Growing Medicinal Plants in Hydroponic Culture

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Abstract

Medicinal plants are increasingly cultivated on a commercial scale to satisfy the large demand for natural remedies. These species are generally grown in open field, which results in large year-to-year variability in both biomass production and content of active principles. Hydroponic technology may be applied to produce highstandard plant material all year-round in consideration of the possibility to control growing conditions and to stimulate secondary metabolism by appropriate manipulation of mineral nutrition. A series of experiments were conducted between 2005 and 2010 at the University of Pisa to investigate the application of the floating raft growing system for the greenhouse cultivation of echinacea (*Echinacea angustifolia* DC) and basil (*Ocimum basilicum* L), which are typically cultivated for their roots and leaves, respectively. Growth and content of distinctive caffeic acid derivatives (CADs), specifically echinacoside in echinacea and rosmarinic acid in basil, were determined. Both species grew rapidly and healthy and in two to four months they accumulated large biomass with minimal contamination. Nevertheless, in echinacea the high biomass production was not associated with high levels of CADs and the concentration of echinacoside (the marker compound used for quality standardization) never reached the minimum standard (1% on a dry weight basis) for the industrial production of dry extract. In contrast, basil accumulated an adequate content of rosmarinic acid. One additional advantage was the possibility to harvest also the root system of basil, which contained higher levels of rosmarinic acid compared to the leaves.

INTRODUCTION

Medicinal and herbal plants are largely used for their contents of bioactive compounds and are increasingly cultivated on a commercial scale to satisfy the large demand for natural remedies.

Echinacea spp. (*Asteraceae*) and *Ocimum* spp. (*Lamiaceae*) are widely employed medicinal plants that contain several bioactive molecules, including caffeic acid derivatives (CADs). These substances are phenolic compounds acting primarily as antioxidants, but also displaying pharmacological properties (Pellati et al., 2005).

Extracts from *Echinacea* spp. are top selling herbal remedies both in North America and in Europe (Zheng et al., 2006a). Among *Echinacea* spp., *E. angustifolia* DC (hereafter referred to as echinacea) is a perennial plant native of North America, whose root tissues are generally employed to obtain herbal products (Zheng et al., 2006a). Echinacea roots contain echinacoside as one of the main CADs, and the standardization of echinacea plant material for the industrial production of dry extract is based on the level of this marker compound, which should not be lower than 1% on a dry weight (DW) basis (Dall'Acqua, 2010).

A lot of species in the *Ocimum* genus are also used in pharmaceutical and cosmetic preparations, due to their high content of essential oils (Makri and Kintzios, 2007) and rosmarinic acid (Juliani et al., 2008). The latter compound is a caffeic acid ester with several important biological properties such as antibacterial, antiviral and antiinflammatory activity (e.g. Juliani et al., 2008). Basil (*Ocimum basilicum* L.) is an important source of rosmarinic acid and is used worldwide, especially as a flavoring agent in food preparation (Makri and Kintzios, 2007).

While basil is traditionally cultivated in soil (Makri and Kintzios, 2007), the field cultivation of echinacea has been promoted during the last decades by the increasing market demand (Dall'Acqua et al., 2010). Anyway, field cultivation of medicinal plants has to cope with several problems: for example, basil is very sensitive to pathogens attack and is strongly affected by soil salinity (Miceli et al., 2003), while echinacea is difficult to grow due to poor germination and field establishment (Dall'Acqua et al., 2010). Moreover, open field cultivation results in large year-to-year variability, as both biomass production and synthesis of secondary metabolites are affected by many factors such as genotype, climatic conditions, soil type, growing practices and the presence of pests and diseases (Aiello et al., 2002; Letchamo et al., 2002; Dall'Acqua et al., 2010). Because of these drawbacks, the

development of suitable artificial systems could represent an effective means for the cultivation of both basil and echinacea.

Hydroponics may allow a cost-effective all-year-round production, as plants generally grow faster and at higher density than in the soil, and are affected by minimal contamination with pollutants and microorganisms (Letchamo et al., 2002; Zheng et al., 2006a). In addition, in greenhouse hydroponic culture the growing conditions can be strictly controlled to produce highly standardized plant material for the industrial extraction of bioactive compounds (Zheng et al., 2006b). Soilless cultivation also allows to regulate the secondary metabolism involved in the accumulation of pharmacological active principles through appropriate manipulation of the nutrient solution fed to the plants (Briskin et al., 2000).

