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Application of low intensity light pulses to delay postharvest senescence of *Ocimum basilicum* leaves

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ABSTRACT

Fresh basil (*Ocimum basilicum* L.) is a highly perishable leafy green vegetable with a storage life of 4–5 d at room temperature. Exposure of basil leaves to temperatures below 12 °C during storage results in chilling injury; therefore, refrigeration cannot be used to extend postharvest life of basil. Typically, leafy vegetables are stored in darkness or extremely low irradiance. Darkness is known to induce senescence, and the initial phase of senescence is reversible by exposure to light. In this work, we studied the effects of low-intensity white light pulses at room temperature on postharvest senescence of basil leaves. Daily exposure for 2 h to 30–37 μmol m^{-2} s^{-1} of light was effective to delay postharvest senescence of basil leaves. Chlorophyll and protein levels decreased, ammonium accumulated and leaves developed visual symptoms of deterioration (darkening) during storage in darkness. Light pulses reduced the intensity of these senescence symptoms. The photosynthesis light compensation point of basil leaves was 50 μmol m^{-2} s^{-1} i.e., higher than the intensity used in this study, and the effect of treatment with red light was the same as with white light, while far red light was ineffective. Light pulses exerted a local effect on chlorophyll loss, but the effect on protein degradation was systemic (i.e., spreading beyond the illuminated parts of the leaf blade). The results of this study indicate that daily treatment for 2 h with low intensity light (30–37 μmol m^{-2} s^{-1} every day) during storage at 20 °C is an effective treatment to delay postharvest senescence of basil leaves. The delay of postharvest senescence by low intensity light pulses seems to be mediated by phytochromes, and it is systemic for protein, and partially systemic for chlorophyll degradation.

1. Introduction

Fresh basil (*Ocimum basilicum* L.) is a highly perishable leafy green vegetable, with a storage life of 4–5 d at room temperature (Cantwell and Reid, 2002). Postharvest senescence of green leaves is induced by detachment and exposure to darkness or very low light, probably as a consequence of the ensuing water and nutrient deficiencies, and lack of photosynthesis (Ella et al., 2003). Senescence involves enhanced chlorophyll and protein degradation, and accumulation of ammonium in detached leaves (Rolny et al., 2011; Chen et al., 1997; Clarke et al., 1994).

Postharvest senescence causes serious commercial losses due to rapid decline in basil leaf quality (Hassan and Mahfouz, 2010). One of the main goals of postharvest technology in green vegetables is to delay senescence symptoms (Page et al., 2001). Basil is a chilling susceptible plant of tropical origin, therefore exposure of leaves to temperatures below 12 °C during storage results in the development of chilling injury, which is manifest by the appearance of dark lesions on leaves, followed by decay (Lange and Cameron, 1994). Therefore, low temperature is not an option for postharvest handling of this vegetable, and it is important to find alternative technologies.

Darkness induces senescence in detached leaves of green vegetables. The initial phase of senescence is reversible, and it can be reversed by light (Zavaleta-Mancera et al., 1999). In the last few years, several publications have reported on the influence of light during storage on the quality of different vegetables. Lester et al. (2010) reported that spinach leaves exposed to continuous light (26.9 μmol m^{-2} s^{-1}) during storage had higher nutritional quality than leaves exposed to continuous darkness. Fluorescent light (21.8 μmol m^{-2} s^{-1}) has been described as promoting weight loss

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but preserving vitamin C of Chinese kale during storage (Noichinda et al., 2007). Light exposure during storage prevented senescence in cabbage (Perrin, 1982). Oms-Oliu et al. (2010) showed that the application of pulsed light at doses of 4.8 J cm^{-2} could extend the shelf life of fresh-cut mushrooms without dramatically affecting texture and antioxidant properties. Brief postharvest exposure to pulsed light stimulates coloration and anthocyanin accumulation in fig fruit, and this treatment seems to be a feasible means of compensating for insufficient sunlight stimulation of color development in figs and possibly other fruit as well (Rodov et al., 2012). The combinations of 24 μmol m^{-2} s^{-1} light exposure with storage at low temperature delayed sensory quality deterioration and prolonged shelf-life of fresh-cut broccoli (Zhan et al., 2012).

However, a negative effect on the quality of different vegetables has been observed during storage under light due to an increase in physiological activity (Sanz et al., 2009). Some authors have reported that light increased respiration of freshly cut green vegetables, which can accelerate browning of the cut edges in leeks (Ayala et al., 2009), accelerated chlorophyll loss in broccoli, and increased transpiration and fermentation in cauliflower (Olarte et al., 2009).

Although several studies have shown that light delays senescence of leaves, the mechanism underlying this effect remains largely unknown. Light effects may be related to its role in photosynthesis and photo-assimilate availability, or light can interact with phytochromes that are involved in several development processes (Paul and Khurana, 2008; Casal et al., 1998). Phytochromes maximally absorb in the red and far-red region of the solar spectrum and play a key role in regulating plant growth and development. Phytochromes exist in two inter-convertible conformations with different absorption spectra: Pfr absorbs far red light and is generally the biologically active conformation, Pr absorbs red light. Absorption of red light converts Pr to Pfr while absorption of far red converts Pfr to Pr. Phytochrome responses are classically defined by their red/far red reversibility (Quail, 2002).

