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Influence of intermittent-direct-electric-current (IDC) on phytochemical compounds in garden cress during growth

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ABSTRACT

The influence of different intensities of applied intermittent-direct-electric-current on the levels of phytochemical compounds in garden cress sprouts was determined. One-week-old treated and non-treated plants were harvested and used to analyse the total phenol content and associated antioxidant activity. The contents of chlorophyll, proteins, in addition to specific elements of macro- and micronutrients, as well as heavy metals, were supplementary determined in order to explain changes in secondary metabolism of the treated garden cress. The results of this study showed that, in contrast to other abiotic elicitors, weak levels of IDC can be applied to promote the biosynthesis of chlorophyll, proteins, and phenolics. An IDC of 1400 mA was effective enough to significantly maximise the total phenol content and other biosynthetic products in garden cress, revealing no signs of damage. The accumulation of heavy metals had no toxic effects in garden cress and did not exceed the legal regulations for human consumption.

1. Introduction

There is much epidemiological evidence that diets rich in fruit and vegetables, e.g. garden cress, have a wide range of positive physiological effects on human health. In Europe and the USA garden cress is mostly consumed as a vegetable or fresh herb. The young sprouts are ready for marketing 7 days after sowing and are commonly used raw as an ingredient of salads or as refinement of soups (Gokavi, Malleshi, & Guo, 2004). Various beneficial effects seem to be associated with the consumption of garden cress, such as prevention for dysentery and diarrhoea (Kirtikar & Basu, 1981). Furthermore, the reduction of the incidence of diseases such as prostate cancer, cardiovascular diseases, and diabetes has been proven in various studies and is widely attributed to secondary metabolites, e.g. phenolic compounds (Nunez Selles et al., 2002; Schijlen, de Vos, van Tunen, & Bovy, 2004; Steinbrecher, Nimptsch, Husing, Rohrmann, & Linseisen, 2009). Considerable amounts of phenolic compounds, e.g. sinapoylglucose (53.1 mg/100 g fresh matter) (Hermann, 2001) have also been detected in garden cress. The beneficial effects of phenolic compounds on human health are ascribed to their antioxidant activity, i.e. the ability to scavenge oxygen radicals and other reactive species depending on their chemical structures. On the other side, the secondary plant metabolites have the same functions in respect to the interaction of plants with their environment and are involved in the regulation of stress situations (Schreiner, 2005). The accumulation of specific proteins and compounds such as plant hormones, reactive oxygen species (ROS) or phenolic compounds are a common response to a variety of biotic or abiotic stressors, e.g. fungi or UV-B irradiation (Josuttis et al., 2010; Padmavati, Sakthivel, Thara, & Reddy, 1997).

To date, numerous research projects have examined various methods to enhance the contents of secondary metabolites in plant cells and intact plants. The screening and selection of cell lines is one of these methods. A major problem with this set up is the genetic modification by mutation or epigenetic changes due to the physiological conditions of the culture, which can lead to reduced productivity (Dornenburg & Knorr, 1995). The use of electricity is another method to influence the secondary plant metabolism. Zhang and Hashinaga (1997) have demonstrated that high electrical fields with a frequency of 60 Hz and intensities from 18 to 105 kV × m^{-1} shortened the mean days of germination for some vegetable seeds. However, an applied pulsed electric field (PEF) above 30 kV × cm^{-1} was used to obtain high content of bioactive compounds in functional food ingredients, e.g. lycopene content of tomato juice (Odriozola-Serrano, Aguilo-Aguayo, Soliva-Fortuny,

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Gimeno-Ano, & Martiin-Belloso, 2007). In contrast, Ward (1996) has found that an applied weak direct electric current (6 V) using wires as electrodes increased the total dry weight and the uptake of magnesium, calcium, and nitrogen of tomatoes grown in a greenhouse. Nevertheless, information on changes in secondary plant compounds in greenhouse crops as affected by weak electric currents is scant. Recently Dannehl, Huyskens-Keil, Eichholz, Ulrichs, and Schmidt (2009) applied different intensities of IDC to radish plants during growth and demonstrated that these treatments increased the phenolic compounds in radish tubers as well as in roots without visible damages to the plants. The radish leaves, which were not in the electrical current flow, remained unaffected. Therefore, in the present study different intensities of IDC were applied to seedlings of garden cress during the initial growth phase using the same experimental set-up described by Dannehl et al. (2009), in order to enhance the level of phytochemical compounds in the above-ground plant segments. The total phenol content and the antioxidant activity in garden cress sprouts were determined. Furthermore, the contents of chlorophyll, proteins, and the content of specific elements of macro- and micronutrients were analysed, in order to explain the change in secondary metabolism of phenolic compounds in the treated plants. Additionally, the content of heavy metals in garden cress was determined, in order to examine a possible accumulation of toxic elements by IDC, which could be caused by electrolysis of the used metal electrodes.

