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Short Communication

Effect of light quality on rosmarinic acid content and antioxidant activity of sweet basil, *Ocimum basilicum* L.

Tomomi Shiga[1,2,a], Kazuhiro Shoji[1,3,a,*], Hiroaki Shimada[2], Shin-nosuke Hashida[1], Fumiyuki Goto[1], Toshihiro Yoshihara[1]

[1] Central Research Institute of Electric Power Industry, Abiko, Chiba 270-1194, Japan; [2] Department of Biological Science and Technology, Tokyo University of Science, Noda, Chiba 278-8510, Japan; [3] Faculty of Horticulture, Chiba University, Matsudo, Chiba 271-8510, Japan

* E-mail: shoji@criepi.denken.or.jp Tel: +81-4-7182-1181 Fax: +81-4-7183-3347

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Abstract Rosmarinic acid (RA), one of the main phenolic compounds in sweet basil (*Ocimum basilicum* L.), has antiviral, antimicrobial, and anti-inflammatory pharmacological properties. As recent studies have shown that UV-B and blue light irradiation stimulate phenylpropanoid biosynthesis, we determined whether light conditions affect RA content and antioxidant activity. Both antioxidant activity and phenolic content were increased by continuous white light irradiation. Ultra performance liquid chromatography analysis of the phenolic content of a methanolic extract of sweet basil revealed the presence of RA, caffeic acid and chicoric acid. Red and white light irradiation induced RA accumulation up to a level of 6 mg g^{-1} fresh weight within 14 days, whereas blue light irradiation only induced RA accumulation up to a level of 3 mg g^{-1} fresh weight, suggesting that the red wavelength at 600–700 nm in both white and red irradiation promoted the RA accumulation. In addition, RA accumulation was dependent on the irradiation period and was greater in the upper leaves than in the lower leaves. These results indicate that continuous white light is effective in increasing the RA content of basil, which results in high antioxidant activity.

Key words: Antioxidant, chicoric acid, light irradiation, rosmarinic acid, sweet basil (*Ocimum basilicum*).

Sweet basil (*Ocimum basilicum* L., Lamiaceae) is one of the most common herbs. It is consumed as a seasoning in dry and fresh form. It is native to tropical Asia, but is now cultivated all over the world. Rosmarinic acid (RA), a phenolic compound, is a well-known constituent of members of the Lamiaceae and Boraginaceae (Hakkim et al. 2007). RA has antioxidant activity and has pharmacological properties such as the ability to reduce pollinosis and allergies (Sanbongi et al. 2004). RA is also insect-repellent and antimicrobial, which protects the plant (Bais et al. 2002). Thus far, RA accumulation has been intensively investigated using root cultures (Tada et al. 1996), additional elicitor preparations, and methyl jasmonate treatment (Szabo et al. 1999). Analysis of the RA biosynthesis pathway in *Coleus blumei* (Petersen et al. 1993; Petersen and Simmonds 2003) and *Lithospermum erythrorhizon* (Mizukami et al. 1993) revealed that RA is an ester of caffeic acid (CaA) and 3,4-dihydroxyphenylalanine, which is derived from L-phenylalanine and L-tyrosine. Recently, it was reported that simultaneous irradiation with blue and UV-B light stimulates the phenylpropanoid pathway from phenylalanine in lettuce (Ebisawa et al. 2008) and affects the generation of phenolic compounds in basil (Nitz and Schnitzler 2004). However, the RA content of basil is lower than that of other Labiatae herbs such as lemon balm and rosemary (Wang et al. 2003). Therefore, it may be possible to increase the RA content of sweet basil by manipulating light conditions. We investigated the effect of light wavelength and irradiation period on RA production in basil.

Seeds of sweet basil were sown on peat moss (Primemix TKS1, Sakata Seed, Japan) in plastic cell trays, placed in a greenhouse, and maintained at an air temperature of 22–24°C and a light period of 16 h d^{-1}. When the seedlings were 2 weeks old, they were transplanted into plastic pots (diameter, 120 mm; depth, 100 mm) filled with a commercial soil mixture (Baiyoudo; Tachikawa Heiwa Nouen, Japan). All the experiments were carried out in a growth chamber (VB1514, W2000×D750×H1400 mm, Vötsch, Germany) maintained at an air temperature of 25°C and a relative humidity of 60%. Before the experimental treatments, the 6-week-old plants were kept in the dark for 24 h to eliminate the effect of circadian rhythm on RA biosynthesis (Eriksson and Millar 2003). Although

[a] These authors contributed equally to this work.