Among the techniques that can be used for hydroponic cultivation, the floating raft system (FRS), which requires relatively low investment and running costs (Pardossi et al., 2006), is commonly employed for short cycle leafy vegetables and seems well applicable to the cultivation of basil. In FRS, bare-rooted plants are grown in stagnant or slowlyrecirculating nutrient solution; therefore, this technique could be properly applied also to species that are cultivated for the roots, like echinacea.

In this study we evaluated the production of echinacea and basil plants in FRS, through the determination of both biomass yield and accumulation of marker compounds (i.e. echinacoside and rosmarinic acid, respectively) in the artificial cultivation system.

MATERIALS AND METHODS

All the experiments were conducted in a glasshouse at the University of Pisa (Pisa, Italy) between 2005 and 2010. *E. angustifolia* seeds (Gold Nugget Seed®) were purchased from Jelitto Staudensamen GmbH (Schwarmstedt, Germany). Basil seeds were purchased from Sais (Cesena, Italy).

For both species, the seeds were sown in rockwool tray plugs and germinated in a growth room at 25°C. After emergence, the trays were transferred into a glasshouse and placed in plastic tanks with 70 L (300 L m^{-2}) of aerated nutrient solution. Eight to 12 tanks, each hosting 30-40 seedlings at transplating, were set up in each experiment. The compositions of the nutrient solutions employed for the cultivation of the two species were reported elsewhere (Maggini et al., 2010; Kiferle et al., 2011).

For both species, plants were randomly sampled from each tank, carefully washed with tap water, rinsed with deionized water, blotted dry on paper towels and sectioned into the

different organs. For growth analysis, the organs of individual plants were weighed and dried to constant weight at 75°C in a ventilated oven.

Echinacea was sampled when about half of the plants were at the flowering stage. The tissues were dried at a temperature of 50°C, and an aliquot of each sample was ovendried at 75°C for moisture determination.

Basil leaves and roots were sampled during the vegetative and the flowering stage (two and four weeks after transplanting, respectively). Fresh tissues were frozen in liquid nitrogen and stored at -80°C before laboratory analysis.

All the samples were extracted and analysed by HPLC according to the protocol reported by Maggini et al. (2010).

HPLC grade solvents were purchased by Carlo Erba Reagenti SpA (Rodano, Italy). Along with echinacoside (Phytolab GmbH, Vestenbergsgreuth, Germany) and rosmarinic acid (Extrasynthese S.A., Genay, France), the following standard substances were used: cynarin, caftaric acid, cichoric acid (Phytolab GmbH, Vestenbergsgreuth, Germany); chlorogenic acid, caffeic acid, ferulic acid, *t*-cinnamic acid, *p*-coumaric acid (Sigma-Aldrich, Milano, Italy).

Peak identification was accomplished by LC-MS and LC-MS-MS. The determinations of CADs were carried out on four replicates and the results were expressed as mg g^{-1} dry weight (DW).

Data were subjected to one-way ANOVA. Statistical analysis was performed using GraphPad Prism® 4.0 (Graph Pad Software Inc., San Diego, CA, USA). Each experiment was repeated three times with similar results.

RESULTS AND DISCUSSION

Echinacea

The FRS produced clean, bare-rooted echinacea plants. The growing cycle from transplanting to harvest lasted at most 16 weeks, to avoid the occurrence of root rot. Crop density at harvest was about 100 plants m^{-2}, that is ten times higher than that of typical field crops (Aiello et al., 2002; Berti et al., 2002).

At harvest about half of the plants had developed the typical inflorescences, which consisted of a 20- to 30-long stem bearing sessile leaves and flowering head. Each individual plant had 20-30 basal leaves and 1-3 inflorescences.

Basal leaves or root dry weights were similar in vegetative and flowering plants, and the total biomass production was about three times higher in the latter due to the presence of inflorescences (Fig. 1). The total dry biomass of individual plants at the vegetative stage was about 3 g on average.

Based on measured data collected in our experiments, we estimated that two consecutive greenhouse FRS cultures could produce almost 3000 kg ha^{-1} of dry roots within eight months. A similar production can be obtained from field-grown plants only after 3 or 4 years of cultivation: for instance, Hobbs et al. (1989) reported for echinacea a root yield of 2500 kg ha^{-1}.

