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Influence of nitrogen nutrition on growth and accumulation of rosmarinic acid in sweet basil (*Ocimum basilicum* L.) grown in hydroponic culture

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Abstract

Sweet basil (*Ocimum basilicum* L. cv. Genovese) was grown in hydroponic culture (floating raft system) for the extraction of rosmarinic acid (RA). Two experiments were undertaken to investigate the influence of nitrogen (N) nutrition on biomass and RA production. Sweet basil seedlings were cultivated for seven weeks from April to July of 2009, under the typical greenhouse conditions of Mediterranean regions. The nutrient solutions contained different NO_3^- concentrations (0.5, 5.0 and 10.0 mol m^{-3}) or NO_3^-/NH_4^+ molar ratios (1:0, 1:1 and 0:1; total N concentration was 10.0 mol m^{-3}). The concentration of other nutrients were as follows: 1.0 mol m^{-3} P-H_2PO_4, 10.0 mol m^{-3} K^+; 3.0 mol m^{-3} Ca^{2+}; 1.5 mol m^{-3} Mg^{2+} plus trace elements. Plants were harvested at full bloom and RA was quantified by HPLC in roots, stems, inflorescences and leaves. In both experiments, sweet basil produced a large amount of biomass with relatively high RA concentration, which ranged from approximately 10 to 97 g kg^{-1} DW in leaf tissues, depending on leaf age and N nutrition. The use of a total NO_3^- concentration of 5.0 mol m^{-3} resulted in optimal plant growth and RA production; this suggests that the standard N concentration used in hydroponic culture (10.0 mol m^{-3} or higher) could be reduced considerably, with important implications from the environmental point of view. In contrast, the addition of NH_4^+ to the nutrient solution was detrimental to both growth and RA production.

Keywords*:* Ammonium; caffeic acid derivatives; floating raft system; nitrate; soilless culture.
Abbreviations: CAD_caffeic acid derivative; EC_electrical conductance; PPFD_photosynthetic photon flux density; RA_ rosmarinic acid.

Introduction

Sweet basil (*Ocimum basilicum* L.) in the *Lamiaceae* is largely employed as a flavouring agent for food and is cultivated worldwide (Makri and Kintzios, 2007). Sweet basil is also used for cosmetical and pharmaceutical preparations, as it contains large amounts of essential oils (Makri and Kintzios, 2007) and rosmarinic acid (RA; Juliani et al., 2008), which is a caffeic acid ester (Petersen and Simmons, 2003).

Medicinal plants, including sweet basil, are generally cultivated in open field and this results in year-to-year variability in both biomass production and the content of active principles (Bourgaud et al., 2001). Hence, there is an increasing interest for greenhouse hydroponic (or soilless) culture, where growing conditions can be strictly controlled and the production of the metabolites of interest can be maximized (Pardossi et al., 2006; Prasad et al., 2010). Hydroponic culture offers several advantages over traditional soil culture such as higher yield per unit ground area, all-year round production, higher quality and ease of processing of harvested material on account of minimal contamination from pollutants, pests and pathogens (Pardossi et al., 2006). Among different hydroponic techniques, the floating raft system is quite suitable for the greenhouse production of leafy vegetables and herbs (Pardossi et al., 2006). This technique has been tested also for the cultivation of medicinal plants (Zheng et al., 2006; Maggini et al., 2012). In these crops, hydroponic culture makes it possible to stimulate the synthesis of secondary metabolites by modifying the composition of the nutrient solution (e.g. Zheng et al., 2006; Montanari et al., 2008). Ammonium (NH_4^+) and nitrate (NO_3^-) are the main sources of nitrogen (N) for crop plants. In soilless culture, N is generally supplied as NO_3^- at concentration close or higher than 10.0 mol m^{-3} (Pardossi et al., 2006). Some authors (e.g. Munoz et al., 2008; Massa et al., 2010) proposed new fertigation practices based on reduced NO_3^- concentration in the feeding solution in order to minimize the environmental impact of soilless culture associated with NO_3^- leaching. A balance between NH_4^+ and NO_3^- provides some degree of pH control in hydroponic nutrient solutions, as NH_4^+ limits the rise in pH associated with NO_3^- assimilation (Savvas, 2001). Decreasing the NO_3^-/NH_4^+ ratio in the nutrient solution also reduces the accumulation of NO_3^-, which is potentially toxic to human health, in leafy vegetables (Santamaria et al., 1998). However, the use of NH_4^+ as the sole or dominating N form generally results in growth suppression, leaf chlorosis and even plant death (Britto and Kronzucker, 2002). On the other hand, NH_4^+ promoted growth in some species such as rice (Britto and Kronzucker; 2002), ryegrass (Cao et al., 2011), lettuce (Savvas et al., 2006) and strawberry (Cárdenas-Navarro et al., 2006). Work is in progress in our laboratory to develop a cost-effective hydroponic production system of sweet basil for the extraction of RA. In a previous work, sweet basil seedlings grown in floating raft system produced a large amount of biomass (including root tissues) with RA concentration ranging from 4 to 29 g kg^{-1} DW (Kiferle et al., 2011). In the present study, we investigated the effects of N concentration and form (NO_3^- and NH_4^+) on biomass and RA production. In particular, we checked the possibility to cultivate sweet basil in floating raft system with 0.5 or 5.0 mol m^{-3} NO_3^- concentration in the nutrient solution. These

