Antioxidant capacity reduced in scallions grown under elevated CO₂ independent of assayed light intensity

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Abstract

Long-duration manned space missions mandate the development of a sustainable life support system and effective countermeasures against damaging space radiation. To mitigate the risk of inevitable exposure to space radiation, cultivation of fresh fruits and vegetables rich in antioxidants is an attractive alternative to pharmacological agents. However, it has yet to be established whether antioxidant properties of crops can be preserved or enhanced in a space environment where environmental conditions differ from that which plants have acclimated to on earth. Scallion (Allium fistulosum) rich in antioxidant vitamins C and A, and flavonoids was used as a model plant to study the impact of a range of CO₂ concentrations and light intensities that are likely encountered in a space habitat on food quality traits. Scallions were hydroponically grown in controlled environmental chambers under a combination of 3 CO₂ concentrations of 400, 1200 and 4000 μmol mol⁻¹ and 3 light intensity levels of 150, 300, 450 μmol m⁻² s⁻¹. Total antioxidant activity (TAA) of scallion extracts was determined using a radical cation scavenging assay. Both elevated CO₂ and increasing light intensity enhanced biomass accumulation, but effects on TAA (based on dry weight) differed. TAA was reduced for plants grown under elevated CO₂, but remained unchanged with increases in light intensity. Elevated CO₂ stimulated greater biomass production than antioxidants, while an increase in photosynthetic photon flux promoted the synthesis of antioxidant compounds at a rate similar to that of biomass. Consequently, light is a more effective stimulus than CO₂ for antioxidant production.

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1. Introduction

During past two decades, carbon dioxide (CO₂) may be the most studied environmental stimulus for plants due to concerns with the impact of its rising concentration on agro-ecosystems. A wide range of physiological and molecular responses has been observed and mechanisms for various responses have been postulated (Ainsworth and Rogers, 2007). It is generally agreed that increasing atmospheric [CO₂] enhances water use efficiency because of reduced stomatal conductance that stimulates light-saturated photosynthesis (Aₘₐₓ), particularly in C₃ plants. It is also known that [CO₂], light intensity, and other environmental parameters have a significant influence on greenhouse vegetable quality attributes such as color and taste (Gruda, 2005), and can affect the antioxidant content and antioxidant activity of the harvested fruits (Wang, 2006). However, reports regarding elevated CO₂ effects on crop quality as is related to small molecule antioxidants are relatively scarce and far from reaching a consensus. For instances, growth under elevated CO₂ resulted in an increase in ascorbic acid (vitamin C) in sour orange (Idso et al., 2002), flavonoids in wheat (Estiarte et al., 1999; Levine et al., 2008) and anthocyanins in strawberry (Wang et al., 2003), but a decline in vitamin C and glutathione in barley (Robinson and Sicher, 2004). Therefore, it is still

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difficult to predict how elevated CO₂ affects the biosynthesis of antioxidants in a specific crop and even more so in a specific tissue. High light intensity has the tendency to increase photogeneration of reactive O₂ species, consequently, increases in the accumulation of antioxidant compounds and enzymes. There is evidence that increasing light intensity increases concentrations of total ascorbate and SH-compounds, especially in Mg-deficient (20 μM) bean leaves (Cakmak and Marschner, 1992). Likewise, low light intensity and shading reduced pigment synthesis in tomato (Dorais et al., 2001) and anthocyanin level in lettuce (Kleinhenz et al., 2003). However, the interaction of elevated CO₂ and light on crop antioxidant biosynthesis is less well known.

The oxidative environment that most organisms experience accentuates the essential role of antioxidants in nature. Plants synthesize and accumulate antioxidants for the purpose of detoxifying reactive oxygen species (ROS), while human often need dietary intake of antioxidative compounds to boost the capacity to combat ROS afflicted diseases such as cancer, cardiovascular disease, neurodegeneration, and diabetes (Fang et al., 2002). A number of epidemiological studies have demonstrated an inverse relationship between consumption of fresh fruits/vegetables and incidence of certain cancers. This health benefit is often attributed to phytochemicals that have direct radical scavenging activity and/or prime antioxidative stress defense systems. Recently there has been a surge of public interest in health promoting foods (so-called functional foods) such as those with high antioxidative activity leading to a paradigm shift in the food industry from an emphasis on quantity to more on nutritional composition; this consumer shift demands innovative horticultural practices to enhance quality traits in crops (Wang, 2006). High-energy radiation in space introduces an additional source of oxidative damage to crew members during long-term Space missions. Rodent and human studies have shown that oxidative damage is elevated after space flights; while increasing the supply of dietary antioxidants may lessen the severity of post-flight oxidative stress (Stein, 2002). A diet supplemented with either strawberry or blueberry extracts for 8 weeks before exposure to elevated radiation protected rats from some of the reductions in brain function, which suggests that addition of antioxidiant-rich foods to the diet can increase protection against the deleterious effects of high-energy and charge particles in long-term space flights (Shukitt-Hale et al., 2007). Under this premise, the growing of antioxidiant-rich fruits and vegetables in space habitats has been proposed for long-duration missions not only to increase diet diversity, but also provide potential countermeasures to mitigate risks associated with space radiation (Smith and Zwarts, 2008).

