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The Isogene *1-Deoxy-D-Xylulose 5-Phosphate Synthase 2* Controls Isoprenoid Profiles, Precursor Pathway Allocation, and Density of Tomato Trichomes

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ABSTRACT Plant isoprenoids are formed from precursors synthesized by the mevalonate (MVA) pathway in the cytosol or by the methyl-D-erythritol 4-phosphate (MEP) pathway in plastids. Although some exchange of precursors occurs, cytosolic sesquiterpenes are assumed to derive mainly from MVA, while plastidial monoterpenes are produced preferentially from MEP precursors. Additional complexity arises in the first step of the MEP pathway, which is typically catalyzed by two divergent 1-deoxy-D-xylulose 5-phosphate synthase isoforms (DXS1, DXS2). In tomato (*Solanum lycopersicum*), the *SlDXS1* gene is ubiquitously expressed with highest levels during fruit ripening, whereas *SlDXS2* transcripts are abundant in only few tissues, including young leaves, petals, and isolated trichomes. Specific down-regulation of *SlDXS2* expression was performed by RNA interference in transgenic plants to investigate feedback mechanisms. *SlDXS2* down-regulation led to a decrease in the monoterpene β-phellandrene and an increase in two sesquiterpenes in trichomes. Moreover, incorporation of MVA-derived precursors into residual monoterpenes and into sesquiterpenes was elevated as determined by comparison of ^{13}C to ^{12}C natural isotope ratios. A compensatory up-regulation of *SlDXS1* was not observed. Down-regulated lines also exhibited increased trichome density and showed less damage by leaf-feeding *Spodoptera littoralis* caterpillars. The results reveal novel, non-redundant roles of DXS2 in modulating isoprenoid metabolism and a pronounced plasticity in isoprenoid precursor allocation.

Key words: Isoprenoid biosynthesis; methyl-D-erythritol 4-phosphate (MEP) pathway; 1-deoxy-D-xylulose 5-phosphate synthase 2 (DXS2); RNA interference (RNAi); trichomes; cross-talk; feedback regulation; GC–C–IRMS.

INTRODUCTION

In spite of their tremendous diversity in structure and function, all isoprenoids are synthesized from the common C_5 building blocks isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). In plants, these building blocks are produced by two separate routes, located in the cytosol and the plastid compartment, respectively (Lange et al., 2000). The long-known cytosolic mevalonate (MVA) pathway provides precursors for the biosynthesis of sesquiterpenes, polyterpenes, sterols, dolichol, and for ubiquinone formation in mitochondria. The more recently discovered plastidial methyl-D-erythritol 4-phosphate (MEP) pathway supports mainly C_5 supply for the formation of mono- and diterpenes, carotenoids as well as the prenyl moieties of chlorophyll, plastoquinone and tocopherol (Chappell, 2002). The two pathways are thought to be largely independent, but cross-talk has been shown to occur under certain circumstances. For example, the extent of the exchange can be increased by feeding of intermediates of the MVA or the MEP pathways, and by the impairment of one of the pathways with chemical inhibitors,

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leading to altered precursor pathway allocations (Jux et al., 2001; Nagata et al., 2002; Hemmerlin et al., 2003; Laule et al., 2003; Schuhr et al., 2003; Bartram et al., 2006). In addition, the synthesis of abundant sesquiterpenes in flowers or trichomes involving strong demand on C_5 supply in the cytosol is supported in part by MEP pathway-derived IPP (Dudareva et al., 2005; Towler and Weathers, 2007; Besser et al., 2009).

The MEP pathway is composed of seven enzymatic reactions starting from pyruvate and glyceraldehyde 3-phosphate (Rodríguez-Concepción and Boronat, 2002; Phillips et al., 2008; Cordoba et al., 2009). The first step is catalyzed by 1-deoxy-D-xylulose 5-phosphate (DXP) synthase (DXS), a transketolase-like enzyme (Lange et al., 1998; Lois et al., 2000). Lack of DXS activity prevents formation of plastidial isoprenoids and strongly impairs plastid biogenesis. The result is an albino phenotype as exemplified by the *Arabidopsis thaliana cla1* mutant, which can be rescued by supplying the dephosphorylated enzyme product (Mandel et al., 1996; Estévez et al., 2000). Overexpression of plant *DXS* genes or introduction of bacterial *DXS* copies can enhance levels of isoprenoid end products (Estévez et al., 2001; Enfissi et al., 2005; Carretero-Paulet et al., 2006; Muñoz-Bertomeu et al., 2006), but can also lead to unexpected perturbations of the isoprenoid metabolic network (Morris et al., 2006). Recently, a *DXS* gene from grapevine (*Vitis vinifera* L.) was found to co-localize with a major quantitative trait locus (QTL) for monoterpene content, important for grape and wine aromas (Battilana et al., 2009; Duchêne et al., 2009). Thus, DXS constitutes a key and bottleneck step in plastidial isoprenoid formation.