2. Materials and methods

2.1. Experimental set-up and plant material

Investigations of the influence of IDC on phytochemical compounds in garden cress during growth were conducted in a climate chamber. The climate conditions were maintained constant at a temperature of 22.5 °C and the relative humidity was set at 70% during the experimental phase. Five high pressure sodium lamps (Philips SON-T AGRO 400 W) were installed one meter above the crops and were used to establish constant light conditions with a light:dark cycle of 18:6 h, respectively. Two separate rows of rock wool cubes were used to cultivate the garden cress plants. Each row consisted of three cubes (1000 × 200 × 80 mm) placed in series and were separately positioned on a non-conductive U-shaped PVC-nutrient solution channel. In addition, two stainless steel-plates (EN-standard: X5CrNi18-10; ThyssenKrupp Nirosta GmbH; Krefeld, Germany) with the dimensions of 3000 × 80 × 1 mm were laterally attached to one row of cubes and acted as electrodes to which a laboratory power supply (Voltcraft, VLP 1303 pro; Hirschau, Germany) was connected. Three temperature sensors were positioned into each row and connected to a data analyser (Fluke, Hydra 2620A; Kassel, Germany). The temperature changes and the electric current were monitored and recorded with a computer programme, developed at the Humboldt-Universität zu Berlin.

All used rock wool cubes were thoroughly moistened with nutrient solution before sowing 8000 seeds per row and experiment. After sowing, two tunnel-shaped constructions (ethylene-tetrafluoroethylene foil) were placed over each row to protect the plants from evaporation. During the investigations, the cultivated plants were irrigated *via* drip irrigation 1 min/h and 18 times per day (Table 1). Preliminary experiments showed that the applied circulating nutrient solution represented a good electric conductor (Dannehl et al., 2009), so that the IDC passed horizontally through the cubes as well as through the roots of garden cress.

The experiment was performed with five treatments of different intensities of IDC (200, 600, 1000, 1400, and 1800 mA) and each treatment was repeated twice. During each test series, the respective intensity of IDC was applied to the test row for 1 h/day during the growth period of 7 days. The non-treated row with garden cress was used as a control and was cultivated parallel for the respective intensity of IDC.

2.2. Sampling and analysis

Fresh plant material of the respective IDC-treated and non-treated rows was collected 7 days after sowing, when the garden cress sprouts were ready for consumption. The sample material was randomly harvested from three sample collections per row. Three replications per sample collection were used for the analysis of each of the phytochemical compounds. One part of the harvested sprouts was immediately used for the determination of the dry matter ($n = 200$) and chlorophyll content ($n = 200$). The other part of the collected material ($n = 1000$) was shock-frozen with liquid nitrogen, kept at −20 °C, and afterwards freeze-dried for 48 h (Christ Alpha 1–4, Christ; Osterode, Germany). The freeze-dried samples were ground and mixed to a fine homogenised powder and stored in a desiccator until further analysis of total phenol content, antioxidant activity, protein content, the contents of macro- and micronutrients, and heavy metals.

2.2.1. Inductively coupled plasma-optical emission spectrometry (ICP-OES) analysis

For the microwave digestion, 0.2 g of each freeze dried sample was weighed into specific deionized containers. After the addition of 5 ml HNO_3 (65%) and 3 ml H_2O_2 (30%), the containers were packed and placed into a microwave (MARS Xpress, CEM; North Carolina, USA) and digested according to the following programme: step 1, 20 min to reach 200 °C; step 2, 5 min at 200 °C; step 3, 1 min to reach 210 °C; step 4, 5 min at 210 °C; step 5, 1 min to reach 220 °C; step 6, 5 min at 220 °C; and step 7, 30 min to cool down. After cooling to room temperature, the resultant solution was transferred into 50 ml volumetric flasks using distilled water and finally filtrated into plastic flasks. Thereafter, the analysis of the elements in the digestion solution was conducted *via* ICP-OES with an ICP Emission Spectrometer (iCAP 6300 Duo MFC, Thermo; Waltham, USA). The operating conditions employed for ICP-OES were 1150 W RF power, 0.55 l/min nebulizer gas flow with argon employed as plasmogen as well as carrier gas and performed with a cross-flow nebulizer (MIRA MIST, Thermo Scientific; Cambridge England), in addition to radial (Ca, Mg) and axial (Fe, Cu, Al, Cd, Cr, Ni, Pb) view. For each element, a single-element standard solution (Roth, Karlsruhe, Germany) of 1000 mg/l was used to prepare the reference analytic solutions in 1.4 mol/l HNO_3. The calibration curves were established with the following reference solutions: blank 1.4 mol/l HNO_3; 1–200 mg/l of Ca; 0.5–50 mg/l of Mg; 0.5–10 mg/l of Al; 0.5–5 mg/l of Cu and Fe; 0.01–1 mg/l of Cd, Cr, Ni, and Pb. The respective element in the digestion solutions was analysed in duplicate at the following wavelength: Ca = 317.9 nm, Mg = 279.0 nm, Fe = 259.9 nm, Cu = 324.7 nm, Al = 369.1 nm, Cd = 214.4 nm, Cr = 267.7 nm, Ni = 231.6 nm and Pb = 220.3 nm. The contents of macro- and micronutrients, as well as heavy metals, in garden cress were expressed as gram per kilogram dry matter (g/kg DM).

2.2.2. Determination of carbon and nitrogen content

The carbon and nitrogen contents of the freeze-dried samples were analysed using an elemental analyser (vario MAX, Elementar Analysensysteme GmbH; Hanau, Germany) according to DIN-ISO-10694 (1995) and DIN-ISO-13878 (1998). Without pre-treatment, 0.3 g of the sample was weighed into individual crucibles and was catalytically combusted at 900 °C with pure oxygen. After this procedure, the combustion products and helium (as the carrier gas) passed through specific adsorption columns at a temperature of 830 °C to separate carbon and nitrogen. Based on the differences