Onion, an important agricultural crop, has strong antioxidation, anti-proliferation and anti-platelet activities. It is high in antioxidiant flavonoids, vitamins C and A as well as organosulfur compounds that are responsible for the distinctive pungency and flavor found in the Allium family (Block et al., 1992). Scallion (or green onion) is a less studied member of the Allium family, known to have almost 4 times the vitamin C and up to 5000 times the vitamin A (beta carotene) that is found in red, white, or yellow onions. Furthermore, it is unknown whether these quality traits may be maintained under the environmental conditions of elevated CO₂ and low light intensity expected in Space agriculture. Previous studies have found that elevated CO₂ results in an increase in photosynthesis (Richards et al., 2004), but a small decrease or no change in onion pungency per gram fresh weigh (Levine et al., 2005). The objective of this study was to evaluate how elevated CO₂ affects the antioxidiant capacity of scallions at a range of light intensities including those most likely encountered in space habitats. This study addressed the central question of how CO₂ enrichment and light regime may be used to enhance a crop’s nutraceutical value.

2. Materials and methods

2.1. Plant materials

Scallions (Allium fistulosum L. cv Kinka) were hydroponically grown from seeds in half strength Hoagland’s solution under cool white florescence lamps in an environmental growth chamber (Model M-48, EGC, Chagrin Falls, OH) with 50% relative humidity, a photoperiod of 16/8 light/dark, and constant day and night temperature of 25 °C (Richards et al., 2004). Approximately 250 seeds were evenly dispersed between fourteen 1 × 50 cm parallel slits in a 0.3 m² plant support tray. With 90% average germination rate, this resulted in a planting density of 750 plants m⁻². Each slit was fitted with a nylon Nitek® wicking material to support germination and growth. Plant support trays were then placed in 0.3 m² hydroponic trays. Seeds were sprayed with distilled water twice daily and covered with translucent covers for 5 days. Subsequently, newly germinated seedlings were exposed to 9 treatments: three light intensity levels of 150, 300, and 450 μmol m⁻² s⁻¹ (constant during light period) and three CO₂ concentrations of 400, 1200, or 4000 μmol mol⁻¹. These light intensities with a photoperiod of 16/8 light/dark resulted in a daily light integral (or daily PAR) of 8.6, 17.3 and 25.9 mol m⁻² d⁻¹, respectively. Experimental treatments and analyses performed are outlined in Fig. 1. Each experimental treatment was repeated twice in time. Thirty-four days after planting (DAP), eight plants from each treatment were sampled and divided into two groups to serve as technical replicates. Consequently, there were four sample replicates per treatment (2 experimental replicates × 2 technical replicates). Roots were removed, while shoots were immediately immersed in liquid nitrogen and subsequently lyophilized. The lyophilized tissue was ground using a Wiley mill to pass through a 40 mesh screen and stored in −80 °C over desiccants until analysis. For comparison, red and sweet white onion bulbs (Allium cepa L.), purchased from a local grocery store were also...
analyzed. The dried shell and the immediately adjacent layer were removed. The remaining material was quartered, lyophilized and prepared in the same way as for scallions.

2.2. Total antioxidant activity (TAA)

TAA was determined based on a diammonium 2,2’-azino-bis(3-ethylbenzothiazolin-6-sulfonate) (ABTS) assay (Re et al., 1999). Briefly stated, 30 mg of freeze-dried tissue was quantitatively extracted three times with one of three solvents (i.e. water, 50% or 75% ethanol (v/v)) and immediately assayed. ABTS (Sigma–Aldrich, Saint Louis, USA) was prepared as a 7 mM aqueous solution and reacted with 2.45 mM potassium persulfate to form an ABTS radical cation. The reaction stock was incubated up to 4 min. There was no significant difference in TAA of onion extracts between 1 and 4 min, thus 1 min was chosen as the end point. The percentage inhibition of absorbance was directly proportional to the extract volume or the antioxidant activity as determined by the ABTS assay, an end-point test to assess the capacity of samples for scavenging radical cations.

