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Commercial advantages on basil architecture by ultraviolet-B irradiation

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Abstract: Sweet basil (*Ocimum basilicum* L.) is one of the most important herbs widely used for its medicinal properties and as food ingredient. The marketing of this product highlights the problem that these plants have long and slender stems, which are easy to break off and thus making difficult their market distribution. In this work, two cultivars of basil (Genovese and Profumo) at the adequate development stage for sale were used. We evaluated the effect of supplemental ultraviolet (UV)-B irradiation (15 W m^{-2}; 3 h day^{-1}) on plant growth and market quality. Both cultivars of basil plants under UV-B irradiation resulted in increased leaf size and biomass, and decreased shoot length in comparison to that of under control growth conditions. These results indicate that the application of UV-B irradiation beneficially influenced plant architecture in basil improving their greenhouse production for fresh market.

1. Introduction

Many species in the genus *Ocimum* (Labiatae) are ranked among the most important herbs for their medicinal properties, that are associated to high content of secondary metabolites including essential oils and caffeic acid derivatives (Gülçin *et al.*, 2007). The most significant species of the genus is sweet basil (*Ocimum basilicum* L.), which originated from tropical areas, such as India, Africa and southern Asia. Sweet basil is an annual herbaceous species that is usually cultivated as an aromatic plant. The minimum temperature for the growth of sweet basil has been determined to be 10.9°C (Vågen *et al.*, 2003; Chang, 2004). It is typically used in Italian and Asian cuisines because of the pronounced scent of its leaves, which depends on genotype (Chang *et al.*, 2009), nitrogen nutrition (Sifola and Barbieri, 2006) and harvesting system (May *et al.*, 2008). Sweet basil is not only cultivated for the use of aroma additives in food but also for other house-hold purposes, pharmaceuticals, cosmetics and folk medicine. Numerous different chemo-types exist in both wild and cultivated basil. For instance, sweet basil contains high levels of phenylpropanoids, e.g. eugenol and methyleugenol, and terpenoids e.g. linalool and 1,8-cineole (Lachowicz *et al*., 1997).

In recent years, the consumption of fresh basil has been expanded in supermarkets where young seedlings are directly sold in pots obtained from greenhouses. These plants are intended for family use, which after to be transplanted, are placed on balconies, vegetable gardens or gardens. The marketing of fresh basil highlights the problem that these plants have long and slender stems, which are easy to break off and thus making difficult their market distributions. Therefore, the objective of this study was to facilitate the sale of fresh basil in pots resulting not only in plants more compact and resistant to movements along the supply chain, but also healthy products in the market because of the avoidance of chemicals to control the plant height (Körner and Van Straten, 2008; Nagashima *et al*., 2011).

The interest on the effects of ultraviolet (UV) irradiation on plants has considerably increased in the last ten years due to the continuous depletion of the ozone layer (Ballaré *et al*., 2011). The stratospheric ozone layer completely absorbs solar UV-C (200-280 nm) which is extremely active and biologically lethal. UV-B (280-320 nm) is the most susceptible to ozone layer depletion because it is efficiently absorbed although small proportion is transmitted to the Earth surface. UV-A (320-400 nm) is hardly absorbed by ozone and thus passes almost unaltered through the stratospheric layer reaching the Earth surface (Houghton *et al*., 2001; Solomon *et al*., 2007). Despite the small proportion of UV-B in the natural daylight and higher energy than UV-A, UV-B has substantive effects on plant growth and metabolism (Kolb *et al*., 2001). Moreover, plants are differently sensitive to UV-B levels which strongly depend on the latitude, hours of direct sunlight and variation in the thickness of the ozone layer (Ballaré *et al*., 2011). For instance, plant species grown in Mediterranean and Tropical environments and/or at high altitudes have developed defensive mechanisms to protect themselves against UV radiation (Zheljazkov *et al*., 2008).

