

Abstract

Terpenes, the largest group of secondary metabolites, are well known as constituents of essential oils, floral scents and defensive resins of aromatic plants, to which they impart their characteristic aromas and flavors. Terpene volatiles defend many species of plants, animals and microorganisms against predators, pathogens and competitors. Moreover, those compounds seem to serve as advertisements to attract pollinators and seed-dispersal agents as well as pest predators. The study of VOCs emitted during fruit development and after challenge with different biotic agents may help to determine the interactions of fleshy fruits not only with legitimate vertebrate dispersers and predators, but also with insects and microorganisms.

Fleshy fruits are particularly rich in volatiles. In citrus fruits, monoterpenes are the main components of the essential oil glands of the peel, being D-limonene the most abundant one (up to 95% in orange fruits). This characteristic makes citrus a good model system for studying the function of terpenes in plants. Modern molecular biology now enable experiments to test terpenoid function by the use of genetically transformed organisms in which terpene levels have been manipulated. In this work, a plasmid harboring the complete cDNA of a citrus limonene synthase gene (*CiTMTSE1*) in antisense (AS) or sense (S) orientation was used to modify the expression and accumulation of D-limonene of sweet orange (*Citrus sinensis* L. Osb) plants. D-limonene accumulation in AS fruits was dramatically reduced but the accumulation of other terpenoids was also modified, such as monoterpene alcohols, whose concentration increased in the peel of fruits. Genetically transformed plants were morphologically indistinguishable from wild-type (WT) and empty vector (EV) control plants.

Transgenic fruits were challenged against a pest and different pathogens to test whether volatile profile alteration results in an improvement in the response of the fruit flavedo against them. Males of the Mediterranean fruit fly (*Ceratitidis capitata*) exposed to AS fruits versus EV in wind tunnel assays were significantly more attracted to the odor of EV control fruits. In separate experiments with the green mould rot of citrus fruits and citrus canker caused by *Penicillium digitatum* and *Xanthomonas axonopodis* subsp. *citri*, respectively, transgenic fruits with a reduced content in D-limonene showed resistance to both pathogens. High D-limonene content in mature orange peels may be a signal for attractiveness of pests and microorganisms which might be likely involved in facilitating the access to the pulp of seed dispersal frugivores.

A global gene expression analysis of the flavedo of AS transgenic fruits linked the decrease of D-limonene and monoterpene metabolism to the up-regulation of genes involved in the innate immunity response, including transcription factors together with Ca^{2+} entry into the cell and activation of MAPK cascades, contributing to activation of jasmonic acid (JA) signaling, which triggered the up-regulation of JA metabolism and drastically increased the accumulation of JA in orange peels upon fungal challenge, explaining the resistance to necrotrophic fungi observed in AS fruits.

These results indicate that limonene accumulation in the peel of citrus fruit appears to be involved in the successful trophic interaction between fruits, insects, and microorganisms and provide a much more comprehensive view of roles of terpenes in nature. It also represents a very promising alternative for increasing resistance or tolerance of plants to pathogens.