NO_3^- levels are fairly lower than the standard concentration used in hydroponic culture, which is generally 10.0 mol m^{-3} or higher (Pardossi et al., 2006; Sonneveld and Voogt, 2009).

Results

Plant growth

Large volume (1.5 L $plant^{-1}$), bicarbonate ($H_2CO_3^-$) content (about 1.2 mol m^{-3}) and frequent discharge of the nutrient solution minimized the variation in pH, electrical conductivity (EC) and ion composition in all hydroponic cultures (data not shown), although in some occasions the pH of NO_3^--containing nutrient solutions was adjusted to 5.8 - 6.2 with diluted sulphuric acid. In both experiments, all the plants developed normally and were healthy, with no symptoms of N deficiency or NH_4^+ toxicity (e.g. tip burn, leaf chlorosis and/or necrosis; root tip burn), while growth rate was influenced significantly by N nutrition (Table 1). In experiment 1, leaf and shoot biomass increased with N concentration in the nutrient solution, although the differences in shoot FW or DW were not significant between 5.0 and 10.0 mol m^{-3} NO_3^- (Table 1). Plant height was not affected by NO_3^- availability (Table 1). Increasing N concentration enhanced leaf formation (Table 1), while reducing the DW/FW ratio of both stem and leaf tissues (data not shown). Root DW increased significantly at 0.5 mol m^{-3} NO_3^- as compared to other concentrations (Table 1). In experiment 2, all measured growth parameters, in particular root DW, decreased significantly when the plants were fed with NH_4^+, regardless of the presence of NO_3^- (Table 1). Leaf and shoot DW/FW ratio increased in the presence of NH_4^+ (data not shown). In experiment 1, leaf chlorophyll and N content did not differ appreciably in the plants grown at 5.0 and 10.0 mol m^{-3} NO_3^- (they averaged 1.03 µg g^{-1} FW and 30.0 g kg^{-1} DW, respectively), while both quantities decreased significantly at the lowest N concentration (0.61 µg g^{-1} FW and 18.5 g kg^{-1} DW, respectively). In experiment 2, N form did not affect significantly the leaf content of both chlorophyll and N, which averaged 0.87 µg g^{-1} FW and 32.2 g kg^{-1} DW, respectively.

Rosmarinic acid

Sweet basil plants were harvested at bloom stage (seven weeks after transplanting), when RA was found to accumulate at the highest level (Kiferle et al., 2011). In all plant samples, RA was the only caffeic acid derivative (CAD) detected at higher concentration than the detection limit; its content ranged approximately from 10 to 97 g kg^{-1} DW (Table 2; Fig 1). The production of RA was generally much higher in the inflorescences and in the leaves (especially in the apical ones) than in other organs (Table 2).

The content of RA in apical and medium leaves was influenced significantly by both N concentration and form, while the content of this CAD in basal leaves was unaffected by N nutrition (Fig 1). Rosmarinic acid accumulated to a large extent in younger (apical) leaves, reaching 97 g kg^{-1} DW (nearly 10%) in the plants grown at 10.0 mol m^{-3} NO_3^- (Fig 1, top). The content of RA increased significantly in the roots when NO_3^- concentration was diminished, while it was not affected by the NO_3^-/NH_4^+ ratio (Table 2). The content of RA in the stems and the inflorescences was scarcely influenced by N nutrition (Table 2). Lower NO_3^- levels resulted in higher RA content of leaf tissues (Table 2); in the plants grown at 0.5 mol m^{-3} NO_3^- leaf RA content was nearly double than in the plants grown at 10.0 mol m^{-3} NO_3^-. However, RA production per plant was significantly reduced at the lowest NO_3^- level (Fig. 2, top). The supply of NH_4^+ decreased significantly the level of RA in leaf tissues (Table 2; Fig. 1, top) and the RA production per plant (Fig. 2, bottom).