2.3. Total phenolics content

Total phenolic content was determined colorimetrically using an optimized Folin–Ciocalteu method (Prior et al., 2005 and references therein) and expressed as tannic acid equivalents.

2.4. Data analyses

Plant cultivars were arranged in growth chambers in a randomized design under stated light levels and carbon dioxide concentrations. Data were analyzed by the general linear model (GLM) procedure in the SAS statistical program and means separated using a least significant difference t-test at the 5% level.

3. Results

3.1. Plant development

Both elevated CO₂ and increasing light intensity enhanced plant biomass (Table 1). A doubling of biomass accumulation was observed when light intensity increased from 150 to 300 μmol m⁻² s⁻¹ for all CO₂ concentrations tested. Increasing light intensity from 300 to 450 μmol m⁻² s⁻¹ provided little growth stimulation for plants at 400 and 4000 μmol mol⁻¹ CO₂ although enhanced biomass was observed at 1200 μmol mol⁻¹ CO₂. Furthermore, CO₂ enrichment beyond 1200 μmol mol⁻¹ provided little or no benefit in terms of plant biomass under the range of light intensity examined, which has been repeatedly observed in other plant species (Levine et al., 2008 and references therein). The optimal vegetative growth was achieved under the highest light level (450 μmol m⁻² s⁻¹) and elevated CO₂ gave rise to slightly higher yield than conditions of high light (450 μmol m⁻² s⁻¹) and ambient CO₂ (400 μmol mol⁻¹). This suggests that CO₂ enrichment can be used to mitigate the low growth rates obtained at low light intensity.

3.2. Total antioxidant activity (TAA)

Onion extracts demonstrated a dose-dependent antioxidant activity as determined by the ABTS assay, an end-point test to assess the capacity of samples for scavenging radical cations in vitro. Antioxidants of varying chemical properties exist in onions; neither water nor alcohol extraction will provide a comprehensive assessment of the

<table>
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<tr>
<th>Light intensity (μmol m⁻² s⁻¹)</th>
<th>Atmospheric CO₂ concentration (μmol mol⁻¹)</th>
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<tbody>
<tr>
<td>150</td>
<td>1.4 ± 0.1</td>
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<tr>
<td>300</td>
<td>3.4 ± 0.2</td>
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<tr>
<td>450</td>
<td>4.1 ± 0.4</td>
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* Means ± SE (n = 16).
antioxidant capacity of the whole plant. In order to assess onion’s antioxidant activity more comprehensively, three extracts with increasing solvent strengths (water → 50% ethanol → 75% ethanol) were initially examined. No significant difference in TAA was found between the water and 50% ethanol extracts, while the 75% ethanol extract had twice the TAA of either water or 50% ethanol extract (Fig. 2). Subsequently, only the water and 75% ethanol extracts were examined in experimental tissues. The interaction CO2 and light on TAA is shown in Fig. 3 with several trends observed. First, ethanol (75%) extracts generally had twice the TAA of the water extracts of the same treatment. Second, elevated CO2 resulted in a reduction in the total antioxidant capacity of onion, but this negative effect was partially mitigated by higher light intensity (e.g. 450 μmol m−2 s−1). Third, similar to the impact of CO2 on biomass production, increasing CO2 from 1200 to 4000 μmol mol−1 (from elevated to super elevated) had little effect on TAA (5 out of 6 data sets). Lastly, increases in light intensity did not impact antioxidant capacity of water extracts at any given CO2 treatment, but resulted in changes in TAA of the 75% ethanol extracts to differing extents depending upon the CO2 concentration.

A comparative investigation of TAA among different types of onions showed that TAA of scallions is higher than that of sweet white onion, but significantly lower than that of red onion (Table 2). Antioxidant activities of six Allium members were previously evaluated for their inhibition of lipid oxidation using a liposome model and were found to be in the order of Chinese leek = shallot bulb > scallion = garlic bulb > onion bulb = garlic greens (Yin and Cheng, 1998). This is consistent with our findings that scallions have a higher antioxidant activity than sweet white onions. The freeze-drying process used in this study did not introduce any apparent artifacts with regard to the TAA (Table 2).

