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Aroma characterisation and UV elicitation of purple basil from different plant tissue cultures

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ABSTRACT

Exposure to stressful environmental conditions can induce severe metabolic variations in basil (*Ocimum basilicum*) aroma. The aromatic profiles of Dark Opal and Red Rubim varieties (*in vivo* plants, *in vitro* shoots, callus, and suspension cultures) were investigated for the first time. The established calli represented the most interesting miniaturised aromatic plant systems, as they were able to emit many typical basil volatiles with very low amounts of phenylpropanoids (1–2%). The hydrocarbon monoterpenes and oxygenated volatiles emitted from calli of both varieties were greatly and conversely affected by UV-C and UV-B, in comparison with the non-irradiated samples. As calli of both varieties still maintained very low levels of phenylpropanoids even after UV elicitation, they might be regarded not only as efficient *in vitro* plant models to study volatile compounds under UV stress conditions, but also as safe aromatic biomass in comparison with *in vivo* basil plants.

1. Introduction

Ocimum genus contains between 50 and 150 species of herbs and shrubs from the tropical regions of Asia, Africa, Central and South America (Lawrence, 1985; Lawrence, Hogg, Terhune, & Pichitakul, 1972). Sweet basil (*Ocimum basilicum*) is one of the most valuable aromatic plants. It is also used in folk medicine for its antiviral, antioxidant and antibacterial properties (Chiang, Ng, Cheng, Chiang, & Lin, 2005; Hussain, Anwar, Hussain, Syed, & Przybylski, 2008; Lawrence, 2001; Opalchenova & Obreshkova, 2003; Rattanachaikunsopon & Phumkhachorn, 2010; Simon, Quinn, & Murray, 1990). The interspecific hybridisation and polyploidy have often created taxonomic confusion within the genus. Moreover, taxonomy is complicated by the existence of chemotypes for both wild and cultivated basil, which do not differ significantly in morphology (Grayer et al., 1996; Lachowicz et al., 1997; Lawrence, 1985, 2001).

The variety of commercially exploited chemotypes (or chemocultivars) is so numerous that the characteristic basil aroma and taste are determined by a pool of several compounds and not by a single one (Grayer et al., 1996; Klimánková et al., 2008; Margotti, Piccaglia, & Giovanelli, 1996; Simon et al., 1990).

Regarding *in vitro* basil tissue cultures, Banthorpe, Branch, Njart, Osborne, and Watson (1986) evaluated the accumulation of terpenoids in different culture cell systems of *O. basilicum* and the liquid cultures showed inferior values, due to the undetectable terpenoid accumulation into the cells or to the direct secretion of volatiles into the medium.

Purohit and Khanna (1983) studied the production of essential oil related specifically to linalool and methylchavicol in *O. basilicum* callus. Other studies have been already performed also on the effects of UV irradiation on the volatiles emitted from *in vivo* basil plants. Johnson, Kirby, Naxakis, and Pearson (1999) reported that UV-B not only changed the total content but also the essential oil (EO) composition. In contrast, other authors (Chang, Alderson, & Wright, 2009; Nitz & Schnitzler, 2004) reported that the total content of volatiles was significantly increased by UV-B, but there was no effect on their relative composition. In addition, Ioannidis, Bonner, and Johnson (2002) stated that neither the quality nor the quantity of the volatiles was affected by UV-B. However, UV exposure had a basic effect on the development of glandular trichomes, which strongly depended also on basil genotypes. UV irradiation below 260 nm (UV-C) is very rare in the environment because it is almost completely absorbed from the Earth's atmosphere. On the other hand, it is very effective as it interacts directly with DNA, resulting in high lethality against microbes (Cockell, 1998). In fact, the application of UV-C has been accepted especially for food, air, and water sterilisation since the mid-20th century.

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No reports have been concerned with plant volatile emissions under UV-C elicitation, even if it is well known that many plant metabolites such as flavonoids and phenolics have a typical UV-C absorbance and they are considered natural sunscreens (Markham, 1989; Shimazaki, Igarashi, & Kondo, 1988).

The present work represents an original investigation on the volatile fingerprint emitted from two basil purple varieties (Red Rubin (RR), Dark Opal (DO)) under different culture conditions and UV irradiations. In particular, the established RR and DO calli, which resulted in significantly enriched basil-type volatiles, were studied after UV-B and UV-C exposure, to study the influence of this elicitation process on the emission of basil volatiles under specific *in vitro* condition.

2. Materials and methods

2.1. In vivo young plants (YP)

The seeds of Basil (*O. basilicum* L.) cultivars "Red Rubin" (RR) and "Dark Opal" (DO), supplied by a commercial seed producer (SAIS S.p.A, Cesena, Italy), were sown in pots in a greenhouse at Albenga (Liguria Region, Italy). The *in vivo* young plants (YP) were obtained from seedlings grown in a greenhouse for 20 days and used as a control to compare their EO composition with that of established shoots, callus, and suspension cultures.

2.2. In vitro shoots, callus, and suspension cultures

The seeds of Basil (*O. basilicum* L.), cultivar "Red Rubin" (RR) and "Dark Opal" (DO), above described were transferred to a water solution of 15% sodium hypochlorite with two drops of Tween-20 with continuous shaking for 10 min; then they were rinsed three times with sterile distiled water.