The samples used for the determination of CADs were dried at 50°C, because the drying treatment resembles the conditions used by echinacea growers or collectors (Aiello, 2002); besides, dried tissues were found to contain higher levels of CADs compared to the fresh ones (Maggini et al., 2010).

Among the CADs under investigation, echinacoside was the only one that was contained in two distinct organs, that is in root tissues along with cynarin and chlorogenic acid, and in the inflorescences along with caftaric acid. The mean root concentrations of the CADs in FRS-grown echinacea were similar in vegetative and in flowering plants
(Fig. 2).

Because of the dissimilatity in populations, growing conditions and analytical protocols, the levels reported in the literature for root CADs in spontaneous or fieldcultivated echinacea plants are spread over a wide range (e.g. Li and Wardle, 2001; Pellati et al., 2005; Berti et al., 2002). However, the concentrations that we determined in FRSgrown echinacea plants generally fell within these limits.

Although echinacoside has only a slight antimicrobial and antibacterial activity (Aiello, 2002) and is not the most valuable pharmaceutical compound among echinacea CADs (Dall'Acqua et al., 2010), it is the marker compound used for the chemical standardization of echinacea extracts and for the quality assessment of echinacea roots. The content of echinacoside that we found in root tissues was lower than 5 mg g^{-1} DW (Fig. 2), which is the minimum concentration required by the European Pharmacopoeia (European Directorate for the Quality of Medicines and Health Care, 2002). Even this value is well below the minimum quality standard (10 mg g^{-1} DW) for industrial production of the root dry extract of echinacea (Dall'Acqua et al., 2010). In FRS-grown echinacea, Zheng et al. (2006b) also found root levels of echinacoside much lower than 10 mg g^{-1} DW.

Basil

Basil plants grew healthy and vigorously in FRS and flowered abundantly within one month after planting. Both fresh and dry biomass accumulation in leaves and roots was much higher at the flowering stage than in the vegetative phase. Figure 3 reports the fresh biomass in the shoot or leaves of basil plants at the vegetative and at the flowering stage. It was estimated that four to five crops per year could be performed with annual leaf fresh biomass production of about 30 kg m^{-2}, in agreement with previous findings (Miceli et al., 2003). This result suggested that the FRS could be an efficient system for producing large amounts of plant material to be easily processed on industrial scale. In contrast with echinacea, basil is mainly consumed as a fresh product; therefore, only fresh samples of each organ were processed and analyzed for the content of RA. Fresh tissues contained much more RA than those that had been desiccated at 70°C. Many authors found several CADs in basil tissues, although in most cases RA was the most abundant one (e.g. Javanmardi et al., 2002; Juliani et al., 2008). In contrast, in all our samples only RA was present in considerable concentrations among the CADs of interest; the other metabolites were present in trace quantities (e.g. ferulic and caffeic acid) or below the detection limit of the analytical method (0.05 mg g^{-1} DW). In addition to the peak of RA, the chromatograms of all the analyzed samples showed one further peak, which, according to the LC-MS analysis, corresponded to a methylated derivative of RA. Further work is in progress for a more accurate structure elucidation of this compound.

In our experiments we detected RA both in shoots and in roots; the level of RA was much higher in the roots than in the leaves in both phenological phases (Fig. 4). Thus, one advantage of FRS is the possibility to harvest also the root system, which in our plants accounted for 10% of the total biomass. The levels of RA reported in the literature for sweet basil tissues are very dissimilar, ranging from less than 0.1 mg g^{-1} DW (Sgherri et al., 2010) to nearly 100 mg g^{-1} DW (Javanmardi et al., 2002) as a result of differences in plant genotype, growing conditions or analytical method. In our experiments the highest concentration of RA was found in root tissues at full bloom (Fig. 4). Juliani et al. (2008) also reported that in basil the leaf concentration of RA increased during the flowering stage. In contrast, in other *Lamiaceae*, such as spearmint and peppermint, the leaf RA content declined with flowering (Fletcher, 2010). In conclusion, the FRS proved to be an effective method to obtain a large biomass production for both echinacea and basil. This outcome was expected in basil, which is a short-cycle plant that is cultivated for the leaves. However,

also echinacea grew quickly in short-cycle, high-density greenhouse hydroponics, although it is a slow growing plant in open field, which is collected after a few years of cultivation. On the other hand, in hydroponically grown echinacea the content of the marker echinacoside was much lower than the minimum quality standard required for the industrial production of dry extract. Echinacea plants, which had to be collected after only a few months of cultivation, at harvest were much younger than those cultivated in open field and had not accumulated sufficient amounts of CADs in root tissues. In contrast, in basil a high biomass production was associated to an adequate degree of RA accumulation, and FRS proved to be a proper technique to obtain plant material that contained satisfactory amounts of this active principle. The highest levels of RA were found in the roots of hydroponically-grown seedlings at full bloom, and with the FRS the root system can be easily harvested together with the aerial part.

