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# Pre-harvest calcium application increases biomass and delays senescence of broccoli microgreens

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ABSTRACT

Microgreen consumption has been steadily increasing in recent years due to consumer awareness of their unique color, rich flavor, and concentrated bioactive compounds. However, industrial production and marketing is limited by their short shelf-life associated with rapid deterioration in product quality. This study investigated the effect of pre-harvest calcium application on the post-harvest quality and shelf-life of broccoli microgreens. Broccoli microgreen seedlings were sprayed daily with calcium chloride at concentrations of 1, 10 and 20 mM, or water (control) for 10 days. The fresh-cut microgreens were packaged in sealed polyethylene film bags. Package headspace atmospheric conditions, overall visual quality and tissue membrane integrity were evaluated on days 0, 7, 14, and 21, during 5 °C storage. Results indicated that the 10 mM calcium chloride treatment increased the biomass by more than 50%, and tripled the calcium content as compared to the water-treated controls. Microgreens treated with 10 mM calcium chloride spray exhibited higher superoxide dismutase and peroxidase activities, lower tissue electrolyte leakage, improved overall visual quality, and reduced microbial growth during storage. Furthermore, calcium treatment significantly affected expression of the senescence-associated genes BoSAG12, BoGPX6, BoCAT3 and BoSAG12. These results provide important information for commercial growers to enhance productivity and improve postharvest quality and shelf-life, potentially enabling a broadening of the retail marketing of broccoli microgreens.

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## 1. Introduction

Microgreens are young and tender cotyledon greens harvested within 7–14 d of vegetable seedling emergence. In recent years, consumption of microgreens has increased along with consumer awareness and appreciation for their tender texture, distinctive fresh flavors, and concentrated bioactive compounds, such as vitamins, minerals, antioxidants, etc., as compared to mature leafy greens (Chandra et al., 2012; Kou et al., 2013; Xiao et al., 2012). Thus they are considered to be "functional foods", which contain health-promoting or disease-preventing properties beyond the basic function of supplying nutrients (Xiao et al., 2012). For example, broccoli (*Brassica oleracea* L. var. *italica*) is a very important economic dietary crop for consumption of both florets and young seedlings. They are highly prized for their health benefits, as they are rich in vitamins, trace elements, amino acids, antioxidants, protein, etc. (Fahey et al., 1997; Finley et al., 2001; Han et al., 2006). Broccoli sprouts contain about 50 times more sulfurophane by weight than mature broccoli (Mewis et al., 2012). However, microgreen consumption is limited by their low productivity, rapid senescence and a very short shelf life, usually 3–5 d at ambient temperature (Chandra et al., 2012; Kou et al., 2013).

Accumulated evidence has shown that calcium plays a pivotal role in plant growth, development and response to external and internal signals (Kudla et al., 2010; Poovaiah and Reddy, 1987; Reddy, 2001). Calcium treatment was also found to have a beneficial effect on the storage of fruits and vegetables by retarding fruit ripening and leaf senescence (Holb et al., 2012; Martin-Diana et al., 2007; Poovaiah and Leopold, 1973). It has been suggested that calcium can retard ripening and senescence by crosslinking with pectic polymers in cell wall (Liu et al., 2009) and protecting cell membrane integrity (Cheour et al., 1992; Guimaraes et al., 2011).

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However, the molecular mechanism for calcium action in delaying plant senescence is not clear. Senescence in plants is a complex but highly regulated process (Breeze et al., 2011; Guo and Gan, 2012; Lim et al., 2007). For normal plant organs, senescence is a natural development and age-dependent deterioration process leading to programmed cell death. The changes in senescence-associated gene expression patterns in tissues occur well before any visible signs of senescence are observed. These genes include *SAG12*, *GPX6*, *CAT3* and *EIN3* which are involved in protein degradation, oxidative stress and ethylene signal transduction pathway (Buchanan-Wollaston and Ainsworth, 1997; Li et al., 2012; Page et al., 2001). As compared to fruits and mature green leaves, fresh-cut microgreens harvested in the very early stage are very tender and subjected to much more stress leading to rapid senescence and a very short shelf-life. The physiological, biochemical and molecular events occurring during microgreen storage deserve more attention, since most studies have focused on the postharvest changes in mature fruit tissues and mature leaves that senesce more slowly. Here we report the effects of pre-harvest application of calcium on broccoli microgreen yield, senescence and postharvest quality.

## 2. Materials and methods

### 2.1. Plant materials and treatments

Broccoli seeds were obtained from Living Whole Foods Inc. (West Springville, USA). Hydroponic 'Sure to grow' pads (0.208 m × 0.254 m, Growers Supply, USA) were set evenly in 0.54 m × 0.28 m × 0.06 m trays (vacuum-formed standard 1020 open flats) containing 600 mL water (pH 5.6 acidified water). The seeds were then spread evenly over the damp pad. The trays were kept in a growth chamber at 25 °C in the dark for 4 d after seed sowing before exposing to light with the light intensity of 42 μmol $s^{-1}$ $m^{-2}$ for 12 h/12 h (light/dark). The seedlings were sprayed on a daily basis for 10 d with $H_2O$ (pH 5.6) only, 1 mM, 10 mM, and 20 mM $CaCl_2$ or $MgCl_2$ or 5 mM EGTA (Sigma–Aldrich, USA). Microgreens, including hypocotyl and cotyledons, were harvested on 10 d after sowing (harvest day) by cutting near the bottom of each hypocotyl with a pair of sterilized scissors. Microgreens with no obvious damaged hypocotyl and cotyledons were used for all postharvest analyses.

