



Identification of putative genes to manipulate flavors in sweet orange fruit (*Citrus sinensis*)

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Abstract

Fruit flavors have always played a *significant role* in consumer acceptability. Although fruit flavors consist of complex mixtures of volatile and soluble substances, certain sesquiterpenes, such as valencene, beta-sinensal, alpha-sinensal, and nootkatone are highlighted as key flavor compounds in citrus. Valencene is an important component of the flavor of the ripe, sweet orange fruit (*Citrus sinensis*). *TPS1* (XP_006477784.1) encodes valencene synthase, a sesquiterpene synthase catalyzing the generation of the orange aroma compound valencene. In the present study, protein-protein interactions (PPIs) network analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were applied on *TPS1* to discover other components that have relationships with *TPS1*, as well as pathways which probably are associated with *flavors* biosynthesis. The PPIs network revealed that *TPS1* is part of an interaction network that included 11 nodes and 49 edges. According to KEGG pathway enrichment analysis the genes included in the PPIs network were significantly enriched in the 3 pathways including sesquiterpenoid and triterpenoid biosynthesis ([cit00909](#)), metabolic pathways ([cit01100](#)), and biosynthesis of secondary metabolites ([cit01110](#)), which have been reported to be related to the biosynthesis of the flavor. To the best of our knowledge, there were no investigations about their role of the predicted genes in the biosynthesis of *flavors* in the *Citrus sinensis*. Given that a very few genes are reported to be directly implicated in the biosynthesis of flavor fruit, these predicted genes may be considered potential candidates for improving the flavor of sweet orange and other fruits through metabolic engineering.

Keywords: *Citrus sinensis*, flavor, *TPS1*

Introduction

Flavors have always played an increasingly important role in fruit quality and consumer perceptions (Klee 2010). Citrus fruits contain particular aromas seldom detected in other fruit species (Sharon Asa *et al.*, 2003). Although the fruit flavor is a critical factor in fruit quality, this is often genetically complicated and laborious to score and quantify, making it a challenging objective for breeding (Galili *et al.*, 2002). The prevailing public concern in flavorful plant products along with the effective potential for metabolic engineering, accentuate the significance of enlightening the regulatory mechanisms, biosynthetic pathways, genes, and enzymes implicated in the fruit flavor formation (Galili *et al.*, 2002; Broun and Somerville, 2001).

Although the fruit flavor consists of complex mixtures of volatile and soluble substances, certain sesquiterpenes, such as valencene, beta-sinensal, alpha-sinensal, and nootkatone are highlighted as key flavor compounds in citrus (Sharon Asa *et al.*, 2003). Valencene was known to be a critical component of the aroma of the ripe sweet orange fruit. Sharon Asa and colleagues (2003) suggested that in Valencia oranges, valencene accumulates continuously throughout fruit maturation. Furthermore, valencene is considered as a substrate for the nootkatone synthesis, which confers a dominant citrus aroma. *TPS1* encodes valencene synthase, a sesquiterpene synthase, which catalyzes the production of the orange aroma compound valencene. Based on pieces of literatures reviewing, valencene synthase transcript increased in the ripening *Citrus sinensis* (Sharon Asa *et al.*, 2003; Yu *et al.*, 2013).

In the present study, protein-protein interactions (PPIs) network analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis using web-based STRING database (<http://string-db.org>) were applied on *TPS1* to discover other components that have relationships with *TPS1*, and pathways which probably are associated with *flavors* biosynthesis. Given that a very few genes are reported to be directly involved in biosynthesis of flavor fruit, metabolic engineering of fruits via these genes would be of significance. So, the predicted genes may be considered potential candidates for improving flavor of sweet orange and other fruits (Sharon Asa *et al.*, 2003).



Material and methods

Network analysis

The Search Tool for the Retrieval of Interacting Genes/Proteins, STRING 10.5 (<http://www.mybiosoftware.com/string-9-0-search-tool-retrieval-interacting-genesproteins.html>) (Szkarczyk *et al.*, 2014) database was used to acquire PPIs of DEGs. STRING calculates a confidence value for those relations from 0.15 to 0.9, as the lowest to the highest score, respectively. In the current study, protein-protein interactions (PPIs) were predicted with a minimum required interaction score of 0.4 (medium confidence).