Many studies found that UV-B irradiation significantly affects secondary compounds such as the biosynthetic pathway of phenylpropanoids, which are antioxidant agents (Korkina, 2007) that also act as protection against UV (Johnson *et al*., 1999; Ioannidis *et al*., 2002). Previous studies reported that UV-B irradiation also increases essential oils and total phenolic compounds content in plants (Kumari *et al*., 2009; Kumari and Agrawal, 2011). Moreover, it has been demonstrated that the UV-B irradiation stimulates the production of volatiles organic compounds in fresh herbs which usually are depleted when plants grown under glass or plastic greenhouses conditions in the absence of natural levels of UV-B (Johnson *et al*., 1999; Ioannidis *et al*., 2002). In the case of plant morphology, several studies have been demonstrated the role of UV-B in controlling the growth of various plants such as *Lycopersicon esculentum* Mill. and *Salvia splendens* L. (Garner and Björkman, 1996; Giannini *et al*., 1996; Del Corso and Lercari, 1997). Additional techniques to control plant growth have been proposed: temperature manipulation, induction of mild water or mechanical stress, and the use of chemical compounds, however all these methods present many drawbacks (Moe and Mortensen, 1992; Garner and Björkman, 1996; Barreiro *et al*. 2006; Sun *et al*., 2008). Therefore, we focused on the role of UV-B as a growth regulator of potted basil intended for fresh consumption.

2. Materials and Methods

Plant material and growth conditions

Two cultivars of basil were used in this study, Genovese and Profumo. Basil seeds were sowed into peat-based growing medium in 8 cm diameter pots (10 seeds each pot). Pots were covered with non-woven fabric and incubated in a growth chamber at 23±1°C, relative humidity 60-65%, 12 h light (150 µmol m^{-2} s^{-1}). After one week of incubation, the coverage was removed and half of the pots were kept at the same conditions while the other half were under supplementary UV-B irradiation (fluorescent tubes with UV-B 10%, 15 W m^{-2}, *Exo Terra*). UV-B was applied for 3 h starting 1 h before darkness, and lamps were placed at 0.5 m above plants. Basil plants were regularly irrigated. Control and UV-B treated plants were sampled 11 and 22 days after the onset of UV treatment.

Morphological analysis

Leaf length and width, shoot length, fresh and dry weight of shoot and leaves were determined upon treatment completion. All measurements were the mean of three independent experiments.

Analysis of pigments

Pigments were extracted and analysed from full expanded leaves as previously described (Pompeiano *et al*., 2013). All the analyses were conducted in triplicate.

Statistical analysis

The statistical analyses of biometric and physiologic traits were subjected to an analysis of variance (ANOVA). Differences between treatments were assessed using the F-test, and the least significant difference (LSD) was calculated at *P*≤0.05. All computations were performed with R 2.14.2 R Development Core Team 2012.

3. Results

In this study, we analyzed the plant architecture and photosynthetic pigments content of two basil cultivars, Profumo and Genovese, at time zero (T0) and after treatment. Two different time points of treatment were analyzed: 11 and 22 days after UV-B treatment (UV) and their respective control (C) conditions.

Morphological analysis was determined on leaves and shoots of both basil cultivars (Fig. 1, 2). Length and width of cotiledonary leaves did not show differences between cultivars at T0, and also between treated and control plants during all the experimental time (Fig. 1). The first true leaves, which appeared at the first time point of the experiment, showed a significant increased size from 11 to 22 days at control conditions in both cultivars (Fig. 1). It was also observed that UV treated plants significantly increase, about two times, the leaf expansion in both cultivars in comparison with their respective control (Fig. 1). Interestingly, only 22 days UV treated seedlings showed the second leaves pair without morphological differences between the cultivars (Fig. 1). In the case of shoot length evaluation, no differences were observed between cultivars at T0, although ‘Genovese’ showed longer shoot than ‘Profumo’ at 11 and 22 days control conditions (Fig. 2). Moreover, the UV irradiated plants showed a considerably shorter shoots than that of control plants at both 11 and 22 days after treatment, and in all cases ‘Genovese’ still longer than ‘Profumo’ (Fig. 2).

