses and the importance of sweet basil and its essential oils, little is known about the biosynthesis and developmental regulation of the compounds responsible for the flavor quality of the fresh and dried herbs (Wang and Pichersky, 1999).

Biosynthesis of phenylpropanoid compounds is produced from the shikimate pathway and regulated by several groups of enzymatic reactions through metabolic channels (Dixon et al., 1992). All phenylpropanoids are derived from cinnamic acid, which is formed from phenylalanine by the deamination action of phenylalanine ammonia-lyase (PAL) (Fig. 1). PAL is one of the most important enzymes with a key role in regulation of phenypropanoid production in plants (Achnine et al., 2004; Iijima et al., 2004). Cinnamate 4-hydroxylase (C4H), is a member of the cytochrome P450 monooxygenase superfamily, catalyzes the hydroxylation of trans-cinnamic acid to 4-hydroxycinnamate (*p*-coumaric acid), and is the second key enzyme of phenylpropanoid biosynthetic pathway (Chen et al., 2007). C4H plays a pivotal role at the interface between the cytosolic phenylpropanoid pathway and membrane-localized electron-transfer reactions (Koopmann et al., 1999). The third enzyme of the general phenylpropanoid pathway is 4-coumarate: CoA ligase (4CL), which may play a central role in regulating overall flux of the hydroxycinnamic acids into subsequent biosynthetic pathways (Rastogi et al., 2013). The 4CL is positioned at the metabolic branch point that connects general phenylpropanoid metabolism with different end-product-specific pathways (Stuible and Kombrink, 2001). The last biosynthetic step involved in the formation of estrogole (methylchavicol) is catalyzed by enzyme chavicol *O*-methyltransferase (CVOMT) (Gang et al., 2001; Lewinsohn et al., 2000).The availability of the sequences of these genes essentially facilitates the identification of the conditions under which their expression levels are enhanced (Nasrollahi et al., 2014).

Environmental and ecological factors are known to remarkably affect the essential oil content and composition in aromatic plants (Nasrollahi et al., 2014). The production of the plant specialized metabolites and the expression level of the genes involved in their biosynthesis are strongly associated with growth conditions. Plants exposed to drought stress generally produce higher levels of specialized metabolites (Selmar and Kleinwächter, 2013). Drought stress may also change the essential oil yield and composition in plants (Khorasaninejad et al., 2011; Nowak et al., 2010; Petropoulos et al., 2008). The increase of essential oil percentage and its main constituents under drought stress have been reported in both *O. basilicum* and *O. americanum* (Khalid, 2006). Linalool and methylchavicol contents of sweet basil, as well as the percentage of total essential oil, increased under water stress (Simon et al., 1992). Successful and efficient use of deliberate drought stress can directly enhance specialized metabolite production. This enhancement can be achieved by applying special irrigation regimes that are both simple and inexpensive, but this approach requires extensive examination to optimize metabolite production (Selmar and Kleinwächter, 2013).

Because of the economic importance of essential oil production, basil is considered as a target for bioengineering. Little is known about the gene regulation of phenylpropanoids in aromatic plants. Understanding the expression pattern of the genes involved in phenylpropanoid biosynthesis pathway and their association with the accumulation of essential oil compounds under different environmental conditions could facilitate the production of economically valued varieties of medicinal plants through genetic engineering techniques. To our knowledge, control of phenylpropanoid biosynthesis regarding essential oil production in basil has not been investigated and published. Hence, the current study was aimed to examine the effect of drought stress on the expression levels of the genes *C4H*, *4CL*, cinnamyl alcohol dehydrogenase (*CAD*), eugenol *O*-methyl transferase (*EOMT*) and *CVOMT*, essential

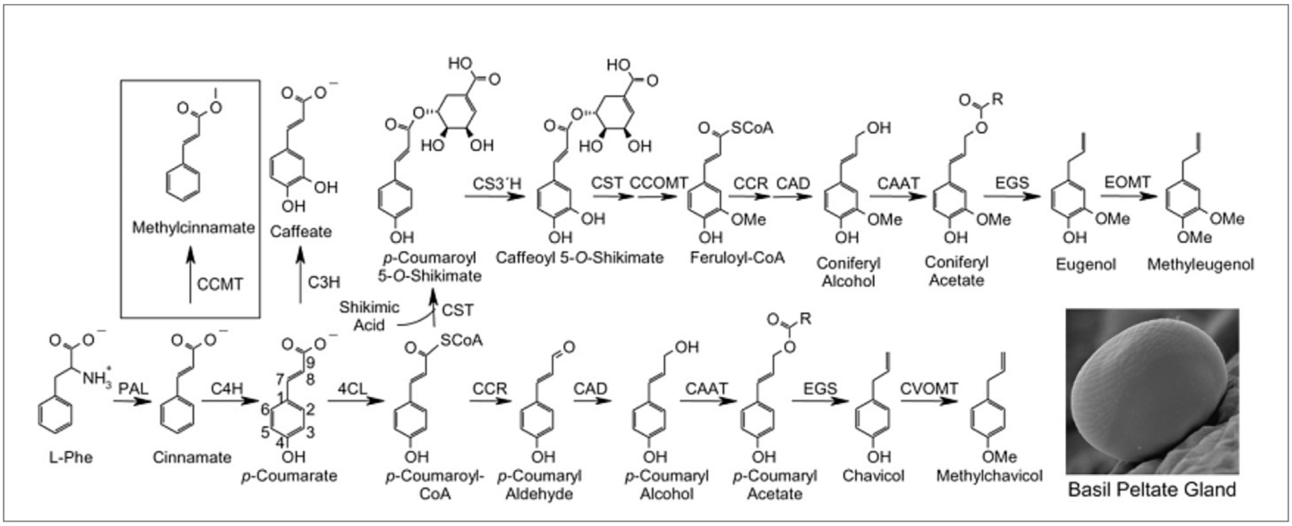

Fig. 1. Methylchavicol and methyleugenol biosynthesis pathway in basil glandular trichomes, enzyme abbreviations are as follows: PAL, phenylalanine ammonia lyase; CCMT, *p*-coumarate/cinnamate carboxyl methyltransferase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate:CoA ligase; C3H, *p*-coumarate 3-hydroxylase; CST, *p*-coumaroyl shikimate transferase; CS3′H, *p*-coumaroyl 5-*O*-shikimate 3′-hydroxylase; CCOMT, caffeoyl-CoA *O*-methyltransferase; CCR, cinnamoyl-CoA reductase; CAD, cinnamyl alcohol dehydrogenase; CAAT, coniferyl alcohol acetyl transferase; EGS, eugenol (and chavicol) synthase; EOMT, eugenol *O*-methyltransferase; CVOMT, chavicol *O*-methyltransferase (Kapteyn et al., 2007).