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the phenolic content, including that of RA, decreased after being kept in the dark, the reduction was less than 30% (data not shown).

To determine the effect of light quality on the antioxidant activity and phenolic content of sweet basil, seven replicates of 6-week-old seedlings were cultivated under continuous white, red, or blue light (FLR900T6, Nippo Electric, Japan). The photosynthetic photon flux densities (PPFDs, 400–700 nm) of the fluorescent lamps were all set to 100 μmol m^{-2} s^{-1} for each light condition according to the light intensity at the top of the plantlet. The spectral photon distributions of radiation from each light source are shown in Figure 1. The plants were grown under the light conditions for 14 days and the top leaves were sampled 7 days and 14 days after starting irradiation. Ten milliliters of methanol or ethanol was added to 2 g (fresh weight) of leaves, which had been cut into pieces. Polyphenols in the mixture were kept in the dark overnight at 4°C. The mixture was homogenized (Polytron, Kinematica, Switzerland) and filtered through glass filter paper (GF/A, Whatman, Japan). The volume of the supernatant was made up to 50 ml with methanol or ethanol and an aliquot was used for further analysis.

For analysis of antioxidant activity, we assessed the ability of the ethanol extracts to scavenge the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical using spectrophotometry according to Gülçin et al. (2007). Extracts from light-irradiated plants had significantly greater DPPH free radical scavenging activity than those that were not irradiated (Figure 2A). No significant differences between the three light conditions were observed for 7 days after the start of irradiation. However, after 14 days, the red and white lights induced greater radical scavenging activity than the blue light. At that time, the maximum activity in plants exposed to white light was 5.4 times higher than that of plants exposed to the dark treatment. Total phenolic content was estimated by the Folin–Ciocalteu method (Dicko et al. 2002). The total amount of phenolic compounds increased continuously over time. The greatest increase was evident under the white light treatment, followed by the red and blue light treatments (Figure 2B). The highest value for total phenolic content was 8.4 gallic acid equivalents (mg g^{-1} fresh weight) after continuous white light exposure for 14 days, but exposure to white light for 12 h d^{-1} only increased total phenolic content to 6.7 gallic acid equivalents (mg g^{-1} fresh weight) (data not shown). The level of antioxidant activity in the basil extracts was correlated with the total amount of phenolic

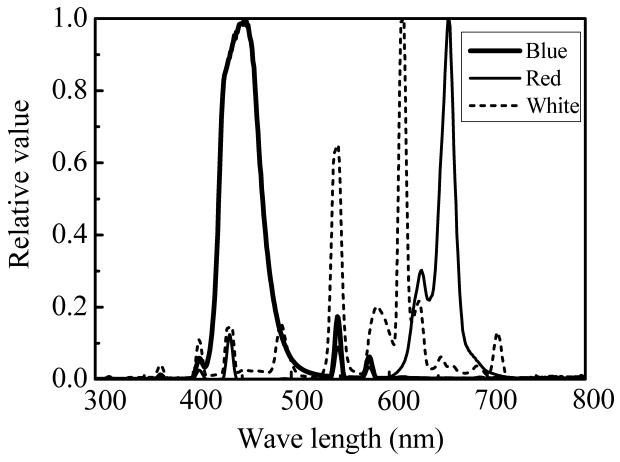

Figure 1. Spectral photon distributions of fluorescent lamps. The relative spectral photon flux of each lamp was normalized to 1.0 at 451 nm (blue), 613 nm (white), and 660 nm (red).