Discussion

Among the CADs of interest, RA was the only compound detected at higher concentration than the detection limit. Other authors reported that RA was the most important CAD in sweet basil (Javannardi et al., 2002; Jayasinghe et al., 2003). The levels of RA determined in this work (10 to 97 g kg^{-1} DW; Table 2, Fig. 1) were similar to those found in a previous work (Kiferle et al., 2011) and within those reported in the literature for sweet basil. These values range from less than 0.1 g kg^{-1} DW (Sgherri et al., 2010) to nearly 100 g kg^{-1} DW (Javanmardi et al., 2002). This variability probably results from differences in plant genotype, growing conditions, or in the method used for RA determination. In our experiments, apical, medium and basal leaves were sampled separately for the determination of RA level, which generally decreased from top to bottom (Fig 1). Although in some plants the amount of secondary compounds increases with leaf maturity (e.g. Forkner et al. 2004), in most cases the opposite was found, for instance in *Lantana camara* (Bhakta and Ganjewala, 2009) and *Psidium guajava* (Nantitanon et al., 2010). In *Rosmarinus officinalis* leaves, the highest accumulation rate of RA was associated with the young stage of development (Del Baño et al., 2003). Accumulation of secondary metabolites in young leaves may be part of a defence strategy against biotic stresses (Mazid et al., 2011). It is known that RA has antibacterial, antiviral and insecticidal activity (Petersen and Simmonds, 2003; Regnault-Roger et al., 2004). Young leaves have a tender structure and high content of sugars and proteins, and thus they are more vulnerable to pests and pathogens than old leaves (Kouki and Manetas, 2002). Nitrogen fertilization is a major factor affecting basil yield (e.g. Adler et al., 1989; Biesiada and Kuś, 2010) and may influence the overall content and composition of essential oils (Zheljazkov, 2008). However, to our knowledge no paper has been published on the influence of N concentration and NH_4^+/NO_3^- ratio on the accumulation of RA in sweet basil grown hydroponically, apart from the work conducted by Nguyen and Niemeyer (2008). In our experiments, both biomass and RA production of sweet basil plants grown in floating raft system was strongly influenced by N nutrition. The reduction of NO_3^- concentration and the supply of NH_4^+ restricted shoot biomass accumulation while having opposite effects on both root growth (Table 1) and RA accumulation in plant tissues (Table 2; Fig. 1). The content of this metabolite increased in root and leaf tissues, especially in apical leaves, when the NO_3^- level in the nutrient solution was diminished; in contrast, RA content decreased in the presence of NH_4^+, at least in leaf tissues (Table 2). It is known that N deficiency inhibits shoot growth while stimulating root growth (Clarkson, 1985); this is an adaptive mechanism that enhances the plant's ability to absorb nutritive ions from the growing medium. Conversely, one of the main effects of NH_4^+ toxicity is a lower root/shoot ratio, although the opposite effect was observed in some species (Britto and Kronzucker, 2002). In our experiments, the supply of NH_4^+, even in the presence of NO_3^-, impaired considerably plant growth (Table 2). In contrast, other authors did not observe any significant difference in shoot FW (Tesi, 1995) or leaf DW (Adler et al., 1989) in sweet basil grown in peat-filled pots with salt fertilizers containing NO_3^- or NH_4^+. Nitrogen deficiency reduces photosynthesis and primary metabolism, thus biomass production, but it may have opposite effect on secondary metabolism. According to the C/N balance hypothesis (Bryant et al., 1983), the concentration of C-based

Table 1. The effect of nitrogen (NO_3^-) concentration or the NO_3^-/NH_4^+ molar ratio in the nutrient solution on the growth of sweet basil (*O. basilicum* L. cv. Genovese) grown hydroponically and harvested at full bloom seven weeks after transplanting. In experiment 2, total nitrogen concentration was 10.0 mol m^{-3}. Mean values of four replicates (± SE), each consisting of four plants. Values followed by different letters differed significantly at 5% level.

