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Effects of Calcium Fertilization on Growth, Yield, and Nutrient Content of Hydroponically Grown Radish Microgreens

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**EFFECTS OF CALCIUM FERTILIZATION ON GROWTH, YIELD, AND NUTRIENT
CONTENT OF HYDROPONICALLY GROWN RADISH MICROGREENS**

A Master's Thesis

Presented to

The Graduate College of
Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree
Master of Science, Plant Science

By

Cady Carson Goble

December 2018

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EFFECTS OF CALCIUM FERTILIZATION ON GROWTH, YIELD, AND NUTRIENT CONTENT OF HYDROPONICALLY GROWN RADISH MICROGREENS

Environmental Plant Science and Natural Resources

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Cady Carson Goble

ABSTRACT

Radish microgreens were grown in a hydroponic wicking system and subjected to a range of calcium chloride (Ca) solutions to evaluate growth, yield, and mineral content. A solution range of 0 to 160 mM Ca was applied to determine upper limits of Ca fertilization. Solutions above 20 mM Ca showed toxic effects to germination and growth, while 5 and 10 mM Ca resulted in the greatest percent of shoots that grew to a desired size for harvest (%H), as well as greater average hypocotyl length per plant (HL) and cotyledon surface area per plant (CSA). For subsequent experiments, Ca solution range was narrowed to 0 to 10 mM Ca. A single best rate of Ca was not identified in this range, but trends suggested that added Ca can increase average fresh weight per plant (FW), HL, CSA, and the percent of shoots that develop a first true leaf (%TL). Capmat II growing media resulted in a low %H across multiple experiments, but when vermiculite was added as a growing media, average percent germination (%G) and %H increased in all treatments. Differences in plant size and biomass did not occur until higher Ca concentrations when microgreens were grown in vermiculite. In general, Ca content of plant tissues increased with Ca solution concentration; however, the level of increase was dependent on the media and whether treatments were applied at the time of planting or delayed until after germination.

KEYWORDS: microgreens, radish, hydroponics, calcium, plant nutrition, fertilization

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In the interest of academic freedom and the principle of free speech, approval of this thesis indicates the format is acceptable and meets the academic criteria for the discipline as determined by the faculty that constitute the thesis committee. The content and views expressed in this thesis are those of the student-scholar and are not endorsed by Missouri State University, its Graduate College, or its employees.

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INTRODUCTION

Microgreens are a specialty crop that have been gaining popularity in recent years (Xiao et al., 2012). They are vegetable seedlings that are grown and harvested in a short amount of time, usually within 7 to 21 days after planting (DAP) (Sun et al., 2015). Microgreens are ready to be eaten when the cotyledons are fully expanded, and the first true leaves are just beginning to emerge. Many species of vegetables, herbs, and grains can be grown as microgreens. They are often used in the culinary industry for their wide range of colors, textures, and flavors (Xiao et al., 2012). In addition to their flavor and aesthetic value, they also have potential to contribute nutritional quality to the human diet. Researchers have even suggested that microgreens could contribute to functional diets for astronauts spending extended time in space (Kyriacou et al., 2017).

The importance of microgreens as a food crop ultimately lies in their high concentration of nutrients that are beneficial to human health. These tender, young plants often contain more phytonutrients, antioxidants, vitamins, and minerals than their mature counterparts (Pinto et al., 2015; Xiao et al., 2016). Their value as health-promoting foods has increased consumer demand. As awareness grows, commercial vegetable growers are embracing this niche market (Xiao et al., 2012), but there are unique challenges to growing microgreens when it comes to post-harvest handling and storage. This is due to the tendency of tender shoots to degrade quickly in storage conditions (Kou et al., 2015; Mir et al., 2016). Since shelf life is an extremely limiting factor in microgreens production, much of the research has focused on this aspect of production.

Research pertaining to microgreens production is limited, however studies are beginning to determine that there are a variety of pre-harvest factors that can affect growth rates, yield,

nutrient content, and post-harvest quality in microgreens. Some of these include various fertilization methods, lighting conditions, pre-germination methods, and growing media composition. Furthermore, the responses to the different factors mentioned above have been shown to vary between species and varieties, along with nutrient composition (Di Gioia et al., 2017; Mir et al., 2016; Xiao et al., 2016). Despite the recent research, and growing popularity, there is still a lack of standardization in the industry.