KEGG Pathway enrichment analysis

To find important pathways, the pathway enrichment analysis of genes included in the network was performed based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database using web-based STRING database (<http://string-db.org>).

Results and discussion

The PPIs study is an effective approach to uncover unknown functions of proteins and gain insight into sophisticated cellular networks and understanding different plant physiological phenomena at the molecular level (Fukao 2012). The PPIs network revealed that *TPS1* is part of an interaction network that included 11 nodes (genes) and 49 edges (interactions) (Fig. 1). The list of genes and their functions are presented in Table 1. To the best of our knowledge, there were no investigations about their role of the predicted genes included in the network in *flavors* biosynthesis in the *citrus sinensis*. So, we propose these genes as a useful resource of potential candidate genes for engineering aroma in *citrus sinensis* and other citrus fruits.

KEGG pathway enrichment analysis revealed that genes included in the PPIs network were significantly enriched in the 3 pathways, including sesquiterpenoid and triterpenoid biosynthesis ([cit00909](#)), metabolic pathways ([cit01100](#)), biosynthesis of secondary metabolites ([cit01110](#)). According to KEGG pathway enrichment analysis, all predicted genes in the PPIs network except XP_006475060.1 are involved in the sesquiterpenoid and triterpenoid biosynthesis. This pathway is validated to exhibit involvement in flavor biosynthesis in attention to other reports.

XP_006475060.1 belongs to the FPP/GGPP synthase family. Multiple geranylgeranyl diphosphate synthases (GGPPS) for biosynthesis of geranylgeranyl diphosphate (GGPP) exist in plants (Coman *et al.*, 2014). Geranylgeranyl diphosphate (GGPP) is the precursor for the gibberellins, geranylgeranylated proteins, chlorophylls, carotenoids, and isoprenoid quinones biosynthesis in plants (Okada *et al.*, 2000). It was also reported that gibberellic acid foliar application influences growth, volatile oil and some physiological characteristics of lavender (*Lavandula officinalis* Chaix.) (Hassanpouraghdam *et al.*, 2011). So, XP_006475060.1 may serve as a novel candidate gene implicated in the flavor biosynthesis in fruits through gibberellins biosynthesis.

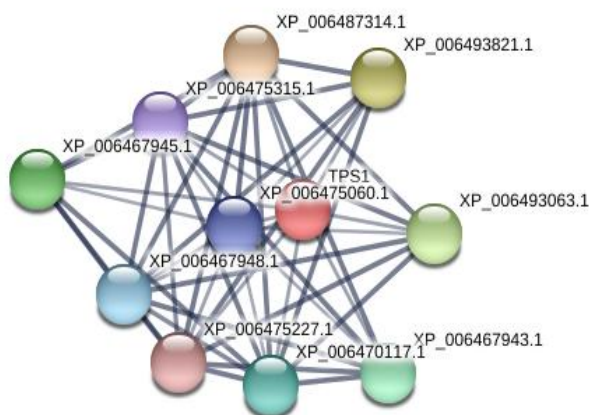


Fig. 1- PPIs network of *TPS1* using pathway STRING 10.5.



Table 2- the list of genes and their function in the PPIs network.

Predicted Functional Partners	Functions
XP_006487314.1	Uncharacterized protein; Squalene synthase isoform X1 (413 aa)
XP_006493821.1	Uncharacterized protein; (3S,6E)-nerolidol synthase 1-like isoform X1; Belongs to the terpene synthase family (579 aa)
XP_006493063.1	(3S,6E)-nerolidol synthase 1-like (579 aa)
XP_006467945.1	Uncharacterized protein; Belongs to the terpene synthase family
XP_006467943.1	Uncharacterized protein; (3S,6E)-nerolidol synthase 1, chloroplastic-like isoform X1 (586 aa)
XP_006470117.1	Uncharacterized protein; Alpha-farnesene synthase-like (605 aa)
XP_006467948.1	Uncharacterized protein
XP_006475060.1	Uncharacterized protein; Belongs to the FPP/GGPP synthase family
XP_006475315.1	Uncharacterized protein; (E)-beta-farnesene synthase-like; Belongs to the terpene synthase family (334 aa)
XP_006475227.1	

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