3.3. Polyphenols

Since a positive correlation between antioxidant activity and total phenolics or anthocyanin content has been observed in strawberry (Wang et al., 2003 and references therein) and fresh plums (Kim et al., 2003), total phenolics were also determined in the water and 75% ethanol extract of scallions. There were no differences in total phenolics contents between the water extract and aqueous ethanol extract of the same treatment in spite of the fact that the water extracted TAA was about half that of the ethanol extract.
extract (Fig. 4). The range of light intensities examined did not affect the total phenolics. The only significant effect was exerted by super-elevated CO₂, which caused the total phenolics level to decline (Fig. 4). A direct correlation was observed between total phenolics and the antioxidant activity ($R^2 = 0.5–0.6$) within the same extraction type but not across different solvent extracts (Fig. 5). This indicates that the antioxidant species that contribute to the TAA differ in these two scallion extracts.

### 4. Discussion

#### 4.1. Interaction of elevated CO₂ and light intensity on TAA

At each CO₂ concentration examined in this study, increasing the light intensity enhanced biomass accumulation, but did not significantly alter TAA with either water or 75% ethanol extraction (Fig. 3). Constant TAA levels with the increase of light intensity indicate that plants accumulated antioxidants and biomass at comparable rates. This is consistent with findings by Kleinhenz et al. (2003), in which there was no significant difference in anthocyanin concentration for two out of the four lettuce varieties tested between ambient light (250–450 μmol m⁻² s⁻¹) and 50% shading. Increases in light intensity provide more energy for plant photosynthesis on one hand, but has the potential to induce photo-oxidation or oxidative stresses in plants on the other hand. The unchanged TAA level is the result of increased synthesis of antioxidants in keeping with the increased need for antioxidant protection with an increase in light intensity. The range of light intensities examined in this study is relatively low compared to solar radiance that can reach 2000 μmol m⁻² s⁻¹ for full sun. It is likely that antioxidant accumulation rate would exceed that of the biomass accumulation resulting in an increase of TAA if the light intensity increased above 450 μmol m⁻² s⁻¹. Furthermore, the effect of light intensity may be more pronounced with the higher CO₂ levels.
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protection from oxidative damage, the antioxidant content may increase among plant species, but also among cultivars of the same species. Moreover, since plants synthesize and accumulate antioxidant compounds for the protection from oxidative damage, the antioxidant content may depend upon the status of plants’ oxidative stresses. As implied by decreases in activity of several key antioxidative enzymes such as superoxide dismutase, guaiacol peroxidase, and catalase, plants exposed to elevated CO₂ experience less oxidative stress (Vurro et al., 2009) and thus have a reduced need for antioxidant protection. On the other hand, elevated CO₂ allows for greater antioxidant synthesis via enhanced rates of photosynthesis and carbohydrate production, provided the plant is under oxidative stresses. For instance, there was a greater increase in activity of antioxidative enzymes with elevated CO₂-grown plants than ambient air-grown plants when grown under ozone (Rao et al., 1995), cold (Schwanz and Poll, 2001), or water stress (Lin and Wang, 2002). Previous reports on increased antioxidants under elevated CO₂ are mostly derived from field studies where plants are exposed to high solar radiation, and may experience greater photo-oxidative stress as well as other stresses such as UV, water and nutrient deficiencies. Indeed, sun-acclimated leaves contain higher ascorbate, glutathione and higher catalase activities than shade-acclimated leaf of sour orange trees after long-term exposure to elevated CO₂ (Schwanz et al., 1996), which might be due to increased photo-oxidative stress. Our study was conducted in environmentally controlled growth chambers via hydroponics technique where water and nutrient stresses were absent, and additional carbon from CO₂ enrichment is primarily funneled into plant growth with minimum investment in antioxidant compounds to maintain a basal defense level. Consequently, elevated CO₂ reduces the level of antioxidant compounds in the absence of stress.