The results of this study suggest that the suitability of the FRS for growing medicinal plants is strongly dependent on the species, as the fast growth observed in hydroponics does not necessarily bring about a sufficient accumulation of phytochemicals in plant tissues. The FRS seems properly applicable to short-cycle species, but it does not ensure a good chemical quality in crops which normally require a long cultivation time. Further work is necessary to develop suitable growing protocols for these species.

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Figures

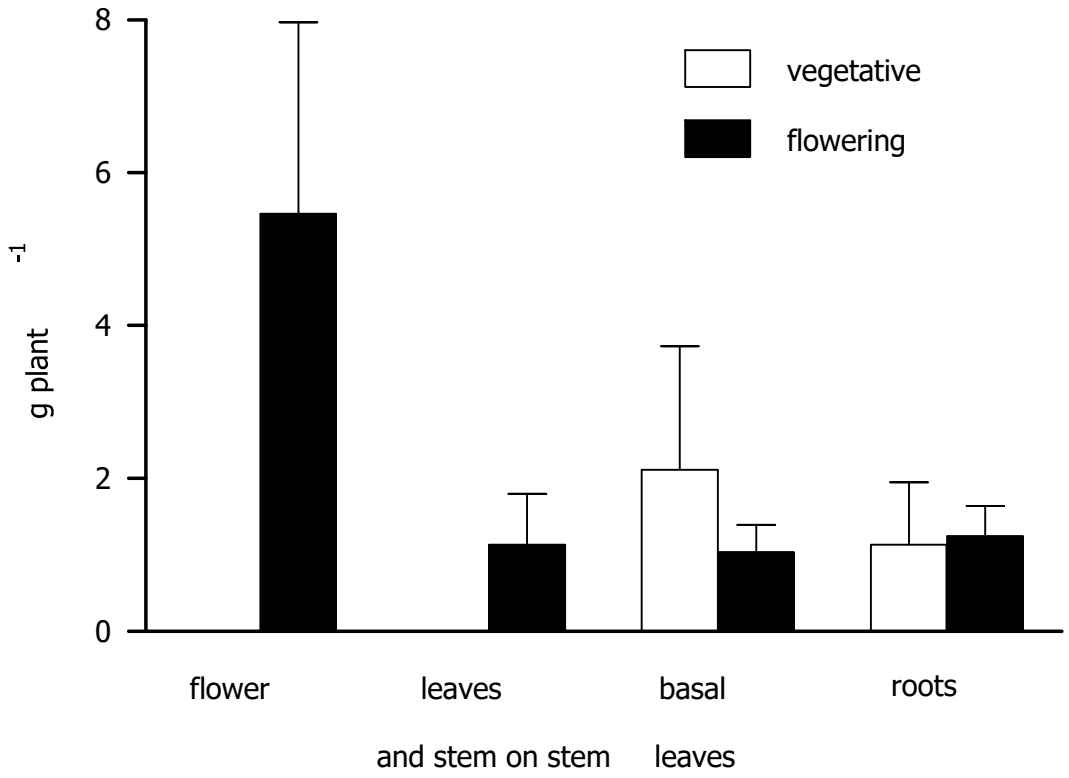

Fig. 1. The dry biomass production of echinacea (*Echinacea angustifolia* var. *angustifolia*) plants grown in hydroponic culture and sampled at the vegetative or flowering stage. Mean values and standard errors of four replicates; each replicate consisted of the organs detached from two or three individual plants. At harvest, nearly half of the plants under observations were at the flowering stage. For basal leaves or roots in distinct phenological phases, mean values are not different at 5% level of significance.