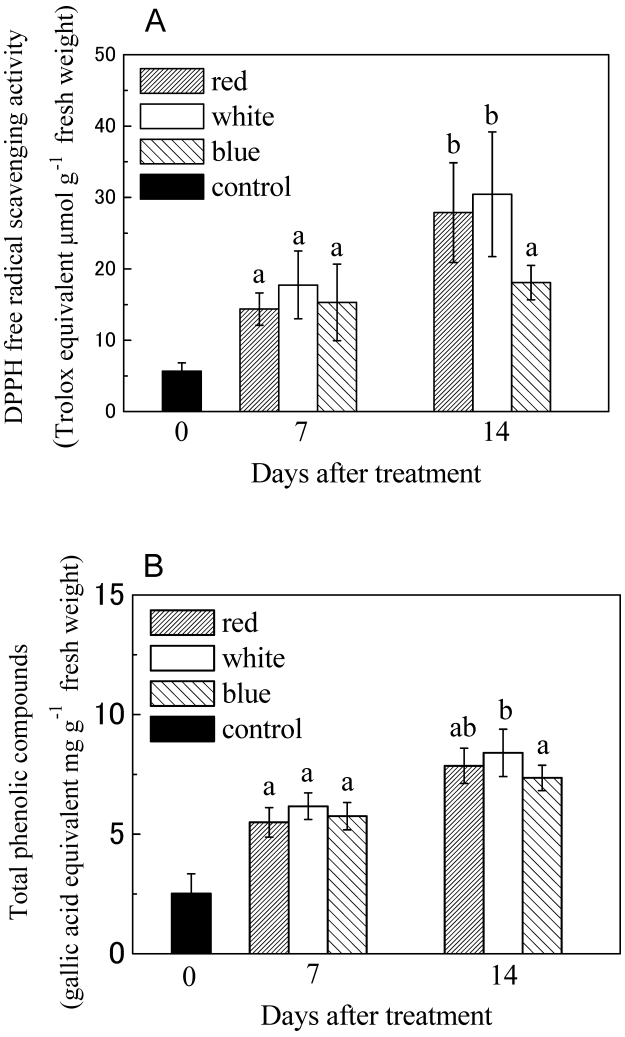

Figure 2. Effects of light irradiation on antioxidant activity and phenolic contents. (A) Free radical scavenging activity according to the DPPH assay of basil leaves exposed to 100 μmol m^{-2} s^{-1} of red, white, or blue light. The 6-week-old basil plants were kept for 24 h in the dark condition before light irradiation. (B) Soluble phenolic compounds extracted from basil leaves grown under various colors of light for 14 days. Phenolic contents were determined by Folin–Ciocalteu's method. Values are the mean±standard deviation for each group. Data are the mean and SD of seven replicate experiments. Means with different letters are significant at $P>0.05$ as determined by the Tukey and Kramer multiple range tests for the 7th and 14th experiments, respectively.

compounds. A correlation between the level of phenolic compounds and antioxidant activity in sweet basil after methyl jasmonate treatment has also been reported (Kim et al. 2006).

For detailed analysis of phenolic compounds, the methanol extracts were resolved using an ACUITY ultra performance liquid chromatography (UPLC, Waters, USA) with a UPLC BEH C18 column (ϕ1.7 μm, 2.1×50 mm, Waters, USA). The column oven was maintained at 40°C. The mobile phase consisted of methanol–water–formic acid (70 : 426 : 4), A, and methanol–water (65 : 35), B. The gradient program was as follows: from 0% B to 50% B (1.5 min), 50% B isocratic (0.5 min), and from 50% B to 100% B (1 min) at a flow rate of 0.5 ml min^{-1}. The total run time was 6 min. When the extract of basil grown under white light was resolved, three major peaks were detected at an absorbance of 310 nm (Figure 3A). Two of the peaks, at elution times of 0.9 min and 1.9 min, were identified as RA and CaA, respectively, based on comparison with the retention times of a standard; however, the peak at 1.4 min could not be identified. Then, mass spectra were measured using a QSTAR apparatus (Applied Biosystems, USA) coupled with UPLC. The capillary voltage was set to 5500 V and the source temperature to 400°C, and the data were analyzed using Analyst 1.4 software (Applied Biosystems, USA). Distinct positive ion mass spectra of the 1.4 min peak were observed at mass-to-charge ratios of 163.0, 295.0, and 497.1 m/z (Figure 3B). These were estimated to be $C_{22}H_{18}O_{12}$, a precursor ion $(M+Na)^+$ of chicoric acid (CiA), dicaffeoyl-tartaric acid, using Analyst 1.4 software. To confirm this result, a CiA standard (Funakoshi, Japan) was resolved with UPLC and a peak was detected at 1.4 min (Figure 3C). CiA, a CaA derivative, is known for its potency against purified HIV-1 integrase (King and Robinson 1998; Bailly and Cotelle 2005). The order of antioxidant activity is CiA>CaA>RA (Dalby-Brown et al. 2005).