The finding that elevated CO₂ favored the accumulation of biomass to antioxidant compounds and increasing light intensity up to 450 μmol m⁻² s⁻¹ stimulated the production of both biomass and antioxidants at a similar rate suggests that light is a more effective stimulus than CO₂ for antioxidant production. This result not only provides insight to the crop quality in term of antioxidative properties that would result from currently anticipated international space station environmental conditions (high CO₂, low light intensity due to the power constraints), but also constitute a first step in guiding the development of future plant production systems with both crop productivity and quality in mind. For instance, space agriculture could take the advantages of light emitting diodes (LED) because of their high electric efficiency, long lasting, safety and versatility in providing any wavelength combination. It has been demonstrated that Lettuce plants grown at 19.4 mol m⁻² d⁻¹ PAR under LED lights had approximately 30% greater total biomass than plants grown at the same light intensity under fluorescent lighting (Stutte et al., 2009). LED lighting has the potential to enhance anthocyanin and other antioxidant production by manipulating the proportions of different wavelength diodes including blue and ultraviolet (UV) (Caldwell and Britz, 2006; Stutte et al., 2009). More work is warranted on whether light intensity greater than those tested in this study and altered light spectrum such as the use of supplemental blue and/or ultraviolet (UV) light will offset the drawback of elevated CO₂ in declined TAA.

4.2. TAA contributing phytonutrients

The health promoting benefits from consumption of onions are generally agreed to be attributable to the presence of a class of organosulfur compounds (thiosulfinates, formed upon the disruption of onion cells followed by the action of alliinase on flavor precursors, alk(en)yl cysteine sulfoxides), generous amount of flavonoids (primarily quercetin), and vitamin C. Although scallion is also rich in vitamin A, because it is practically insoluble in water and only sparingly soluble in ethanol, it is not discussed here. Whether thiosulfimates possess antioxidant activity is still debatable in spite of the other claimed biological activities (e.g. anti-inflammatory). Both quercetin and vitamin C are potent antioxidants, having 2.27 and 0.45 μM Trolox equivalent, respectively, indicating that quercetin is about five times more potent than vitamin C as an antioxidant. Onion bulbs (Allium cepa L.) ranked highest in quercetin content in a survey of 28 vegetables and nine fruits (Hertog et al., 1992). In onion, quercetin occurs mainly as mono- and diglucosides, with highest levels in red and yellow onions and lowest in white onions ranging from 10 to 100 mg/100 g FW (Sellappan and Akoh, 2002; Smith et al., 2003). Lower quantities of quercetin (ca. one fifth of the level in white onions) and kaempferol are found.
in scallion leaves but not in bulb tissue (Bilyk et al., 1984). On the other hand, scallions have almost four times the vitamin C that is found in red, white, or yellow onions. Although the chemical composition in two extracts were not directly measured, the proportion of quercetin and other phenolic compounds in 75% ethanol extract may be higher than water extract because the extraction efficiency for quercetin and other phenolic compounds are higher in aqueous alcohol solution that water alone (Wach et al., 2007). This, along with the fact that quercetin has higher Trolox equivalent antioxidant capacity than ascorbic acid, explains the overall higher TAA in 75% ethanol extract than water extract. More quercetin and other phenolics in the 75% ethanol extract were expected to result in higher total phenolics value. In contrary to this expectation, total tannic acid equivalents in the water extract were found to be similar to that in the 75% ethanol extract (Fig. 4). It is known that total phenolics estimated by the Folin–Ciocânteau method is affected by the presence of sugars and ascorbic acid (Prior et al., 2005) that are readily soluble in water. Abundant sugars and ascorbic acid in scallion may contribute to an over estimation of total phenolics in the water extract.

Two major organosulfur flavor precursors, methyl cysteine sulfoxide (MCSO) and propenyl cysteine sulfoxide (PrenCSO), were detected in scallion (data not shown). The proportion of these two compounds differed in tissue type. Pseudobulbs had not only higher total flavor precursors, but higher ratio of MCSO to PrenCSO than leaves. Authentic compounds of these flavor precursors were tested for their capacity to scavenge the ABTS radical cation in vitro and exhibited no activity. Since flavor precursors can be rapidly converted to thiosulfinates in the presence of alliinase (EC 4.4.1.4), the question arises as to whether thiosulfinates have direct antioxidant activity. Allicin, a predominant thiosulfinate in garlic derived from alliin upon the action of alliinase is reported to be responsible for the antioxidant activity of garlic bulb (Prasad et al., 1995), but Yin and Cheng (1998) found that allicin concentration was not strongly correlated to the antioxidant activity against lipids in foods of Allium family. Furthermore, although the flavor precursors are readily extracted by water and aqueous ethanol, aqueous ethanol inhibits alliinase activity and makes the formation of thiosulfinate impossible. Consequently, regardless of whether thiosulfinates have antioxidant activity or not, organosulfur compounds should have no contribution to the TAA of the 75% ethanol extract. It is concluded that the higher TAA in 75% ethanol extract relative to that of the water extract most likely originated from polyphenolics.

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