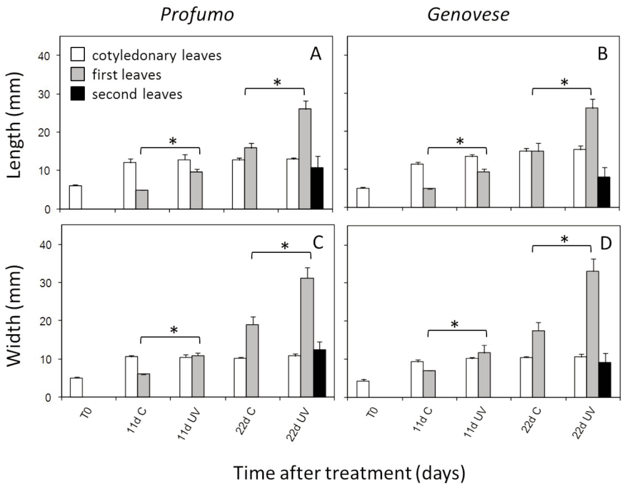

Fig. 1 - Effect of UV-B radiation on the morphology of basil leaves. Two cultivars of basil were use: (A, C) cv. Profumo and (B, D) cv. Genovese. (A, B) Length and (C, D) width of basil leaves were measured at time zero (7 days old basil seedlings, T0) and after treatment. Two different time points of treatment were evaluated: 11 and 22 days (d) after UV-B treatment (UV) or control (C) conditions. Cotyledonary (white bars), first (grey bars) and second leaves (black bars) were measured separately. Each value is the mean ± SD of three independent experiments. Asterisk indicates significant differences among treatments (*P*≤0.05).

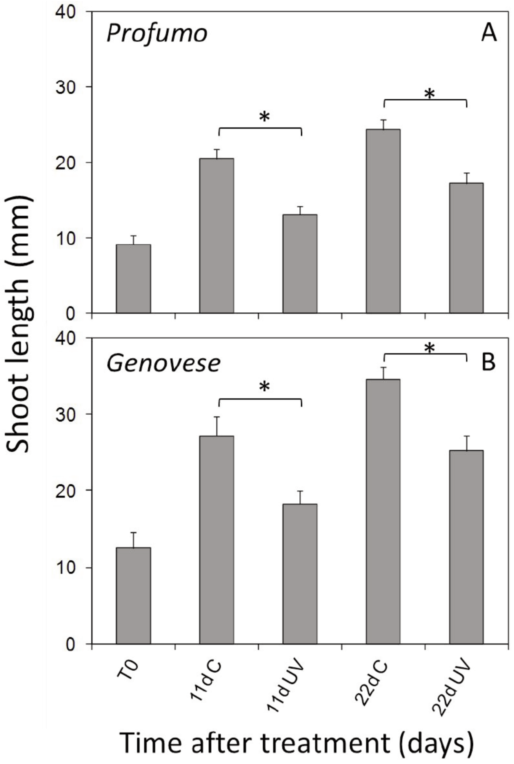

Fig. 2 - Effect of UV-B radiation on the length of basil shoots. Two cultivars of basil were use: (A) cv. Profumo and (B) cv. Genovese. Shoot length of basil cultivars was measured at time zero (7 days old basil seedlings, T0) and after treatment. Two different time points of treatment were evaluated: 11 and 22 days (d) after UV-B treatment (UV) or control (C) conditions. Each value is the mean ± SD of three independent experiments. Asterisk indicates significant differences among treatments (*P*≤0.05).

The effect of UV irradiation on basil biomass of both cultivars was also analyzed (Fig. 3). At T0, no differences were exhibited in fresh weight (FW) and dry weight (DW) leaves (only cotyledon) between cultivars, while FW and DW shoots were almost double in ‘Genovese’ in comparison with ‘Profumo’ (Fig. 3 A-B).

At 11 days after treatment, no differences were reported in FW and DW of shoot or leaves (cotyledon plus first pair) between cultivars (Fig. 3 C-D). Both cultivars treated with UV irradiation showed significant increase of FW and DW leaves in comparison with that of the control conditions, while no differences were reported in shoots (Fig. 3 C-D). At 22 days after treatment the biomass in both cultivars was increased in comparison with that of 11 d, while similar pattern was maintained when it was compared the biomass before and after UV irradiation (Fig. 3 E-F).