It is common for commercial microgreens producers to use hydroponic systems, but research to evaluate the influence of certain mineral nutrient concentrations in solution on plant growth rates, yields, and nutrient content is also limited. Hydroponic growing methods do however provide a method to precisely control nutrient availability when growing microgreens and provides an ideal method for studying the effects nutrition on microgreen production. The following literature review will examine existing research relating to factors influencing microgreens production, especially pertaining to mineral nutrition.

LITERATURE REVIEW

Assessment of Nutritional Value of Microgreens

Many species and varieties of vegetables, and herbs can be grown as microgreens. The nutrient content and composition of the mature stages of vegetable, herb, and grain crops are well documented. However, nutrient composition and concentrations at later stages of growth have been shown to differ from early growth stages when microgreens are harvested for consumption. Even though some studies have begun to contribute to the base of knowledge in microgreens nutrition, only a narrow range of species and varieties have been evaluated.

Xiao et al. (2012) evaluated the concentrations of certain vitamins and carotenoids in microgreens. Concentrations of phyloquinone (vitamin K₁), ascorbic acid (vitamin C), β -carotene (provitamin A), lutein/zeaxanthin, violaxanthin, and tocopherol, were measured in 25 different varieties of microgreens from different families, genera, and species. These were also compared to known concentrations in the mature forms of these plants. The concentrations of each of these vitamins and carotenoids varied greatly between species, and even within species. Despite the variation, 23 of the 25 varieties assayed proved to be good sources of these phytonutrients, and some were found to have higher concentrations of these when compared to their mature counterparts. For example, red cabbage microgreens possessed six-fold greater vitamin C than that of mature cabbage leaves (Xiao et al., 2012).

Pinto et al. (2015) compared the mineral profile of microgreen lettuce to that of mature lettuce. While mature lettuces had greater nitrogen (N), phosphorus (P), and potassium (K) content, microgreen lettuce was greater in other nutrients including 2.0x calcium (Ca), 1.9x iron (Fe), 9.3x manganese (Mn), 1.6x zinc (Zn), 2.5x molybdenum (Mo), and 5.3x selenium (Se). Furthermore, microgreen lettuce also exhibited lower concentrations of nitrate and ammonium,

making them safer for consumption, especially for children, who have higher risk of nitrate toxicity (Pinto et al., 2015).

More recently, Xiao et al. (2016) reported the mineral element composition and content of 30 microgreens varieties from the Brassicaceae family. They quantified the amounts of the macronutrients Ca, K, P, magnesium (Mg), and sodium (Na), along with the micronutrients Fe, Mn, Zn, copper (Cu), and heavy metals cadmium (Cd) and lead (Pb). Potassium was found to in greatest concentration of the macronutrients (176 to 387 mg/100 g fresh weight), followed by P (52 to 86 mg/100 g fresh weight), and then Ca (28 to 66 mg/100 g fresh weight). Iron was generally the most abundant micro element (0.47 to 0.84 mg/100 g fresh weight), on average, followed by Zn, Mn, and Cu. These results indicated that Brassicaceae microgreens are an overall good dietary source of macro-and microelements (Xiao et al., 2016).

The species and varieties of microgreens that have been analyzed for nutrient content have shown that they are good sources of nutrition in the human diet. Many species, such as lettuce and red cabbage, even have higher concentration of key nutrients than that of the mature leaves. Still, much more work is needed to identify nutrient profiles of the many varieties of microgreens currently available to growers and consumers.

Lighting Influence on Nutrient Content of Microgreens

Several researchers have evaluated the effects of light intensity, quality, and duration on the nutrient content of various species of microgreens. They have shown that light conditions can not only influence growth and morphology, but also concentrations of phytochemicals, vitamins, and minerals. The effects of lighting factors on microgreens are varied and species-specific (Kyriacou et al., 2016). Much research in lighting effects on nutrient content of microgreens

focuses on the use of light emitting diodes (LED), as this technology is quickly becoming a standard for indoor growers in many fields. LED lighting gives growers the ability to provide specific spectral wavelengths to meet specific requirements of desired horticultural crops (Morrow, 2008).

