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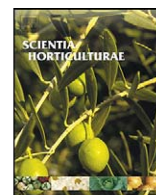

Effects of nutrient solution electrical conductivity and sulfur, magnesium, and phosphorus concentration on sesquiterpene lactones in hydroponically grown lettuce (*Lactuca sativa* L.)

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ABSTRACT

The effect of nutrient solution electrical conductivity (EC) and sulfur (S), magnesium (Mg), and phosphorus (P) levels on the content of the primary sesquiterpene lactones (SLs), lactucin, 8-deoxylactucin, and lactucopicrin, in hydroponically grown lettuce was assessed. Lettuce grown at 4 EC levels (0.5, 1.0, 2.0, and 3.0 dS m^{-1}) displayed significant differences in leaf area index, number of leaves, plant height, fresh weight per plant, and chlorophyll content that were highest at EC 2.0 dS m^{-1}. Lactucin (5.5 $\mu g\ g^{-1}$ dry weight), 8-deoxylactucin (7.5), lactucopicrin (35.8), and total SLs (48.7) concentraions were highest at EC 0.5 dS m^{-1}. Four S (8, 16, 48, and 80 mg L^{-1}) and Mg (6, 12, 36, and 60 mg L^{-1}) levels and 3 P (8, 16, and 48 mg L^{-1}) levels were assessed for their effect on individual and total SLs. S and P had the greatest effect on SL levels. Plants in the lowest S level had significantly higher lactucin, lactucopicrin and total SLs. Each of the SLs was higher in the highest P level while Mg influenced only the lactucopicrin level in a quadratic manner. The results indicate that solution culture conditions can strongly influence the SL concentration and therefore bitterness and acceptability of lettuce.

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1. Introduction

Sesquiterpene lactones (SLs), found primarily in the Asteraceae family, are colorless, lipophilic, relatively stable, and often bitter (Price et al., 1990; Merfort, 2002). Various SLs have been shown to display anti-inflammatory, anti-tumoral, anti-microbial, anthelmintic, and anti-feeding effects (Rodriguez et al., 1976; Merfort, 2002). In addition to their biological and therapeutic attributes, when the concentration is sufficiently high, their bitterness has a negative impact on the taste of chicory (*Cichorium intybus* L.) and lettuce (*Lactuca sativa* L.), affecting consumer purchasing decisions (Tamaki et al., 1995; Park and Lee, 2006). Lactucin, 8-deoxylactucin, and lactucopicrin are the primary bitter SLs in chicory and lettuce (Price et al., 1990; Van Beek et al., 1990).

Genetic and environmental factors affect the concentration of lactucin, 8-deoxylactucin, and lactucopicrin in both species. Assessment of 25 lettuce and 6 chicory cultivars demonstrated a wide range in the concentration of each of the 3 compounds. In general, chicory had higher SL contents than lettuce cultivars (Price et al., 1990). In Korean leaf lettuce cultivars, the total concentration of SLs ranged from 14.6 to 67.7 $\mu g\ g^{-1}$ dry weight, with lactucopicrin being the primary contributor to bitterness due to its higher concentration and lower bitterness threshold (Seo et al., 2009). Variation in the concentrations of the SLs in chicory cultivars grown in different production locations demonstrated that location can significantly affect concentration (Peters et al., 1997; Foster et al., 2006). The SL composition of chicory also varied within cultivars due to temperature, harvest date, and production year (Foster et al., 2006). Nitrogen fertilization rate can also significantly affect the concentrations of lactucopicrin and lactucin-like SLs (Peters et al., 1997).