	Shoot length (cm)	Shoot FW (g $plant^{-1}$)	Shoot DW (g $plant^{-1}$)	Number of leaves	Leaf FW (g $plant^{-1}$)	Leaf DW (g $plant^{-1}$)	Root DW (g $plant^{-1}$)
				Experiment 1			
NO_3^- concentration (mol m^{-3})							
0.5	94.00 ± 1.92 a	70.64 ± 4.02 b	9.04 ± 0.65 b	47.58 ± 3.99 c	27.14 ± 1.99 c	2.96 ± 0.28 c	1.52 ± 0.11 a
5.0	94.67 ± 1.66 a	134.06 ± 16.74 a	14.33 ± 2.05 a	68.92 ± 6.27 b	42.67 ± 4.49 b	4.14 ± 0.46 b	0.71 ± 0.13 b
10.0	92.92 ± 2.39 a	171.03 ± 16.49 a	18.01 ± 2.14 a	114.58 ± 9.14 a	62.83 ± 6.03 a	5.76 ± 0.78 a	0.71 ± 0.01 b
				Experiment 2			
NO_3^-/NH_4^+ ratio							
1:0	94.62 ±2.20 a	183.15 ± 15.17 a	19.04 ± 2.31 a	119.41 ± 8.90 a	64.09 ± 5.87 a	6.03 ± 0.81 a	0.80 ± 0.02 a
1:1	83.61 ± 2.90 b	112.98 ± 15.06 b	13.07 ± 1.53 b	76.61 ± 9.99 b	40.38 ± 5.34 b	4.16 ± 0.49 b	0.31 ± 0.01 b
0:1	72.91 ± 2.08 c	75.77 ± 10.00 c	9.98 ± 1.90 b	62.54 ± 10.48 b	31.63 ± 4.93 b	3.46 ± 0.63 b	0.17 ± 0.01 b

Table 2. The effect of nitrogen (NO_3^-) concentration or the NO_3^-/NH_4^+ molar ratio in the nutrient solution on the content of rosmarinic acid (RA) in sweet basil (*O. basilicum* L. cv. Genovese) grown hydroponically and harvested at full bloom seven weeks after transplanting. In experiment 2, total nitrogen concentration was 10.0 mol m^{-3}. Mean values of four replicates (± SE), each consisting of four plants. The effects of N concentration or the NO_3^-/NH_4^+ ratio, plant organ and their interaction were significant at $P \leq 0.01$ (**) or $P \leq 0.001$ (***) according to ANOVA. Values followed by different letters differed significantly at 5% level.

	RA content (g kg^{-1} DW)					
	Experiment 1					
NO_3^- concentration (mol m^{-3})	Roots	Stems	Inflorescences	Leaves	Significance	
0.5	72.84 ± 2.15 a	20.19 ± 3.00 d	36.72 ± 4.46 c	61.75 ± 7.03 a	N concentration (A)	***
5.0	47.02 ± 2.78 b	20.60 ± 6.34 d	45.77 ± 1.87 b	45.25 ± 4.41 b	Organ type (B)	***
10.0	12.58 ± 2.07 e	14.09 ± 4.49 e	43.25 ± 0.39 b	33.13 ± 5.30 c	A x B	**
	Experiment 2					
NO_3^-/NH_4^+ ratio	Roots	Stems	Inflorescences	Leaves	Significance	
1:0	13.34 ± 2.14 d	15.04 ± 4.52 d	43.35 ± 0.50 a	35.12 ± 5.30 b	NO_3^-/NH_4^+ ratio (A)	n.s.
1:1	17.52 ± 2.55 d	12.21 ± 0.75 d	41.09 ± 1.52 a	25.12 ± 2.73 c	Organ type (B)	***
0:1	17.54 ± 2.49 d	14.09 ± 4.33 d	43.29 ± 0.39 a	17.13 ± 1.73 d	A x B	**