Kopsell and Sams (2013) measured the effects of exposure to either red and blue light ($350 \mu\text{mol m}^{-2} \text{s}^{-1}$) or blue light ($41 \mu\text{mol m}^{-2} \text{s}^{-1}$) on pigments in shoot tissues, as well as GLS and mineral elements. Broccoli microgreens were grown for 13 days in a complete nutrient solution before being exposed to light treatments for five days before harvest. Short term blue light increased concentrations of carotenoids (21%), xanthophylls (28%), and GLS (35%) in plant tissues. There were also increases in the micronutrients Cu (50%), Fe (64%), Mn (47%), Na (61%), Zn (35%), Mo (56%), and B (29%), and macronutrients Ca (38%), P (54%), K (65%), Mg (53%), and S (54%) in the blue light treatment. This study concluded that a specific wavelength of light could influence and even improve nutritional value of broccoli microgreens, when applied for a few days before harvest (Kopsell and Sams, 2013).

Effects of LED red/blue and red/blue/green ratios were further compared to fluorescent/incandescent bulbs on broccoli microgreens (Kopsell et al., 2014). It was concluded that total fresh weight of shoots was 6 to 55% greater in all LED treatments than in the fluorescent/incandescent treatment. All LED treatments increased chlorophyll a and b, total carotenoids, total GLS, as well as macro- and micronutrients, compared to fluorescent/incandescent lighting. A treatment of 20% blue to 80% red resulted in the greatest concentrations of most of the measured metabolites (Kopsell et al., 2014).

Samuoliene et al. (2013) measured the effects of a range of different photosynthetic photon flux densities (PPFD) from LED lighting on four different species of Brassicaceae

microgreens. The treatments included PPFDs of 110, 220, 330, 440, and 545 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The lower PPFD treatments resulted in decreased growth and nutritional value. In general, between 330 – 440 $\mu\text{mol m}^{-2} \text{s}^{-1}$ led to greater leaf surface area, anthocyanins, phenols, and free-radical scavenging capacity. This range also resulted in lower nitrates. The responses to these treatments varied between species, even though all four belonged to the same family. This has been reported to be a common occurrence when one study includes multiple species or varieties of microgreens (Samuoliene et al., 2013).

Brazaitye et al. (2013) investigated the effects of supplementary, short-term red LEDs on shiso (*Perilla frutescens*). For this experiment, the microgreens were grown primarily under natural daylight with supplemental high-pressure sodium (HPS) lamps for 20 days. For the last three days before harvest, red LEDs (200 $\mu\text{mol m}^{-2} \text{s}^{-1}$) were provided in addition to HPS lamps (90 $\mu\text{mol m}^{-2} \text{s}^{-1}$). After harvest, shoots were analyzed for ascorbic acid, flavanols, anthocyanins, alpha tocopherol, and nitrates. The addition of the red LEDs resulted in increased ascorbic acid (21%) and anthocyanins (25%) and decreased nitrates (20%) but did not affect flavanols or free-radical scavenging activity (Brazaitye et al., 2013).

Gerovac et al. (2016) investigated the effects of light intensity and quality from LED sources on growth, morphology, and nutrient content of three microgreens species in the Brassicaceae family. Treatments consisted of combinations of different percentages of red, far red, green, and blue light (light quality) resulting in 105, 210, and 315 $\mu\text{mol m}^{-2} \text{s}^{-1}$ between 400 and 800 nm for 16 hours each day. The results of these experiments revealed that light intensity had a greater overall impact on yield and nutrient content of the different varieties, though the results varied between species. Regardless of light quality, as light intensity increased, dry weight increased, but hypocotyl length decreased in all species. Additionally, as light intensity

increased, leaf area of cotyledons decreased, and relative chlorophyll content increased in Kohlrabi. Lower light intensities generally led to increases in macro- and micronutrient content. These results indicated that light intensity can affect growth, yield, and nutrient content of microgreens, and that LED lighting can provide the ability to customize light treatments to individual crop needs (Gerovac et al., 2016).