Because the RA content of sweet basil was greater than that of CiA or CaA (Figure 3A), we focused on changes in RA content in subsequent experiments. We again grew basil under the three light conditions at the same irradiation strength as described previously. The results showed that RA increased under all conditions after 14 days of irradiation (Figure 4A). RA accumulation in sweet basil was increased to 3 mg g^{-1} fresh weight by continuous blue light. The RA content was increased to 6 mg g^{-1} fresh weight by continuous irradiation with wavelengths between 600 and 700 nm (red or white light) for 14 days. In addition, RA accumulation after exposure to white light for 12 h d^{-1} was about half that after continuous exposure to white light (data not shown). The RA accumulation level in this experiment was notably high compared with the RA

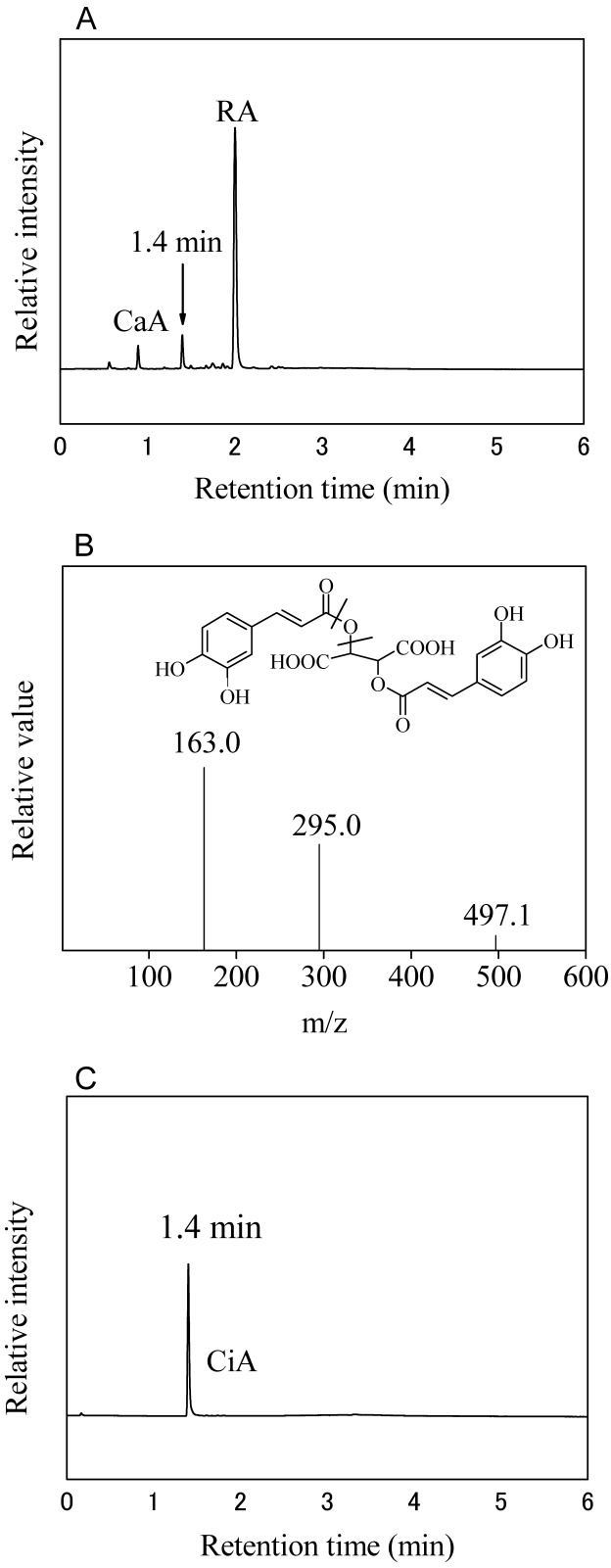

Figure 3. Ultraperformance liquid chromatography (UPLC)/mass spectrometry analysis of basil extract at 310 nm. (A) Liquid chromatography separated caffeic acid (CaA; 0.9 min) and rosmarinic acid (RA; 1.9 min) from the basil extract. (B) The UPLC/MS spectra of a peak at 1.4 min corresponded to chicoric acid and precursor ion $(M+Na)^+$, m/z 497.1. (C) Chicoric acid standard.