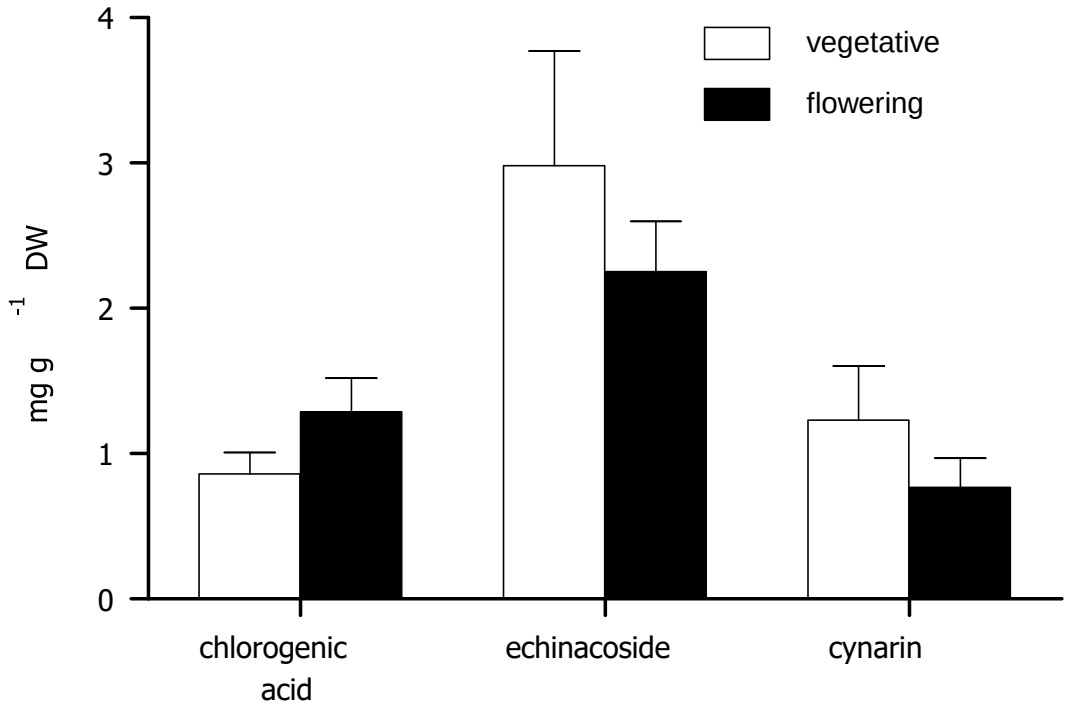

Fig. 2. The root content of caffeic acid derivatives in echinacea (*Echinacea angustifolia* var. *angustifolia*) plants grown in hydroponic culture and sampled at the vegetative or flowering stage. Mean values and standard errors of four replicates; each replicate consisted of the organs from two or three individual plants. For each metabolite, mean values in distinct phenological phases are not different at 5% level of significance.