These studies in lighting factors have shown light quality and intensity can have significant effects on growth, development, yield, and nutrient content of microgreens. By using LED lighting, microgreens growers can customize the light that their plants receive to achieve the best possible growing conditions. They may also see an increase in important antioxidants, and other nutritionally important compounds. However, these studies also show variation in the responses of different species and varieties of microgreens, so further research is needed to determine requirements for specific microgreens varieties.

Cultural Methods in Microgreens Production

Most information on cultural methods of microgreen production is provided from seed suppliers, such as Johnny's Selected Seeds (Winslow, ME, USA). Some research has examined different methods to improve seed germination, establishment and growth by using different soilless growing media, fertilization, and various seeding rates and soaking methods. However, there is a lack of standardization in commercial methods for producing microgreens. Although hydroponic methods are common in microgreen production and are used in research methods, no clear standards or directions have been identified for hydroponic microgreens production.

For seed germination, Lee et al. (2004) evaluated a few methods of pre-germination soaks and matric priming as ways to improve germination, and seedling emergence and

development of microgreen beets and ruby red chard (*Beta vulgaris* L.). Germination was evaluated when seed balls were matrically primed in fine grade vermiculite, or soaked in deionized (DI) water, 0.3% H₂O₂ in DI water, 0.3% HCl in DI water, or 4% NaCl. The matric priming and H₂O₂ soak treatment resulted in the fastest germination. These two treatments were chosen for further comparison of seedling emergence, along with a control treatment where seeds were planted into vermiculite with no treatment. Additionally, seedling development was evaluated when seeds were planted in growing media after being treated with the two soak treatments, the vermiculite control and the combination of matric priming and soaking with pre-germinating in vermiculite. The results revealed that the seeds sown in vermiculite with no priming or soaking emerged at the same time or earlier than those that were treated with priming or soaking (Lee et al., 2004).

Murphy and Pill (2010) expounded upon some of the methods developed by Lee et al. (2004) to evaluate different seedings rates of arugula (*Eruca vesicaria*), as well as pre-planting fertilization of the growing media and post emergence fertilization, and the combination of both pre-planting and post emergence fertilization. Increasing the seeding rate increased total fresh weight of the arugula due to an increase in the number of shoots, but fresh weight per shoot declined. Lower seeding rates may result in larger individual shoots, however overall yield (total fresh weight) would be less. These results indicated it is more economically sensical to adhere to recommended rates of seeding (Murphy et al., 2010; Murphy and Pill, 2010).

These experiments also evaluated different sources of N fertilizers (calcium nitrate, ammonium nitrate and urea) in different states (solid or liquid), and at different concentrations (500 to 4,000 mg N L⁻¹) applied to the growing media before planting. Because liquid-state fertilizers were more evenly dispersed and readily available to be taken up by the plants, they all

resulted in greater fresh weight per plant than the solid-state fertilizers. All fertilizers resulted in increased total fresh weight and dry weight per plant when compared with no fertilizer treatment. Calcium nitrate yielded the highest fresh weight, followed by sodium nitrate, and then urea. Regardless of the N source, increasing the N concentration did increase fresh weight yields up to 2,000 mg N L⁻¹, with 4,000 mg N L⁻¹ decreasing fresh weights (Murphy and Pill, 2010). In the post-emergence fertilization study, a 21-2.2-16.6 fertilizer, diluted to provide 0, 75, or 150 mg N L⁻¹, was provided daily to germinated seedlings as an aqueous solution. Total fresh weight increased with fertilizer concentration, but fresh weight per shoot ultimately declined (Murphy and Pill, 2010).