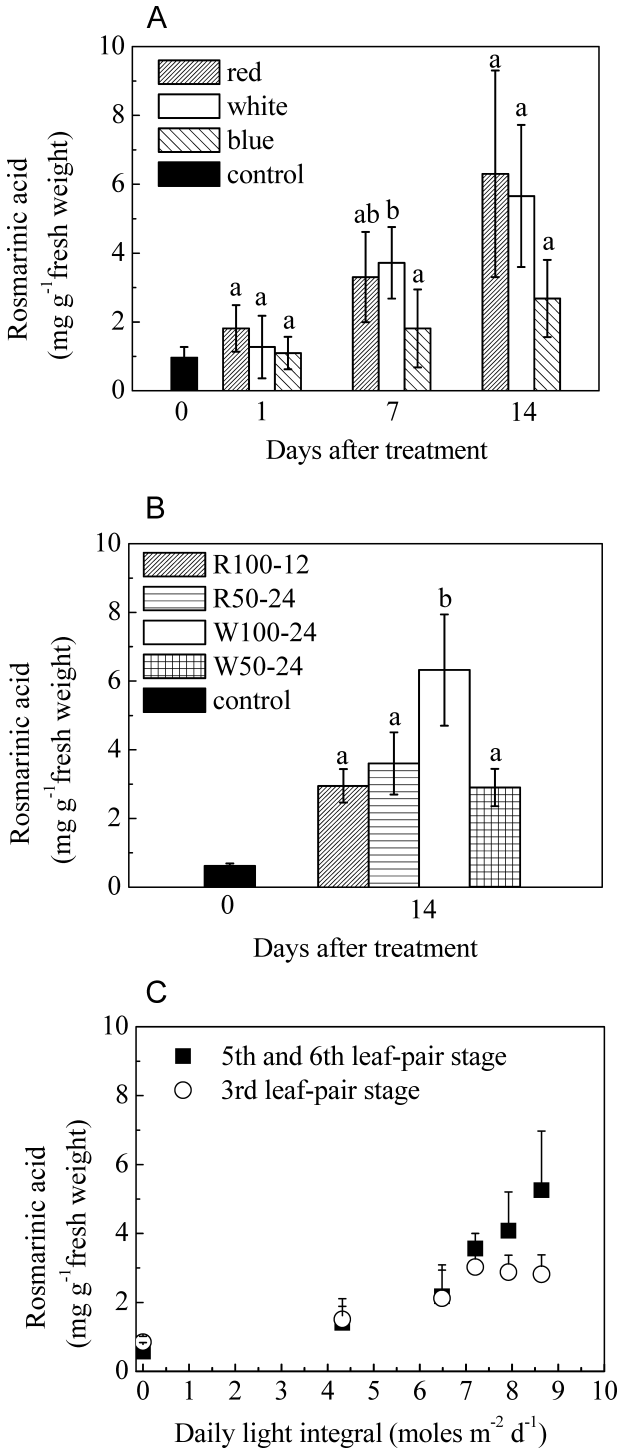

Figure 4. Effects of light irradiation on RA content. (A) Time-course RA accumulation in the top leaves of basil exposed to 100 μmol m^{-2} s^{-1} constant blue, red, or white light. The 6-week-old basil plants were kept for 24 h in the dark before light irradiation. (B) Effect of red light integrals and total light integrals (red or white fluorescent lamps) on RA accumulation in the top leaves of basil. (C) Light integral affected RA content in the upper and lower leaves under white light conditions. The upper leaves were harvested at the 5th and 6th leaf-pair growth stages and the lower leaves were harvested at the 3rd leaf-pair growth stage after 14 days of light irradiation. Statistical analyses are described in Figure 2.

content of basil grown in the presence of chemical inducers of RA such as chitosan or methyl jasmonate (Kim et al. 2005; 2006).