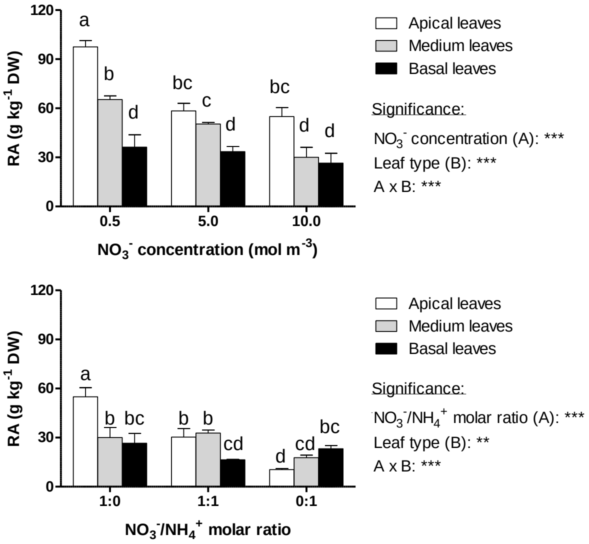

Fig 1. The effect of nitrogen (NO_3^-) concentration (experiment 1; top) and the NO_3^-/NH_4^+ molar ratio (experiment 2; bottom) in the nutrient solution on the content of rosmarinic acid (RA) in different types of leaves in sweet basil (*O. basilicum* L. cv. Genovese) grown hydroponically and harvested at full bloom, after seven weeks from transplanting. In experiment 2, total nitrogen concentration was 10.0 mol m^{-3}. Mean values (± SE) of four replicates, each consisting of four plants. The effects of N concentration or the NO_3^-/NH_4^+ ratio, leaf type and their interaction were significant at $P \leq 0.01$ (**) or $P \leq 0.001$ (***) according to ANOVA. Values followed by different letters differed significantly at 5% level.

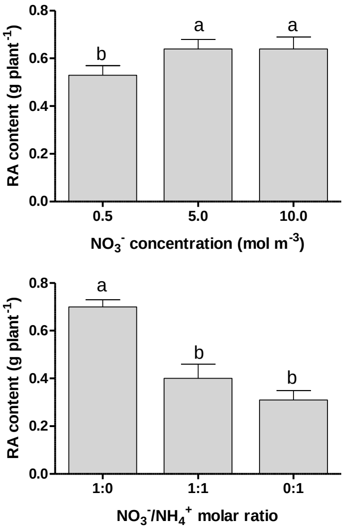

Fig 2. The effect of nitrogen (NO_3^-) concentration (experiment 1; top) and the NO_3^-/NH_4^+ molar ratio (experiment 2; bottom) in the nutrient solution on the total content of rosmarinic acid (RA) in sweet basil (*O. basilicum* L. cv. Genovese) grown hydroponically and harvested at full bloom, after seven weeks from transplanting. In experiment 2, total nitrogen concentration was 10.0 mol m^{-3}. Mean values (± SE) of four replicates, each consisting of four plants. The effects of N concentration and the NO_3^-/NH_4^+ ratio were significant at $P \leq 0.01$ (**) or $P \leq 0.001$ (***) according to ANOVA. Values followed by different letters differed significantly at 5% level.

secondary metabolites like phenolics are positively correlated to the C/N ratio within the plant. A limitation of N supply restricts growth more than photosynthesis, thus resulting in excess carbohydrates that are in part allocated to C-based secondary metabolites, such as phenolics. These compounds play a role in the plant's adaptation to nutrient starvation. Nitrogen deficiency enhances the formation of reactive oxygen species (ROS; Kováčik et al., 2007), which cause several damages to cellular components (Gill and Tuteja, 2010). Plants have evolved a protective mechanism against ROS based on enzymes and antioxidant compounds, such as phenolic compounds (Gill and Tuteja, 2010). An increase in the production of phenolics was observed in N-deficient plants (e.g. barley, Mercure et al., 2004; chamomile, Kováčik et al., 2007). In agreement with our results, an increase in leaf RA content of sweet basil grown under limited N availability was reported by Nguyen and Niemeyer (2008). Rosmarinic acid is a phenylpropanoid with a strong antioxidant activity (Petersen and Simmonds, 2003). A set of genes involved in the early steps of the phenylpropanoid pathway, including phenylalanine ammonia-lyase (PAL), 4-coumarate:CoA ligase (4CL) and cinnamate 4-hydroxylase (C4H), was strongly induced under N starvation (Fritz et al., 2006). Excess NH_4^+ may have several negative effects on plants, although the biochemical mechanisms of NH_4^+ toxicity remain to be further elucidated (Britto and Kronzucker, 2002). Ammonium may alter intracellular pH gradients (Dixon and Paiva, 1995) and this may affect many metabolic pathways including phenylpropanoid biosynthesis. In sweet basil irrigated with a nutrient solution containing 10.0 mol m^{-3} NH_4^+, the total content of essential oil was markedly reduced as compared to the plants fed with NO_3^- (Adler et al., 1989). The application of NH_4^+ reduced the content of phenolics in leaf and/or root tissues in some species such as *Zea mays* (Vuletić et al., 2010) and *Pisum sativum* (Domìnguez-Valdivia et al., 2008). Montanari et. al. (2008) found that feeding hydroponically-grown *Echinacea angustifolia* plants with both NO_3^- and NH_4^+ reduced significantly the accumulation of some CADs in root tissues as compared to the plants fed only with NO_3^-; this decrease was associated to a reduction of PAL activity. A reduction of the NO_3^-/NH_4^+ ratio in the growing medium decreased the production of RA in *Lavandula vera* cell suspension culture (Pavlov et al., 2000), in agreement with our findings. In addition, NO_3^- was more necessary than NH_4^+ for growth and phenolics accumulation in adventitious roots of *Echinacea angustifolia* grown *in vitro* (Wu et al., 2006).