The final experiment compared the combination of pre-germination and ungerminated seeds with the addition of pre-plant incorporated solid calcium nitrate at 2,000 mg N L⁻¹, or daily fertilization of 150 mg N L⁻¹ as 21-2.2-16.6. The other treatments consisted of the two fertilizers combined at full strength and at half concentration. Total fresh weight increased in all treatments when seeds were pre-germinated in vermiculite. All fertilizer treatments resulted in higher fresh weight than those with no fertilizer treatment, regardless of whether the seeds were pregerminated in vermiculite. Overall, the most effective treatment to increase yields of arugula microgreens was 2,000 mg N L⁻¹ calcium nitrate added to the media prior to planting with the addition of daily fertilizer solution of 150 mg N L⁻¹ (Murphy and Pill, 2010).

Another study by Murphy et al. (2010) revealed similar results in microgreen beet (*Beta vulgaris* L.) seeding rates. Sowing seeds at commercially recommended rates was more beneficial in total fresh weight yields than increasing or decreasing the rates. This study also further examined the treatment N fertilizer treatments that showed the best results in the arugula experiments, where seeds were grown in peat media pre-planting application of 2,000 mg N L⁻¹

calcium nitrate with the addition of a daily solution of 150 mg N L⁻¹. This was also tested at half strength (1,000 mg N L⁻¹ calcium nitrate plus 75 mg N L⁻¹). These treatments were evaluated when seeds were planted directly in the media or pre-germinated in vermiculite. The full-strength fertilizer treatment increased fresh weight per plant by 47% when seeds were pre-germinated in vermiculite, and 48% when seeds were not pre-germinated (Murphy et al., 2010).

Additional studies by Murphy et al. evaluated non-treated seeds with seed pre-germination in vermiculite or hydroxyethyl cellulose gel (HEC) and growth in peat growing media versus nutrient film technique (NFT) hydroponic culture. The nutrient solution used for the NFT system was comprised of hydrosol 5-4.7-22 (1 g L⁻¹), calcium nitrate (1 g L⁻¹), magnesium sulfate (0.1 g L⁻¹), and Fe chelate (0.04 g L⁻¹). Nutrient film technique hydroponic culture produced greater fresh weight per plant than peat growing media, ranging from 33% to 98%, depending on seed treatment (Murphy et al., 2010).

The previous two studies showed fertilizer solutions and NFT hydroponic culture can affect the fresh weight yields of a specific variety of microgreens. Another study by Bulgari et al. (2017) assessed the yield and nutrient content of three microgreens varieties grown in a half strength Hoagland's solution in a hydroponic system. Three species: basil (*Ocimum basilicum*), swiss chard (*Beta vulgaris*), and arugula (*Eruca vesicaria*) were grown in vermiculite media-filled polystyrene cell trays in a floating hydroponics system. Microgreens were evaluated for mineral uptake and content, and nitrate, pigment (chlorophyll, carotenoids, phenols, and anthocyanins), and sugar content. The results indicated different responses between varieties in growth, yield, and content of most mineral nutrients, pigments, and sugars. The variation of the results is consistent with other research using multiple species and varieties, and it shows that

there is still much to be learned about individual nutrient requirements and nutrient content of microgreens (Bulgari et al., 2017).

Different growing medias have been compared by Di Gioia et al. (2017) using *Brassica rapa* L. microgreens are often grown in a peat-based growing mix or Sure to Grow® fabric mats (polyethylene terephthalate), however there are presumably more sustainable substrates that can be used. The growing substrates compared in this experiment were recycled textile fiber (TF) mats made from recycled polyester and cotton fibers, jute-kenaf fiber mats (JKF) made from compostable recycled fibers, Sure to Grow® (STG) mats, and a 50/50 mixture of fine black and white peat mosses. Bulk densities of the medias were all within a desirable range, with peat being the highest, followed by TF, JKF, then STG mats. Peat had the highest total pore space and water holding capacity, but the lowest air capacity. Water extracts from water activity tests revealed TF, JKF, and STG had significantly lower concentrations of Na, Cl, and SO_4^{2-} , suggesting that nutrient supply could be controlled and customized more easily in TF, JKF, and STG mats to meet plant needs (Di Gioia et al., 2017).