### 2.2. Total calcium content

Calcium content was measured as described (Codling et al., 2007). Briefly, the harvested microgreens were rinsed three times with double distilled water to remove any surface calcium before drying in 70 °C oven for 48 h. Oven-dried ground plant tissues (0.002 kg) were ashed at 550 °C for 16 h, followed by the addition of 2 mL concentrated nitric acid (trace element grade); samples were then brought to dryness on a hot plate. After drying, 10 mL of 3 M HCl were added and the mixtures were allowed to reflux for 2 h. The digests were filtered through Whatman #40 filter paper and the filtrate volumes were brought to 25 mL with 0.1 M HCl. Calcium concentration in the tissue was determined using Optima 4300 DV Inductively Coupled Plasma Optical Emission Spectrometer (PerkinElmer, USA) with strontium as an internal standard. For quality control, one blank and one peach leaves standard from the National Institute of Standards were added for every 10 samples.

### 2.3. Postharvest package and storage

The fresh-cut broccoli microgreens (0.01 kg each) were packaged in sealed bags (0.1 m × 0.1 m) prepared with polyethylene films of 16.6 pmol $s^{-1}$ $m^{-2}$ $Pa^{-1}$ oxygen transmission rate (OTR). Samples were stored at 5 °C for 21 d. Quality evaluations were performed on 0, 4, 7, 14 and 21 d postharvest (DPH). Antioxidant associated enzyme activities were measured on 0, 7 and 14 DPH.

### 2.4. Postharvest quality and plant physiology assessment

The $CO_2$ and $O_2$ in the headspace of packages containing fresh-cut microgreens were measured using a gas analyzer (Check mate II, PBI Dansensor Co., Denmark) by inserting the needle of the measuring assembly through a septum adhered to the packaging film and into the package headspace.

Sensory quality attributes of off-odor and overall visual quality were evaluated by a highly-trained panel (members had either more than seven-year sensory experience or intensive training and experience in sensory analysis of microgreens) immediately after opening the bags following a modified procedure (Luo et al., 2004). Off-odor was scored on an 1–5 scale, where 1 = no off-odor and 5 = extremely strong off-odor. Overall quality was evaluated with a 9-point hedonic scale, where 9 = like extremely, 5 = neither like nor dislike and 1 = dislike extremely (Meilgaard et al., 1991).

Tissue electrolyte leakage was measured following a modified procedure (Kim et al., 2005). Fresh broccoli microgreens (0.003 kg) were submerged in 150 mL aliquots of distilled water at 5 °C for 30 min. The electrical conductivity of the solution was measured using a conductivity meter (model 135A; Orion Research Inc., USA). Total sample conductivity was determined on the same treatments after freezing at −20 °C for 24 h and subsequent thawing. Tissue electrolyte leakage was expressed as a percentage of the total conductivity.

### 2.5. Postharvest microbiological quality assessment

Each sample (0.003 kg) were macerated in 27 mL 1× PBS (phosphate buffered saline) using a model 80 Lab Stomacher (Seward Medical, UK) for 120 s at high speed in filtration stomacher bags. A 50 μL sample of each filtrate or its appropriate dilution was logarithmically spread on agar plates with an automatic spiral plater (Wasp II, Don Whitley Scientific Ltd., UK). Enumeration of the total aerobic mesophilic bacteria were plated on tryptic soy agar (TSA, Difco Lab, USA) and incubated at 30 °C for 24–48 h. Microbial colonies were counted using an automated colony counter (Protocol SR; Synoptics, UK) and reported as log CFU $mL^{-1}$ of tissue.

### 2.6. Assessment of antioxidant enzyme activity

Antioxidant enzymes peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) were extracted and analyzed as described (Havir and McHale, 1987; Lurie et al., 1997; Prochazkova et al., 2001). Briefly, 0.004 kg samples were homogenized in 10 mL pH 7.8, 50 mM PBS, containing 1% (w/v) polyvinylpolypyrolidone (PVPP) and 1 mM EDTA, then centrifuged at 10,000 × *g* for 20 min at 4 °C. The supernatant was collected for POD, SOD, and CAT assay. The POD activity was expressed as U (unit) $kg^{-1}$, on a fresh weight basis, where $U = 0.01\ \Delta$ absorbance 470 $s^{-1}$. The CAT activity was expressed as U $kg^{-1}$, on a fresh weight basis, where $U = 0.1\ \Delta$ absorbance 240 $s^{-1}$, and the SOD activity was expressed as U $kg^{-1}$, on a fresh weight basis. The volume of enzyme corresponding to 50% inhibition of nitro-blue tetrazolium (NBT) reduction at 560 nm was considered as one enzyme unit.

### 2.7. RT-qPCR

Total RNA was isolated from frozen tissue using the RNeasy Plant Mini Kit following the manufacturer's instructions (Qiagen, USA). One μg of total RNA was used to synthesize cDNA with the oligo-$(dT)_{18}$ primer following the instructions of the Superscript III kit (Invitrogen, USA). Quantitative Real-Time PCR (qPCR) analysis