The photosynthetic pigments analyzed in both cultivars include chlorophyll *a*, chlorophyll *b* and carotenoids (Fig. 4). All pigments were detected at T0 without differences among cultivars. Then, the amount was dramatically reduced after 11 days under control conditions as well as UV irradiation, showing no statistical differences even between cultivars (Fig. 4). At 22 days after treatment, the level of chlorophyll *b* was maintained similar to that of 11 days and no differences were registered between control and UV conditions (Fig. 4). Chlorophyll *a* and carotenoids levels at 22 days under control conditions were increased in respect to that of 11 days, and no differences were detected when compared with that of after UV treatment (Fig. 4).

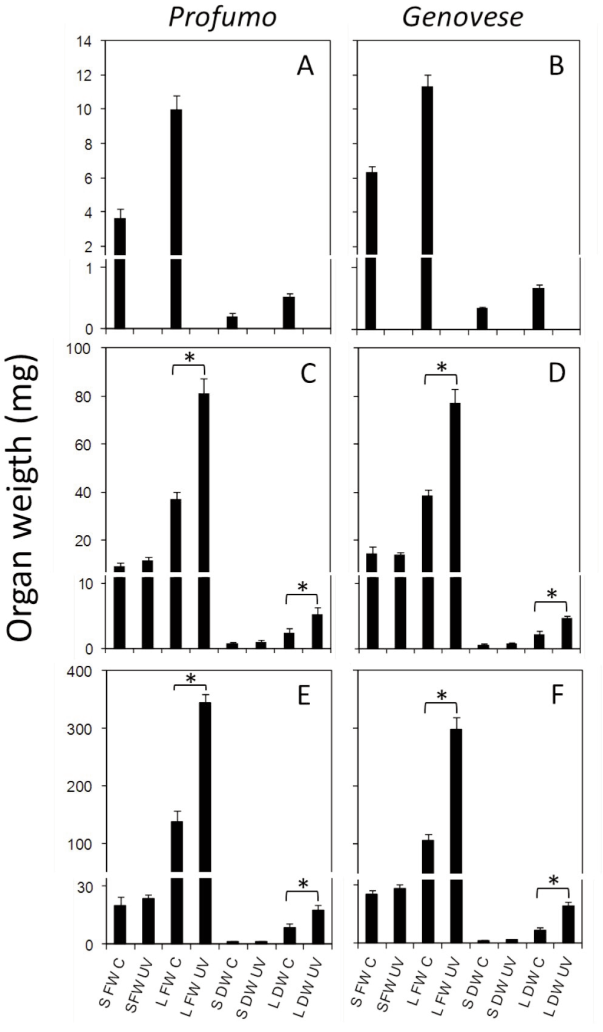

Fig. 3 - Effect of UV-B radiation on fresh and dry biomass of basil. Two cultivars of basil were use: (A, C, E) cv. Profumo and (B, D, F) cv. Genovese. Measurements were monitored at (A, B) time zero (7 days old basil seedlings) and after treatment. Two different time points of treatment were evaluated: (C, D) 11 days and (E, F) 22 days after UV-B treatment (UV) or control (C) conditions. S: shoot, L: leaf, FW: fresh weight, DW: dry weight. Each value is the mean ± SD of three independent experiments. Asterisk indicates significant differences among treatments ($P \leq 0.05$).

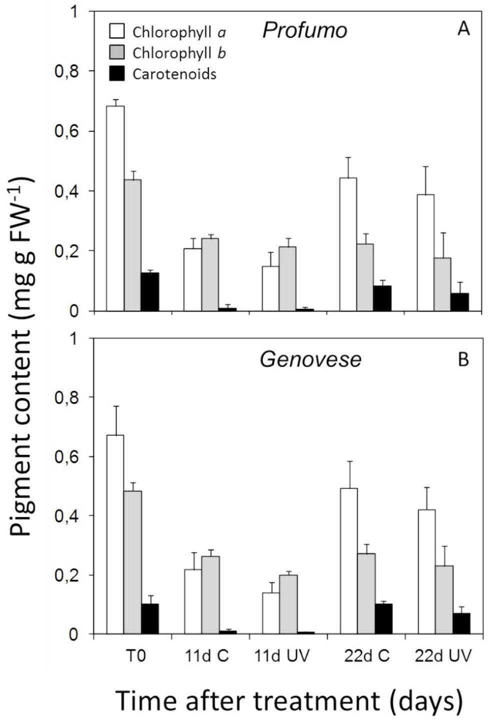

Fig. 4 - Effect of UV-B radiation on photosynthetic pigments in basil leaves. Two cultivars of basil were use: (A) cv. Profumo and (B) cv. Genovese. Measurements were monitored at time zero (7 days old basil seedlings, T0) and after treatment. Two different time points of treatment were evaluated: 11 and 22 days (d) after UV-B treatment (UV) or control (C) conditions. Chlorophyll a (white bars), chlorophyll b (grey bars) and carotenoids (black bars) content are expressed as mg g FW^{-1}. Each value is the mean ± SD of three independent experiments