The nuclear genes encoding MEP pathway enzymes are generally single copy (Rodríguez-Concepción, 2006; Phillips et al., 2008). The most notable exception is the diversification of *DXS* genes into two structurally distinct, differentially regulated isogenes (Walter et al., 2002). A third, *DXS*-like sequence has been mentioned in this early work, and its expression has further been investigated (Vallabhaneni and Wurtzel, 2009), but its DXS functionality has not been proven. The *DXS1*/*DXS2* diversification is widespread among plant genomes excluding currently only *A. thaliana* (Walter et al., 2002; Kim et al., 2006; Phillips et al., 2008; Kim et al., 2009; Cordoba et al., 2009). The DNA-deduced primary structures of class 1 (DXS1) and class 2 (DXS2) isoforms from various plant species are usually only about 70% identical. The importance of the structural differences is elusive, since both isoforms are functional without any biochemical differences known at this point. However, the striking differences in expression correlated with either normal plant development (primary metabolism, DXS1) or with the formation of isoprenoids important for interactions with the environment (secondary metabolism, DXS2) suggest distinct non-redundant functions in plastidial isoprenoid metabolism (Walter et al., 2002; Kim et al., 2006; Okada et al., 2007; Phillips et al., 2007; Sando et al., 2008; Kim et al., 2009). Such strictly differential and frequently complementary expression patterns of *DXS1* and *DXS2* genes were first described from roots colonized by arbuscular mycorrhizal fungi. Only *DXS2* expression was correlated with the accumulation of certain apocarotenoids during mycorrhization (Walter et al., 2002). In *Medicago truncatula*, the expression of a RNA interference (RNAi) construct targeting *DXS2* in transgenic hairy roots resulted in strong reduction of mycorrhizal apocarotenoid accumulation and was correlated with impairment of symbiotic functions, whereas *DXS1* transcript levels were unaffected (Floss et al., 2008). However, expression of the *DXS2*–RNAi construct was limited to roots in these experiments and a potential impairment of shoot functions by a lack or a depletion of DXS2 activity still needs to be investigated.

Roots of tomato (*Solanum lycopersicum*) colonized by mycorrhizal fungi also exhibit a correlation of *DXS2* expression and apocarotenoid accumulation. However, in contrast to *M. truncatula* and *Zea mays*, elevated levels of *DXS2* transcripts have been found also in tomato leaves (Walter et al., 2002). Tomato leaves contain abundant trichomes, whereas trichome formation is less pronounced in the other two species mentioned. The cloning of a *DXS2*-type gene from trichome-containing peppermint leaves (Lange et al., 1998) and the availability of a multitude of *DXS2*-type ESTs from cDNA libraries from trichome-rich material (see http://compbio.dfci.harvard.edu/tgi/) has led to a tentative assignment of the elevated *DXS2* transcript levels in tomato leaves to trichomes (Walter et al., 2002). Leaves and other organs of tomato develop both non-glandular and glandular trichomes in considerable density. The type VI glandular trichomes with their four-celled heads have been shown to accumulate high levels of monoterpenes and sesquiterpenes and to contribute to insect herbivore resistance (Simmons and Gurr, 2005).

Given the various hints for a potential relevance of *DXS2* expression in tomato trichome isoprenoid formation, we have undertaken a more detailed expression analysis of *DXS* isogenes and their roles in the cultivated tomato variety Moneymaker. To tackle the question of their functional significance, we have carried out a specific knock-down approach on *DXS2* expression in stable tomato transformants. The results obtained extend the scope of *DXS2* expression patterns and the spectrum of potential roles of DXS2. Concerning trichomes, the data demonstrate an unexpected contribution of DXS2 to the control of mono- to sesquiterpene ratios associated with altered utilization of either MEP- or MVA-derived isoprenoid precursors and a role in the regulation of trichome density.

RESULTS

Cloning and Characterization of cDNA and Genomic Sequences of *SlDXS1* and *SlDXS2*

To identify tomato DNA sequences for both DXS isoforms, specific primer pairs were derived from a published *DXS1*–cDNA sequence from *Solanum lycopersicum* (AF143812, Lois et al., 2000) and a putative *DXS2* sequence from a trichome-enriched cDNA library of *Solanum habrochaites* (ShDXS2, AY687353).