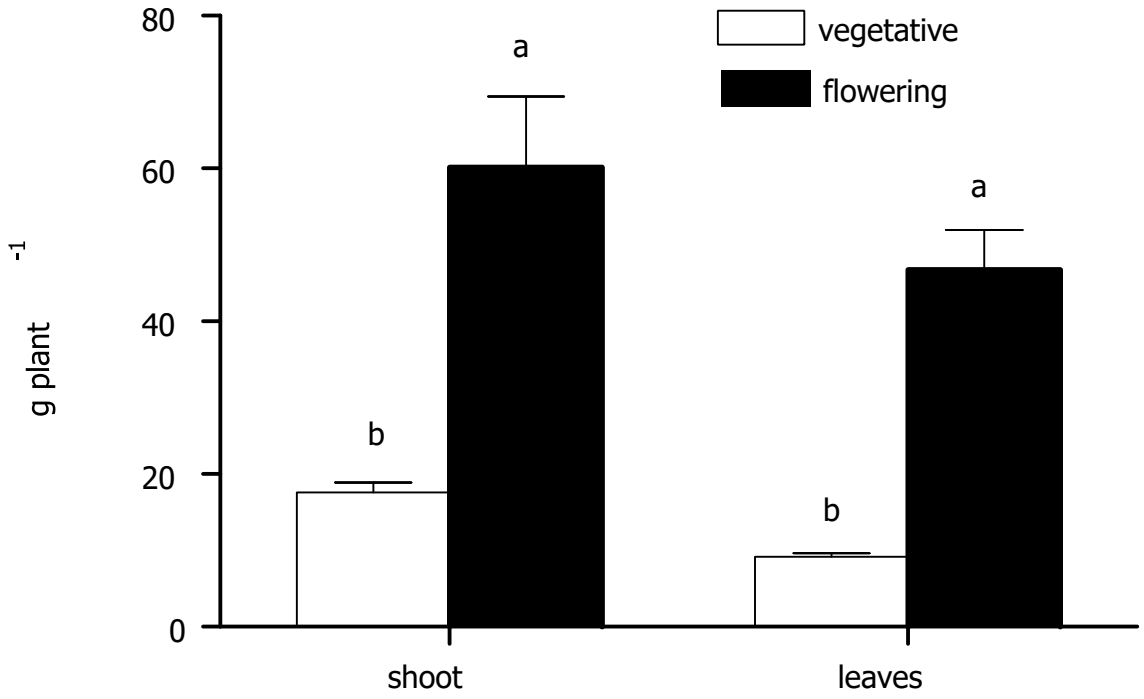

Fig. 3. The fresh biomass production of basil plants grown in hydroponic culture and sampled at the vegetative and at the flowering stage (two or four weeks after transplanting, respectively). Mean values and standard errors of 15 replicates, each consisting of one individual plant. Distinct letters indicate different values at 5% level of significance.

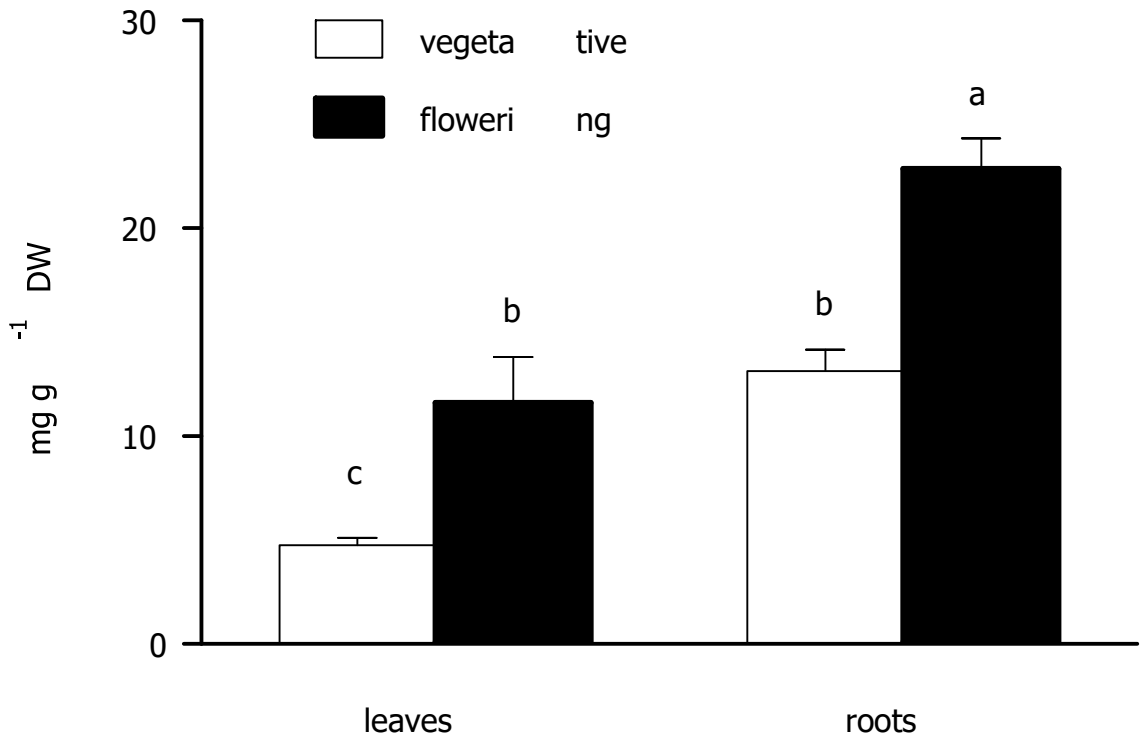

Fig. 4. The content of rosmarinic acid in basil plants grown in hydroponic culture and sampled at the vegetative and at the flowering stage (two or four weeks after transplanting, respectively). Mean values and standard errors of four replicates, each consisting of the organs from one individual plant. Distinct letters indicate different values at 5% level of significance.