In Vitro Cell.Dev.Biol.—Plant (2016) 52:303–314
DOI 10.1007/s11627-016-9755-8

PLANT TISSUE CULTURE

In vitro flowering, glandular trichomes ultrastructure, and essential oil accumulation in micropropagated *Ocimum basilicum* L.

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Received: 30 July 2015 / Accepted: 9 March 2016 / Published online: 8 April 2016 / Editor: Adriana Pinheiro Martinelli

Abstract The aromatic and medicinal properties of *Ocimum basilicum* L. (sweet basil) are related to the unique essential oil chemistry in different cultivars. This study describes efficient micropropagation and *in vitro* flowering protocols from shoot tips and reveals information on seed germination capability, glandular trichomes ultrastructure, and essential oil content and composition at different plant developmental stages from micropropagated *O. basilicum* 'Sweet Thai', chemotype methyl chavicol. Shoot tips from 2-mo-old aseptic seedlings were induced to proliferate shoots on Murashige and Skoog (MS) medium supplemented with 6-benzyl-aminopurine (BAP) and gibberellic acid (GA_3) either alone or in combination with α-naphthaleneacetic acid (NAA). Maximum shoot formation was achieved in MS medium supplemented with 1.0 mg L^{-1} BAP. The micropropagated plants were successfully acclimatized *ex vitro* with an 80% survival rate. All of the micropropagated plants flowered *in vitro* on MS medium supplemented with 1.0 mg L^{-1} GA_3. Relative to the mother plant, *in vitro* plants flowered at a younger stage of plant development but showed a lack of seed formation, fewer fully filled peltate glandular trichomes, lower essential oil content, and higher methyl chavicol content. *Ex vitro* plants flowered at an intermediate stage of plant development and formed seed with nearly the same seed germinability, essential oil content, and methyl chavicol content as the mother plant.

Keywords *Ocimum basilicum* · 'Sweet Thai' basil · Micropropagation · *In vitro* flowering · Essential oil

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Introduction

Ocimum basilicum L. (sweet basil) belongs to the family Lamiaceae and is a multipurpose herb characterized by its rich and aromatic essential oil content. This genus is primarily grown in warm temperate and tropical regions in Asia, Africa, and Central and South America as a culinary herb and an attractive, fragrant ornamental (Simon *et al.* 1999; Carović-Stanko *et al.* 2010). The aromatic leaves, flowers, and seeds are added to foods and beverages for flavor; extracted as active ingredient for use in perfumes, soaps, cosmetics, and dental products; and are included in traditional herbal medicines to treat fevers, headaches, kidney problems, gum ulcers, childbirths, rheumatoid arthritis, and menstrual irregularities (The Herb Society of America 2003). Besides these traditional medical uses, recent scientific studies have demonstrated potent antioxidant (Jayasinghe *et al.* 2003), antiviral (Chiang *et al.* 2005), and anti-proliferative activities (Manosroi *et al.* 2006) of compounds in *O. basilicum* essential oil and leaf extracts.

O. basilicum essential oil is a mixture of numerous compounds, mainly methyl chavicol (estragole), linalool, 1,8-cineole (eucalyptol), eugenol, and methyl cinnamate (Wesołowska *et al.* 2012; Said-Al Ahl *et al.* 2015). These compounds possess several biological activities. For example, methyl chavicol has antispasmodic (Coelho-de-Souza *et al.* 1997), antimicrobial (Friedman *et al.* 2002), and local anesthetic properties (Silva-Alves *et al.* 2013), linalool has anti-inflammatory properties (Peana *et al.* 2002), 1,8-cineole has vasorelaxant properties (Lahlou *et al.* 2002), and eugenol has antioxidant properties (Ogata *et al.* 2000). However, the composition of essential oil varies depending on the cultivar, and the taxonomy is complicated by the existence of chemotypes or chemical races that do not differ significantly in morphology (Simon *et al.* 1990; Carović-Stanko *et al.* 2010).