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Changes in the essential oil content and selected traits of sweet basil (*Ocimum basilicum* L.) as induced by foliar sprays of citric acid and salicylic acid

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ABSTRACT

The experiment aimed to understand how these organic acids are going to affect on essential oil production in sweet basil. Three concentrations of each citric acid (CA; 0, 4, or 7 mM) and salicylic acid (SA; 0.5 or 1 mM) were applied. We added a standard control for comparison. The first spray was started at 2-leaf stage that was continued with four subsequent sprays in a 15 day interval. The plants were harvested 75 days after sowing the seeds, when they had produced seed. SA in 1 mM concentration caused the maximum of the total essential oil production along with the highest essential oil production by both leaves and stems with an increase of 32.8, 38.3, and 25.8% (respectively) when compared to control treatment. A synergism between 7 mM CA and 1 mM SA was observed in flowering parameters, which yielded the tallest inflorescence in CA_7SA_1 along with the highest inflorescence count per plant and floret count in main inflorescence. Despite these quantitative improvements in flowering parameters, the thousand seed weight was still at its maximum in this combination, as well. The seed mucilage, on the other hand, increased significantly by foliar application of 0.5 mM SA. Seed oil content responded positively to applied CA, while SA effect was negligible. The results show the potential for using these compounds in manipulation of plant growth and metabolism toward the intended final use.

1. Introduction

Sweet basil (*Ocimum basilicum* L.) is an herb from the Lamiaceae family that is used as a favorite fresh vegetable together with many traditional foods, dressings, and salads. The aroma of basil due to its essential oil makes a great contribution to its organoleptic quality as well as the medicinal value.

Salicylic acid (SA) is among the most readily available plant growth regulating agents; it is also effective in other forms of acetyl SA and methyl salicylate in the plant (Raskin, 1992). SA acts as a stress messenger that induces the hypersensitive response (HR), helping the plant to resist invading organisms (Klessig and Malamy, 1994). Interactions have been reported between SA and other natural stress management compounds (Cipollini et al., 2004; Metraux and Durner, 2004; Kachroo and Kachroo, 2007; Zottini et al., 2007; De Torres Zabala et al., 2009). The alternative oxidase enzyme activity in mitochondria could be induced by SA, which is involved in the stress alleviation mechanism in plants (Raskin, 1992; Vanlerberghe and McIntosh, 1997). Beneficial effects by application of lower concentrations (less than 1 mM) has been reported for plant growth (Rivas-San Vicente and Plasencia, 2011). SA caused enhancement (Kiddle et al., 1994; Kang et al., 2004; Wang et al., 2007) or decline (Prithiviraj et al., 2005; D'Onofrio et al., 2009) in specific secondary metabolites of the plant. In sweet basil, increase in the content of essential oil in response to sprays of SA at 0.1 mM is reported (Gharib, 2006). Jafari and Hadavi (2012) tested higher SA concentrations and suggested finding the optimum SA concentration between 0.1 mM and 2 mM.

Citric acid (CA) is a six carbon organic acid, featuring a central role in citric acid cycle in mitochondria that creates cellular energy by phosphorilative oxidation reactions. It is created by addition of imported acetyl-CoA from glycolysis to oxaloacetic acid that is converted in later stages to succinate and malate (Wills et al., 1981). Foliar sprays of CA alone or in combination with Fe salts have been used to recover many plants from the Iron chlorosis (Abadía et al., 2002; Álvarez-Fernández et al., 2004; Eidyan et al., 2014; Mengel et al., 1994; Tagliavini et al., 1995, 2000). Later studies revealed that the CA effect is not just due to pH change and there are a variety of physiological responses to applied CA; foliar use of CA alone or in combinations with SA and malic acid increased the essential

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oil production of Basil (Jaafari and Hadavi, 2012) and Dill (Jafari and Hadavi, 2012). Some physiological parameters were improved in Tuberose by application of foliar CA (Eidyan et al., 2014), Lilium (Darandeh and Hadavi, 2012), and Bean (El-Tohamy et al., 2013). The increase in the vase life of cut rose flowers obtained in the soilless culture system by foliar pre-harvest application of the combinations of SA and CA revealed that the obtained results are not necessarily dependent on soil system (Hajreza et al., 2013). A recent study based on current work confirmed that the combination of 1 mM SA with 7 mM CA caused a significant increase in root acquisition of boron in a soilless culture system (Ghazijahani et al., 2014).