The type of growing media did not affect the number of shoots that grew, and fresh weight was only reduced in the STG pads. The highest fresh weight per shoot resulted from peat, followed by TF, JKF, and STG. These results indicate that the type of media used can affect the yield of microgreens. Furthermore, mats made from recycled fibers could potentially provide a sustainable alternative to popular growing medias (Di Gioia et al., 2017).

Recent research lends further evidence that responses to cultural methods of microgreens production is varied by species and variety but does suggest that providing supplemental nutrition to microgreens may improve yields. Still evident after these studies is that the microgreens industry still lacks standardization in production methods.

Factors Influencing Shelf Life of Microgreens

Shelf life of fresh-cut microgreens has proven to be one of the most limiting factors in commercial microgreens production. Fresh cut fruits and vegetables are affected by several post-harvest stress factors, both internal and externally. These include increased respiration rates and ethylene production, along with abiotic factors such as physical injury from processing or chilling injury from low temperature storage (Hodges and Toivonen, 2008). Plants harvested in a young stage of growth are more sensitive to abiotic stress factors that occur during harvest, washing, and storage than that of mature vegetables (Watkins and Nock, 2012). These stress factors, along with microbial populations that speed up the senescence process, can decrease the amount of time that microgreens remain edible after harvest (Chandra et al., 2012). Sanitizers, packaging materials, storage lighting, and Ca foliar sprays have been investigated in order to identify the best post-harvest processing and storage methods to achieve maximum shelf life for microgreens.

Different sanitizers and packaging were evaluated by measuring microbial populations and post-harvest quality of *Brassica campestris* var. *Narinosa* microgreens. Microgreens were washed with tap water (TW), 100 ml L⁻¹ chlorinated water (Cl), 0.25% (w/v) each of citric and ascorbic acid mixed solution (CA + AS), or 0.5% (w/v) citric acid solution followed by 50% (v/v) ethanol spray (CA + E). The microgreens were then packaged in either polyethylene (PE) or polypropylene (PP) bags. They were stored for nine days at 5°C, while being monitored for CO₂ formation within the bags, color, electrolyte leakage, and microbial populations. Sensory evaluations were also performed by a panel to determine levels of off-odor and visual quality. The PE packaging yielded the best results with lower CO₂ formation and less electrolyte leakage, most likely due to increased permeability of PE films compared to PP films. For

sanitizers, CL and CA+E treatments decreased CO₂ formation and exhibited lower aerobic bacteria counts. Regardless of treatment and packaging, off-odor and visual quality declined past marketable quality by day five (Chandra et al., 2012).

Xiao et al. (2014) evaluated the effects of light exposure on the concentration of ascorbic acid, carotenoids and tocopherols, antioxidant activity, as well as visual quality and off-odor of daikon radish (*Raphanus sativus* var. *Longippinatus*) microgreens under storage conditions. In addition to this, they monitored gas composition in package headspace, and weight loss of plant material. They also compared these results in two different types of packaging: polyethylene (OTR), and laser microperforated polypropylene (LMP). The stored microgreens in each packaging type were subjected to a treatment of fluorescent light ($\approx 30 \mu\text{mol m}^{-2} \text{s}^{-1}$) or no light ($\approx 1 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 16 days (Xiao et al., 2014).

Gas composition in the LMP bags remained stable at atmospheric levels and were not affected by the light or dark treatment. In the light treatments, CO₂ level in OTR packaging remained unchanged from day zero to day 12, but slightly increased in the last four days. Under dark conditions, CO₂ levels rose sharply in OTR packaging from day zero to day four and remained constant after that. Visual quality of microgreens stored in dark conditions in OTR packaging proved to be acceptable by a panel even at 16 days, whereas OTR samples stored in light conditions and both light treatments with LMP packaging declined over time. After four days of storage, the LMP packaging resulted in lower off-odor scores than the OTR packaging, which was likely attributed to the permeability of LMP film. Dark treatments maintained fresh weight close to that of day zero but declined significantly in light treatments through the course of the experiment. Samples stored under light conditions generally resulted in higher total ascorbic acid by day 16, whereas dark storage helped maintain higher levels of carotenoids,

antioxidant activity. Other bioactive compounds were unaffected by the treatments. These results indicate that storage conditions such as light and packaging can have effects on the physical and nutritive properties of radish microgreens (Xiao et al., 2014).