Materials and Methods

Plant material and growing conditions

Two experiments were conducted from early April to late July of 2009 at the University of Pisa (Pisa, Italy; latitude 43°43'N, longitude 10°23'E) under the typical climatic conditions of Mediterranean regions. Sweet basil seeds (cv. Genovese) were germinated in rockwool tray plugs in a growth chamber (25 ± 1°C; 250 µmol s^{-1} m^{-2} photosynthetic photon flux density (PPFD); 12 h photoperiod) and, at the second leaf stage, the seedlings were transferred to a glasshouse. Two weeks after sowing, the plants were transferred into 12 separate hydroponic systems, each consisting of a polystyrene plug tray floating in a 60-L plastic tank with stagnant nutrient solution. The solution was continuously aerated in order to maintain an oxygen content higher than 6.0 g m^{-3}. Forty plants were planted in each tank; crop density was approximately 160 plants m^{-2} (on a ground area basis). Climatic parameters were continuously monitored

Table 3. Ion concentration and electrical conductivity (EC) of the nutrient solutions used in the experiments conducted with sweet basil (*O. basilicum* L. cv. Genovese) grown hydroponically. The composition of tap water is also reported. The nutrient solution also contained the following concentrations of trace elements: 40.6 mmol m^{-3} Fe; 35.0 mmol m^{-3} B; 3.6 mmol m^{-3}, Cu; 4.6 mmol m^{-3}, Zn; 10.9 mmol m^{-3} Mn; 1.0 mmol m^{-3} Mo.

Parameter	Tap water	N-NO_3^- concentration (mol m^{-3})			NO_3^-/ NH_4^+ molar ratio		
		0.5	5.0	10.0	1:0	1:1	0:1
N-NO_3^- (mol m^{-3})	0.0	10.0	5.0	0.5	10.0	5.0	0.0
S-SO_4^{2-} (mol m^{-3})	1.0	3.4	3.4	5.7	3.4	5.4	10.4
P-H_2PO_4 (mol m^{-3})	0.0	1.0	1.0	1.0	1.0	1.0	1.0
Cl^- (mol m^{-3})	2.0	3.4	8.4	9.5	9.9	11.9	9.4
HCO_3^- (mol m^{-3})	4.0	1.2	1.2	1.2	1.2	1.2	1.2
N-NH_4^+ (mol m^{-3})	0.0	0.0	0.0	0.0	0.0	5.0	10.0
K^+ (mol m^{-3})	0.0	10.0	10.0	10.0	10.0	10.0	10.0
Ca^{2+} (mol m^{-3})	1.8	3.0	3.0	3.0	3.0	3.0	3.0
Mg^{2+} (mol m^{-3})	0.5	1.5	1.5	1.5	1.5	1.5	1.5
Na^+ (mol m^{-3})	3.4	3.4	3.4	4.5	9.9	5.9	3.4
Total conc (mol m^{-3})	12.7	36.9	36.9	36.9	49.9	49.9	49.9
EC (dS m^{-1})	0.95	2.32	2.32	2.42	2.94	3.03	3.27

by a weather station located inside the glasshouse. The minimum and ventilation air temperature were 16 and 27°C, respectively; maximum temperature reached up to 30–32°C in sunny hours. Daily global radiation and mean air temperature averaged, respectively, 12.3 MJ m^{-2} and 24.8°C.