An increase in the exudation of citrate and malate from roots of calcicole plants (plants growing in alkaline soils) enables them to extract P and Fe from such soils (Lopez-Bucio and Nieto-Jacobo, 2000). Jafari and Hadavi (2012), suggested an increase in mineral absorption by elevated exudation of organic acids in response to foliar organic acids as part of mechanism responsible for improved growth parameters. This was confirmed recently by An et al. (2014), and the mediation of these exudates in mineral absorption in the soil medium is well known (Marschener, 1998; Bais et al., 2006; Arcand and Schneider, 2006). In addition, the exudated organic acids are the main carbon source for rhizobacteria (Ohwaki and Sugahara, 1997). Therefore, a much complex interaction and effect size is expected by use of foliar organic acids in a soil culture system as compared to a soilless culture system (Ghazijahani et al., 2014).

There are reports on changes in leaf/stem ratio in response to applied excess nitrogen in Basil (Frabboni et al., 2011), and Mint (Ram and Kumar, 1997). Therefore, making a distinction between their share in essential oil production might be useful to understand how they could interact on total essential oil production in response to applied treatments.

The present experiment was designed mainly to assess the effect of those promising ranges and combinations of SA and CA, which were suggested by earlier reports on essential oil production of sweet basil.

2. Material and methods

This study was conducted during the summer of 2010. The field had a sandy-loam soil with a pH 7.6, a relatively high EC of 5.2 4 dS/m, total organic carbon of 0.6%, total N of 0.55%, dissolved phosphorus of 9.6 mg kg^{-1} and extractable potassium of 314 mg kg^{-1}.

2.1. Plant culture and foliar treatments

Seeds of the *O. basilicum* 'Karaj' accession were directly planted in the field. Seeds were planted in plots (2 m width by 6 m length) with 50 cm line spacing. All field operations were performed manually using local interns. The experiment was conducted in a randomized block design factorial arrangement (2×3) with four replications. CA (0, 4, and 7 mM) and SA (0.5 and 1 mM) were applied in five consecutive sprays starting at two real leaf stage; the sprays were repeated every 15 days. We added a standard control that was sprayed with distilled water as well with four replications for comparison. A commercial wetting agent was added to enhance the wetting effect of the sprays (citogate).

2.2. Essential oil measurement

Dry samples of 50 g from herb were obtained from each replication in day 75 after sowing the seeds and used for essential oil extraction. Essential oil was extracted by hydro-distillation in a Clevenger apparatus according to the method described by Carvalho Filho et al. (2006), with modifications. Samples were placed in round bottomed flasks containing 1.5 L of water and refluxed for 3 h; the oil weight (g) was recorded. The obtained essential oil content (EOC; %) for stem and leaf, were used together with stem dry weight yield (DWY) and leaf DWY to product essential oil yield (EOY) for stem and leaf using following formula: $\mathrm{EoY(kg/ha)} = (\frac{\mathrm{EoC(\%w/w)} \times \mathrm{DWY(kg/ha)}}{100})$. Next, the stem plus leaf EOY yielded in the total EOY. Furthermore, the total EOC was calculated back by the same formula. The ratio of leaf EOY to stem EOY was also calculated.

2.3. Number of inflorescences per plant

Six plants of each plot were randomly selected and the number of inflorescences in each plant was counted and averaged.

2.4. Number of florets in the main inflorescence

Six plants of each plot were randomly chosen and the florets only on the main inflorescence were counted and then averaged.

2.5. Seed oil content

For estimation of seed oil content, 10 g of dry seed was ground thoroughly using pestle and mortar and put in Soxhlet apparatus containing *n*-hexane solvent. The extract was then placed in a rotary evaporator and the remaining oil was weighted.

2.6. Thousand seed weight

A thousand of pure seed were counted and weighted by a 0.0001 digital scale.

2.7. Seed mucilage

Five gram of clean and dry seeds from each treatment were weighed and the equal amount of 100 mL of distilled water was added to each. After 24 h, excess water drained and weighed again. Then the initial weight of 5 g subtracted from each to yield the amount of absorbed water by mucilage. The amount of water absorbed by 5 g sample was indicative of their mucilage content.

2.8. Statistical analysis

Results were analyzed by the GLM module of the SPSS software (version 20, IBM Inc.) and comparison of means was done by the Tukey HSD test ($p < 0.05$). Multiple regression analysis (MRA) was conducted using linear regression component of the same software to test relationship among selected factors and variable.

3. Results

3.1. Essential oil content (EOC)

The results show that the direct effect of CA and SA and the interaction between CA and SA on the total amount of essential oil, essential oil of the leaves and stems were proved to be significant ($p = 1\%$; Table 1).

With constant 0.5 mM SA, an increase of CA to 4 mM caused a slight decrease in the total, leaf, and stem EOC, while increasing the level further to 7 mM caused them to decline further which suggests an antagonism between the two (Fig. 1A). On the other hand, with constant 1 mM SA, all the EOCs declined sharply by an increase of CA to 4 mM, whereas, unlike the previous trend, by increase of the CA level further to 7 mM, the EOC increased.

The mean comparison test showed the greatest increase in the percentage of total essential oil, essential oil of the leaves and stems,