Nutrient solution electrical conductivity (EC) is known to affect the content and composition of the essential oils in sweet basil (*Ocimum basilicum* L.) which are mainly comprised of mono- and sesquiterpenes (Lee et al., 1993; Carrasco et al., 2007). For example, basil grown in a 1.5 dS m^{-1} nutrient solution had a higher essential oil yield than when grown at higher EC levels (i.e., 3.0 and 4.5 dS m^{-1}). In hydroponically grown sweet basil, the essential oil content on a fresh weight basis decreased as EC increased. Macronutrients are also known to modulate the synthesis of

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essential oils. For example, the essential oil content of sweet basil, purple basil (*O. basilicum purpurascens*), and bush basil (*O. minimum*) increased with increasing S and Mg concentration in the nutrient solution (Suh and Park, 1999a,b) and elevated P fertilization increased the essential oil, camphor and chrysanthenyl-acetate concentrations in feverfew [*Tanacetum parthenium* (L.) Sch. Bip.] (Saharkhiz et al., 2007). Although the effect of EC and certain macronutrients on the synthesis of essential oils has been studied in some aromatic species, little information is available on their effects on the SLs in lettuce. As a consequence, the objective of this study was to determine the effects of EC and the macronutrients S, Mg, and P on the SL composition and content of hydroponically grown lettuce.

2. Materials and methods

2.1. Plant materials and growth conditions

The leaf lettuce cultivar 'Taepung-yeoleum-jeokchukmyeon' (Kwonnong seed Co., Cheongjoo, Korea) was seeded on a polyurethane sponge in the Gyeonggi-do Agricultural Research and Extension Services polyethylene-covered-greenhouses (36°N, 126°E), Hwasung, Republic of Korea on 5 July 2001. The air temperature and relative humidity (RH) were maintained at 29.9 ± 2.5 °C and 76.9 ± 10.3%. Average outside solar radiation was 17.8 ± 6.6 MJ m^{-2} d^{-1}. Seedlings were grown for 20 days using 0.5 strength Yamazaki nutrient solution for lettuce and then transplanted on July 25 into a deep flow culture system at a spacing of 15 cm × 8 cm. The system had 50 L tanks (85 cm × 40 cm × 35 cm deep). To investigate effects of EC on SLs, 4 EC levels were used, i.e., EC 0.5 (0.51 ± 0.01 dS m^{-1}), 1.0 (0.99 ± 0.01 dS m^{-1}), 2.0 (1.98 ± 0.02 dS m^{-1}), and 3.0 (2.97 ± 0.05 dS m^{-1}). The 4 different EC levels were adjusted mixing a 100 times concentrated Yamazaki stock solution with tap water (EC < 0.1 dS m^{-1}). The basal concentrations of macronutrients (NO_3^-, NH_4^+, PO_4^{3-}, K^+, Ca^{2+}, Mg^{2+}, and SO_4^{2-}) were 6.0, 0.5, 0.5, 4.0, 1.0, 0.5, and 0.5 mmol L^{-1} and micronutrients (Fe, B, Mn, Zn, Cu, and Mo) 35, 18, 3.6, 0.3, 0.2, and 0.1 μmol L^{-1}, respectively (Yamazaki, 1982). During growth, the EC of each nutrient solution was measured daily and the nutrient solution replaced weekly, resulting in remaining relatively consistent through harvest with the exception of the EC 3.0 (3.0 dS m^{-1}) treatment that began increasing 5 days before harvest, reaching 3.97 dS m^{-1} at harvest. All samples for SL analysis were harvested on 23 August with the samples randomly selected from within treatments that were arranged in a completely randomized block design with 3 replication of 13 plants. The harvested samples were mixed in a tank, placed in vinyl freezer bags, and frozen (−80 °C). The frozen samples were lyophilized for 48 h using a vacuum freeze drier (PVTFD100A, Ilshin Lab Co., Yangju, Korea), ground to a fine powder using a mortar and pestle, and held at −80 °C until analysis. Chlorophyll contents were estimated with SPAD unit (leaf chlorophyll index) obtained using a SPAD-502 chlorophyll meter (Konica Minolta Sensing Inc., Osaka, Japan).