Nutrient solutions

In experiment 1, three NO_3^- concentrations were tested: 0.5, 5.0 and 10.0 mol m^{-3}. In experiment 2, three NO_3^-/NH_4^+ molar ratios were compared with total N concentration of 10.0 mol m^{-3}: 1:0, 1:1 and 0:1. Electrical conductivity and the concentrations of macronutrients, HCO_3^-, Na^+ and Cl^- in all the nutrient solutions are reported in Table 3. The nutrient solutions were prepared by dissolving in tap water appropriate amounts of technical-grade inorganic salts [$CaCl_2$, $Ca(NO_3)_2$, KCl, K_2SO_4, KNO_3, KH_2PO_4, $(NH_4)_2SO_4$, NH_4NO_3 and $MgSO_4$] plus chelated trace elements. The pH of the nutrient solutions was adjusted to 6.0 with diluted H_2SO_4. In order to compare iso-osmotic nutrient solutions, in Experiment 2 the total ion concentration was adjusted to 49.9 mol m^{-3} by adding small amounts of NaCl to the nutrient solutions containing NO_3^-. Electrical conductance, which depends on the sum of valences (Sonneveld and Voogt, 2009), differed slightly among the nutrient solutions, in particular in Experiment 2 (Table 3). The nutrient solution in each tank was checked for pH and EC almost daily and completely replaced every five or six days. In most cases, the exhausted nutrient solutions were analysed for the concentration of macronutrients.

Determinations

Sweet basil plants were sampled for growth determinations and laboratory analysis at bloom stage seven weeks after transplanting. Fresh and dry (after oven drying at 80°C till constant weight) mass of leaves, stems (including inflorescences) and roots were measured. The contents of RA and other CADs (caffeic acid, caftaric acid, chlorogenic acid, cicoric acid, cynarin, ferulic acid, *t*-cinnamic acid, *p*-coumaric acid) were determined in different organs: leaves, stems, inflorescences and roots. Each sample consisted of all the organs collected from four individual plants. Leaf RA content was also measured in the leaves detached from the lower, middle or upper third of the plants. All samples were rapidly washed in tap water, rinsed in deionised water, gently dried with a towel, frozen in liquid nitrogen and stored at -80°C before analyses, which were performed within a few weeks after sampling. The samples were not dried, as desiccation prior to extraction was found the reduce markedly the RA content of sweet basil tissues (Kiferle et al., 2011). The concentrations of RA and selected CADs in HCl-methanol extracts were determined by means of HPLC, as previously described (Kiferle et al., 2011) and expressed per kg dry weight (DW) on the basis of the dry matter content determined in a sub-sample from each sample. Peak identification was accomplished by LC-MS and LC-MS-MS, as previously reported (Kiferle et al., 2011). The detection limit of the analytical method was 0.05 g kg^{-1} DW. The total amount of RA produced by each individual plant was calculated on the basis of the dry mass of roots, stems, inflorescences and leaves and their RA content. Leaves were also sampled for the determination of N content in dry tissues (Kjeldahl method) and chlorophyll in fresh samples extracted with methanol soon after sampling (Lichtenthaler, 1987).

Statistical analysis

The experimental design was completely randomized with four replicates. Data were subjected to analysis of variance (ANOVA) and the mean values were compared using LSD test at 5% probability level. Statistical analysis was performed with Statgraphics Centurion XV.II (Manugistic Co., Rockville, Maryland, U.S.A.) software. Each experiment was repeated twice with similar findings; the paper reports the results from a representative run.

Conclusions

These findings suggest the potential of greenhouse hydroponic culture of sweet basil for agro-industrial production of RA, as a large amount of biomass with a high concentration of this compound (up to 10% DW in apical leaves) could be produced in a few weeks. A total N concentration of 5.0 mol m^{-3} (as NO_3^-) in the nutrient solution resulted in large biomass production and satisfactory RA content. The plants grown with 5.0 mol m^{-3} NO_3^- contained more RA in root and leaf tissues than those grown with higher NO_3^- concentration. This suggests that the standard N concentration used in hydroponic culture (10.0 mol m^{-3} or higher) could be significantly reduced, with important implications from the environmental point of view. Ammonium, as the only N form or in the presence of NO_3^-, was detrimental to both growth and RA accumulation (especially in apical leaves) and its use should be avoided in the hydroponic culture of sweet basil.

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