4. Discussion and Conclusions

Results of the present study pointed towards a positive impact of supplementary UV-B irradiation on basil architecture, resulting on the reduction of stem elongation, increase of leaf length and width, increase of biomass, induction of second leaf sprouting and with a similar trend of photosynthetic pigments content in comparison to that of plants under control conditions. These UV-B effects on plants are in accordance with previous studies (Giannini *et al*., 1996; Del Corso and Lercari, 1997).

The reduction of stem elongation found in our research was also reported previously in other species such as wheat (Yuan *et al*., 1998). This phenomenon could be related to the impact of UV-B radiation on phytohormones metabolism, such as photo-oxidation of indole-3-acetic acid which plays an important role in stem elongation and lateral shoots (Ros and Tevini, 1995; Mark and Tevini, 1996). In addition, effects of UV-B radiation on leaf thickening, leaf elongation and biomass accumulation are highly dependent on UV-B dose and source, experimental parameters and species of study (Kakani *et al*., 2003). Therefore, in this study, we used growth chamber instead of open fields and greenhouses in order to control all the environmental conditions, making this condition replicable in any situation and avoiding the influence of environmental and seasonal variations.

Our results showed also an increase of leaf thickening, leaf elongation and biomass after UV-B irradiation, which could be related to a morphological architecture strategy to protect plants to deleterious effects of UV-B radiation (Maffei and Scannerini, 2000; Jansen, 2002; Santos *et al*., 2004; Chang *et al*., 2009). Moreover, the generation of second leaves in basil plants was earlier promoted after UV-B treatment which may also involve phytohormones metabolism changes; this effect was also previously reported (Barnes *et al*., 1988). Thus, structural changes on leaves in response to the applied UV-B were observed, although no changes on the content of chloroplast pigments was registered between UV treated and control plants. Photosynthetic pigments are useful indicators of UV-B tolerance or sensitivity (Kataria *et al*., 2014). Lower pigments content was obtained at 11 days of treatment in comparison with T0, which could be due to the stress arising from the removal of the non-woven fabric that protected plants from excessive light. However, this phenomenon was recovered in a tendency to the start point T0, suggesting the acclimatization of plants, as observed similarly in previous report (Teramura and Sullivan, 1994; Radyukina *et al*., 2012). Therefore, our data suggest that Genovese and Profumo basil cultivars are tolerant to the supplementary UV-B irradiation (15 W m^{-2}; 3 h day^{-1}) at chlorophyll level, but producing other strategy to prevent UV-B penetration to the mesophyll cell at plant architecture level.

Although the impacts of UV-B radiation on plant growth and development have been widely studied (Strid *et al*., 1994; Ballaré *et al*., 1995; Giannini *et al*., 1996; Del Corso and Lercari, 1997; Santos *et al*., 2004; Zu *et al*., 2004; Körner and Van Straten, 2008), the detailed mechanism of how UV-B radiation affects plant morphogenesis is still unclear. However, previous evidences found that UV-induced morphological changes are associated with the induction of the phenylpropanoids pathway resulting in accumulation of flavonoids (Jansen *et al*., 1998). Moreover, it has been demonstrated that flavonoids regulate auxin transport affecting plant architecture (Jansen, 2002; Robson *et al*., 2015).

In conclusion, the results of this work have practical implications for greenhouse production of pot basil. In fact, supplementary UV-B radiation reduced plant height but increased biomass and leaf number, width and length with no significant effect on photosynthetic pigments. Therefore, the application of UV lamps could replace chemical growth retardants and/or other techniques adopted for growth control in the production of potted basil for fresh market.

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