After sterilisation, the seeds were transferred for germination on half-strength Linsmaier and Skoog (LS/2) basal medium (Linsmaier & Skoog, 1965) supplemented with 15 g/l sucrose, 500 mg/l MES (4-morpholineethanesulfonic acid), adjusted to pH 5.8 and solidified with agar at 7.5 g/l. The medium was autoclaved at 121 °C, 1 atm for 20 min. The seeds were sown on the medium, which was previously dispensed into Petri dishes (five seeds/dish). After 10 days from root emergence, in order to induce the proliferation phase, the basil seedlings were excised, cutting off the roots from the hypocotyls and transplanted (nine explants/vessel) in Magenta GA3 vessels (Sigma–Aldrich, Italy) with a modified MS medium supplemented with 3 g/l sucrose, 300 mg/l reduced glutathione (GSH), 500 mg/l MES and 0.5 mg/l 6-benzylaminopurine (BA). This medium, named BP medium, was adjusted to pH 5.8 and solidified with agar at 7.5 g/l followed by autoclaving at 121 °C and 1 atm for 20 min. *In vitro* proliferated shoots (VS) were subcultured every 4 weeks on the same medium. In a second instance, in order to stimulate callus productions, 0.5 cm^2 leaf fragments (three pieces/dish) from the *in vitro* proliferating seedlings of RR and DO cultivars were transferred to Petri dishes (∅ 60 mm) containing a medium named BP+ based on the previous described BP composition but with BA and 3-indolebutyric acid (IBA) at 1 mg/l. The callus was repeatedly subcultured to establish stock cultures. These stock callus cultures were employed to set up the UV experiments and to start the suspension cultures by the same BP+ medium. For this purpose, the liquid substrate (10 ml) was dispensed in 100-ml Erlenmeyer flasks. The initial callus inocula (0.52 g/flask) were partially fragmented and placed in the flasks, which were sealed with sterile aluminium foil. The flasks were placed on an orbital shaker (IKA® KS 260 Basic; IKA Werke GmbH & Co. KG, Staufen, Germany) at 100 rpm. At 4-week intervals the liquid cultures were filtered and subcultured in the fresh medium. All *in vitro* cultivation phases were performed in a growing chamber at 21 °C, under 16 h photoperiod provided by fluorescent lamps (80 μmol m^{-2} s^{-1}).

2.3. UV irradiation experiments on callus of RR and DO varieties

The DO and RR calli at the 4th week of culture on BP+ medium, were subjected to UV treatment. The UV-B irradiation was obtained from two Philips TL 12W/40 fluorescent tubes spaced out 40 cm from the Petri dishes containing the plant tissues. The spectrum emission of this kind of lamp (Fig. 1) has λ from 280 to 400 nm, resulting in a maximum at 315 nm, typical for UV-B emission.

The lamp emission was fractionated employing cut-off filters WG (Scott, Mainz, Germany): the UV-C component was removed using cut-off filters WG 280, while UV-B from 280 to 305 nm was removed by cut-off filters WG 305, leaving the main UV-B component of the lamps. The irradiance, i.e. the instantaneous intensity of UV incident on a surface, was 5 W m^{-2} s, and was measured by a radiometer placed under the cut-off filters. The exposure lasted 3 or 6 h.

The UV-C irradiation was performed using a germicidal apparatus (Germigima Plus; GIMA, Milan, Italy) equipped with mercury vapour lamp 8 W (model G8T5, Sylvania; predominant wavelength = 254 nm). The irradiance was of 20 W m^{-2} s and the exposure lasted 3 or 6 h. The lids of the plastic Petri dishes were removed and replaced directly with the sterilised cut-off filters or, for the UV-C experiment, the open dishes were passed directly under the germicidal lamps. Four dishes each with callus derived from stock cultures at the 4th week (three callus pieces per dish) for each different UV irradiation were studied. During the treatments, callus cultures were maintained in a growth chamber at 23 ± 1 °C. After the exposition treatments the Petri dishes were maintained under standard conditions in the growth chamber with a 16 h photoperiod (cool white fluorescent light, 70 μmol m^{-2} s^{-1}) for 24 h.

The control and threatened callus tissues were collected and maintained at −20 °C.

2.4. Extraction and analysis

The essential oils (EOs) from the fresh leaves (100 g) of RR and DO cultivars of *in vivo* plants for each sample obtained from 10 randomly collected YP and the *in vitro* shoots (5.0 g for each sample obtained from five Magenta vessels at the 4th week of culture, VS) were distiled by Clevenger-type apparatus (2 h).

The essential oils were dissolved in Et_2O, dried over anhydrous $MgSO_4$, filtered and the solvent removed by evaporation on a water bath.

The static headspace (HS) of *in vivo* plants (5.0 g for each sample obtained from 10 randomly collected YP) and *in vitro* plant material (2.0 g, for each sample obtained from five vessels of *in vitro* shoots, from three flasks of suspension cultures, and from four dishes of callus cultures) were determined by HS–SPME–GC–MS analysis (PDMS polydimethylsyloxane fibre, 100 μm).

Each plant aliquot was inserted separately into a glass conical flask and after the equilibration time (10 min), the fibre was exposed to the headspace sample (1 min) at room temperature and transferred directly to the injection port of the GC–MS system.

GC/EIMS analyses were performed using a Varian CP-3800 gas chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm; coating thickness 0.25 μm; Agilent, Santa Clara, CA) and connected to a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperatures 220 and 240 °C, respectively; oven temperature programmed from 60 to 240 °C at 3 °C/min; carrier gas helium at 1 μl/min; injection of 0.2 μl (10%, diluted in hexane); split ratio 1:30.