Next, we determined whether the integral of a specific spectrum of red light is essential for increasing RA accumulation. Because white fluorescent light contains approximately 50% of the red photon distribution, we used (1) 100 μmol m^{-2} s^{-1} red light for 12 h (R100–12, red light integral/total light integral=4.3 mol m^{-2} d^{-1}/4.3 mol m^{-2} d^{-1}), (2) 50 μmol m^{-2} s^{-1} red light for 24 h (R50–24, 4.3/4.3), (3) 100 μmol m^{-2} s^{-1} white light for 24 h (W100–24, 4.3/8.6), and (4) 50 μmol m^{-2} s^{-1} white light for 24 h (W50–24, 2.2/4.3). It was hypothesized that the RA increase pattern ratio would be (1) : (2) : (3) : (4)=2 : 2 : 2 : 1 if the RA production depended on only the red light. However, if the RA production depended on the total light integral, the ratio would be (1) : (2) : (3) : (4)=1 : 1 : 2 : 1. After treatment, the RA accumulation was 2.9, 3.6, 6.3, and 2.8 mg g^{-1} fresh weight for R100–12, R50–24, W100–24, and W50–24, respectively (Figure 4B). The (1) : (2) : (3) : (4) ratio was 1 : 1 : 2 : 1, indicating that the total light integral was essential for increasing RA production.

Furthermore, the effects of white light integral and leaf position on the RA production of basil were observed. The upper leaves at the 5th and 6th leaf-pair positions showed an increase in RA content, depending on the light integral, of 4.3–8.6 mol m^{-2} d^{-1} (Figure 4C). RA production became saturated when the light integrals were increased from a PPFD of 100 μmol m^{-2} s^{-1} to 300 μmol m^{-2} s^{-1} (data not shown). Irradiation time also played an important role in RA production. A longer irradiation period enhanced RA production even at a strength lower than that of sunlight. Interestingly, the RA level of the lower leaves peaked at only 3 mg g^{-1} fresh weight, irrespective of daily light integral. That the upper leaves of this type of basil accumulated a greater amount of RA than the lower leaves contrasts with the observation that sweet Thai basil leaves produce more RA in the older leaves than in the younger leaves (Vassão et al. 2006). The productivity or sensitivity of leaves at different positions may differ among plant species. However, upper leaves contain high densities of glandular trichomes (Werker et al. 1993; Gang et al. 2002) and contain essential oil (Gang et al. 2001).

Natural antioxidants protect against the effects of free radicals, promoting human health. Therefore, studies on natural antioxidants have increased in importance. In our trials, the soluble phenolic compound content of sweet basil increased to 8.4 gallic acid equivalents (mg g^{-1} fresh weight). This is a much higher level than that achieved using light reflection from mulch (Loughrin and Kasperbauer 2001). Antioxidant activity after exposure to white light for 14 days was 5.4-fold greater than that after treatment with darkness. Light irradiation

is, therefore, an important and convenient tool for regulating natural antioxidant content. RA production under constant red and white light was correlated with irradiation period, especially in the upper leaves. Phenolic content and antioxidant activity increased with the increase in RA level, indicating that the induction of antioxidant activity in sweet basil was mainly responsible for the accumulation of RA during the light treatment.

Interestingly, CiA has higher antioxidant activity than RA (Dalby-Brown et al. 2005). CiA is produced from phenylalanine, but the complete CiA biosynthesis pathway has not been fully elucidated. Future investigations are warranted to clarify the relationship between CiA biosynthesis and light stimulation.

The constant light treatments increased plant height and dry weight of leaves, stems, and roots, but the plants became a little harder and had a curled morphology (data not shown). Light quality is one of the most important environmental signals that regulates the growth and composition of basil (Chang et al. 2007; Skrubis and Markakis 2008). Further development of methods to increase phenolic content and prevent morphological changes in basil may contribute to human health via an increased intake of natural antioxidants.

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