The aim of this paper was to assess the feasibility of using low intensity light pulses to delay postharvest senescence of *O. basilicum* leaves. The physiological basis of the effects of low intensity light on postharvest senescence is also discussed.

2. Materials and methods

2.1. Plant material and treatments

Basil (*O. basilicum* L.) leaves, harvested early in the morning, were obtained from local producers (La Plata, Argentina). Leaves were placed in trays (ten leaves per tray) and covered with a PVC film to decrease water loss. Trays were maintained at 20 °C in darkness, or given pulses of low intensity white light (30–37 μmol m^{-2} s^{-1}) provided by fluorescent lamps. To illuminate leaves with red and far red light, the respective LEE filters were placed between lamps and samples so that irradiance at leaf level reached 30–37 μmol m^{-2} s^{-1}. To examine if the response to low intensity light pulses is local or systemic, in one set of leaves one half (lengthwise) of the leaf lamina was covered with aluminum foil while the rest of the lamina was treated with low intensity white light pulses. Complete darkness below aluminum foil was confirmed through measurements with a photosynthetically active radiation quantum sensor (RADIAPAR, Cavadevices, Buenos Aires, Argentina). For analytical determinations in this experiment, each half of each leaf was considered as a different sample: A and B. After treatment with low intensity light for 2 h, all trays were returned to darkness. In all experiments the intensity of light was measured with a photosynthetically active radiation quantum sensor.

2.2. Chlorophyll and carotenoid content

Relative chlorophyll content per unit leaf area was determined non-destructively using a SPAD (Soil Plant Analysis Development) analyzer (SPAD-502 Chlorophyll Meter, Konica Minolta, Tokyo, Japan). Alternatively, pigment content was determined in samples of fresh leaves spectrophotometrically according to Lichtenthaler (1987). Five discs (1 cm diameter each) were ground in liquid nitrogen, extracted for 15 min with 1 mL of 95% ethanol (v/v), and then centrifuged at 3000 × *g* for 10 min. The supernatant was used to determine the absorbance (Abs) at 664.2 and 648.6 nm for chlorophylls and 470 nm for total carotenoid content. Total chlorophyll and carotenoid contents were calculated using Lichtenthaler's equations. Three replicates per treatment were analyzed.

2.3. Protein content

Two freshly cut leaf discs (1 cm diameter each) were homogenized in chilled buffer (50 mM Tris hydroxy-methyl aminomethane–HCl, pH 8. 5 mM EDTA, 1 mM PMSF, 1 μM cysteine and 5 nM Leupeptin) and centrifuged at 10,000 × *g* for 10 min at 4 °C. Proteins in the supernatant were determined according to Bradford (1976) with bovine serum albumin as standard. Three replicates per treatment were analyzed.

For SDS-PAGE analysis, one volume of the supernatant from protein extraction was mixed with one volume of 2× solubilization buffer (125 mM Tris pH 6.8; 4%, w/v, SDS; 10%, v/v, glycerol; 10%, v/v, β-mercaptoethanol), boiled for 5 min and separated in 1.5 mm thick, 12% acrylamide concentration minigels as in Laemmli (1970). Proteins were visualized by staining with Coomassie Brilliant Blue R-250. Gels were photographed with a digital camera, and the protein content was calculated by using the SIGMA gel analysis software. Different concentrations of bovine serum albumin were included in each gel to serve as standard. Three replicates per treatment were analyzed.

2.4. Ammonium content in leaves

Eight freshly cut leaf discs (1 cm diameter each) were homogenized with 1 mL of 0.3 mM H_2SO_4. Samples were centrifuged at 10,000 × *g* for 10 min at 4 °C and ammonium was determined with Indophenol blue according to Hung and Kao (2007). $(NH_4)_2SO_4$ was used as standard. Three replicates per treatment were analyzed.

2.5. Glutamine synthetase (GS) activity

Five freshly cut leaf discs (1 cm diameter each) were extracted in 1 mL of extraction buffer (pH 7.6) containing 100 mM HEPES, 1 mM EDTA, 10 mM $MgSO_4$, 5 mM glutamate, 10% (v/v) ethylene glycol, 10 μM leupeptin and 6 mM cysteine. The crude extract was centrifuged at 12,000 × *g* for 30 min at 4 °C. GS activity was measured using a synthetase assay based on the method described by Lea et al. (1990). 100 μL of crude leaf extract was added to 380 μL of assay mix, which consisted of 100 mM HEPES, 80 mM glutamate, 6 mM hydroxylamine HCl, 20 mM $MgSO_4$, 4 mM EDTA at pH 7.6. The reaction was started by the addition of 20 μL of 0.2 M ATP at pH 7.6. After 10 min of incubation at 30 °C, the reaction was stopped by the addition of 500 μL of ferric chloride reagent (0.24 M TCA, 0.1 M ferric chloride, 1.0 M HCl). Samples were then centrifuged at 10,000 × *g* for 5 min and absorbance was read at 505 nm. GS activity was expressed as the increase of $Abs_{505\,nm}$ s^{-1} m^{-2}. Three replicates per treatment were analyzed.