To investigate effects of each S, Mg, and P on SLs, modified Yamazaki nutrient solutions with different S (8, 16, 48, and 80 mg L^{-1}), Mg (6, 12, 36, and 60 mg L^{-1}), and P (8, 16, and 48 mg L^{-1}) concentrations were individually prepared (Table 1). $MgSO_4 \cdot 7H_2O$ and K_2SO_4 were used for adjusting the S concentration, $MgSO_4 \cdot 7H_2O$ and $MgCl_2 \cdot 6H_2O$ for adjusting the Mg concentration, and $NH_4H_2PO_4$, K_2HPO_4, and KH_2PO_4 for adjusting the P concentration. The EC values of modified solution for S, Mg, and P were as followed: S [8 (EC = 1.21 ± 0.01 dS m^{-1}), 16 (1.22 ± 0.01 dS m^{-1}), 48 (1.45 ± 0.02 dS m^{-1}), and 80 (1.82 ± 0.02 dS m^{-1}) mg L^{-1}], Mg [6 (EC = 1.22 ± 0.01 dS m^{-1}), 12 (1.13 ± 0.01 dS m^{-1}), 36 (1.37 ± 0.01 dS m^{-1}), and 60 (1.58 ± 0.01 dS m^{-1}) mg L^{-1}], and P [8 (EC = 1.21 ± 0.06 dS m^{-1}), 16 (1.20 ± 0.03 dS m^{-1}), and 48 (1.35 ± 0.07 dS m^{-1}) mg L^{-1}]. The harvest date and procedures for SL analysis were the same as the experiment for investigating effect of EC on SLs.

2.2. Isolation of sesquiterpene lactones

The samples were analyzed using the method of Price et al. (1990) where powdered lyophilized aliquots (1 g) were extracted with 100 mL of methanol by boiling under reflux at 65 °C for 1 h and filtering through Whatman #2 filter paper. The methanol was evaporated under reduced pressure in a rotary evaporator (ca. 5 mm Hg, 30–35 °C). The sesquiterpene lactone santonin (50 μg) was added as an internal standard to the crude extract due to its similar structure ($C_{15}H_{18}O_3$) and molecular weight (M.W. 246.29) to two of the SLs [lactucin ($C_{15}H_{16}O_5$, 276.3), 8-deoxylactucin ($C_{15}H_{16}O_4$, 260.3), and lactucopicrin ($C_{23}H_{22}O_7$, 410)]. The extract was then partitioned three times between water/chloroform (200 mL; 1:1 mixture by volume), with the chloroform separated, dried over anhydrous magnesium sulfate, and evaporated using a rotary evaporator (ca. 5 mm Hg, 20–30 °C). The residue was dissolved in 0.5 mL methanol/chloroform (1:2 by volume) and separated using high-performance liquid chromatography (HPLC).

2.3. HPLC analysis

The SLs were separated using a HP 1100 series HPLC system (Agilent Technologies, Palo Alto, CA) equipped with an AD 25 Absorbance Detector (Dionex Co., Sunnyvale, CA). A Luna C_{18} column (250 mm × 4.6 mm i.d., 5 μm particles; Phenomenex Co.,

Table 1
Composition of modified Yamazaki nutrient solution with different S, Mg, and P levels.

	mg L^{-1}									
	KNO_3	$Ca(NO_3)_2 \cdot 4H_2O$	$NH_4H_2PO_4$	$MgSO_4 \cdot 7H_2O$	K_2SO_4	$Mg(NO_3)_2$	$MgCl_2 \cdot 6H_2O$	NH_4Cl	K_2HPO_4	KH_2PO_4
S level (mg L^{-1})										
8	404.4	236.0	57.6	66.0	–	64.0	–	–	–	–
16	404.4	236.0	57.6	123.2	–	–	–	–	–	–
48	404.4	236.0	57.6	123.2	174.4	–	–	–	–	–
80	404.4	236.0	57.6	123.2	348.8	–	–	–	–	–
Mg level (mg L^{-1})										
6	404.4	236.0	57.6	61.6	43.6	–	–	–	–	–
12	404.4	236.0	57.6	123.2	–	–	–	–	–	–
36	404.4	236.0	57.6	123.2	–	–	202.8	–	–	–
60	404.4	236.0	57.6	123.2	–	–	406.0	–	–	–
P level (mg L^{-1})										
8	404.4	236.0	28.8	123.2	–	–	–	13.2	–	–
16	404.4	236.0	57.6	123.2	–	–	–	–	–	–
48	404.4	236.0	57.6	123.2	–	–	–	–	116.0	45.2

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