The application of Ca is commonly used to slow the ripening process in fresh fruits and vegetables. This is likely attributed to its role as a second messenger in decreasing the expression of senescence-associated genes (Marschner, 2012; Kou et al., 2014). Because of this, recent research in microgreens production and shelf life have centered around application of Ca salts to microgreen shoots both pre- and post-harvest. Kou et al. (2014) conducted two experiments in Ca application with the objective of improving shelf life and post-harvest quality.

Kou et al. (2014) evaluated the effects of nutrient foliar sprays applied before harvest on shelf-life and quality of broccoli (*Brassica oleracea*) microgreens. Concentrations of 1, 10, and 20 mM CaCl₂ or MgCl₂, or 5 mM ethylene glycol-tetraacetic acid (EGTA) were applied to shoots daily for 10 days before harvest. After harvest and weighing, tissue samples were taken for separate analysis of shoot Ca concentrations. Fresh samples were placed into polyethylene bags and stored at 5 °C for 21 days. Gas composition, electrolyte leakage, microbial populations, as well as antioxidant activity were measured throughout the experiment. This experiment also evaluated the effects of treatments on expression of genes known to be associated with senescence (Kou et al., 2014).

The results of this experiment revealed that 10 mM CaCl₂ foliar spray led to greater hypocotyl length (27%), fresh weight (54%), and dry weight (24%) than the water treated control, and the cotyledons were larger overall. Ca concentration in the shoots also increased 160% in the 10 mM Ca treatment compared to the water treated group. O₂ loss was slower, and electrolyte leakage was less in 10 mM Ca, as well. Results of visual quality and off-odor scores

revealed that 10 mM Ca retained a higher quality than the other treatments after day seven, up to day 21. All Ca treatments led to significantly lower aerobic bacterial growth than the water control. Compared to water, the 10 mM Ca exhibited significantly lower expression of two out of three senescence-associated genes, which slowed degradation of plant tissues in storage. Overall, these results indicate that the degree to which Ca affects growth and development is dependent on dosage, and that pre-harvest Ca sprays can improve the post-harvest quality of microgreens, leading to a longer shelf life (Kou et al., 2014).

Further studies examined different sources of Ca applied to broccoli microgreens. Treatments consisted of pre-harvest foliar sprays of different forms of Ca, post-harvest sprays and dips, and the combination of pre- and post-harvest treatments. The pre-harvest treatments were 1, 10, or 20 mM Ca lactate, Ca amino acid chelate (Ca AA) solutions, or 10 mM CaCl₂. The post-harvest treatments included 0, 25, 50, or 100 μmol L⁻¹ Ca lactate plus 100 μL L⁻¹ chlorine (Cl). The remaining treatments consisted of varying combinations of the pre- and post-harvest treatments. Post-harvest quality, gas composition, electrolyte leakage, and microbial activity were measured, as in the previous experiment (Kou et al., 2015).

Ca lactate and Ca AA pre-harvest sprays significantly reduced microbial populations of harvested microgreens compared to H₂O controls but were still less effective than 10 mM CaCl₂ pre-harvest spray alone. The 10 mM CaCl₂ pre-harvest spray also led to the best scores in visual quality and off-odor along with post-harvest dip in 50 mmol L⁻¹ Ca lactate (+100 μL L⁻¹ Cl), compared to all other treatments. Overall, the CaCl₂ pre-harvest foliar spray led to better results in all aspects having to do with prolonged shelf life. Still, there is a need for less damaging washing and drying procedures, as they can negatively affect shelf life and quality (Kou et al., 2015).

A more recent study by Lu et al. (2018) evaluated the effects of a CaCl₂ foliar spray in addition to short-term, post-harvest exposure to UV-B lighting on the shelf life, and level of Glucosinolates (GLS) in packaged broccoli (*Brassica oleracea* var. *Italica*) microgreens. Microgreens were sprayed daily with H₂O (control), 1 or 10 mM CaCl₂ foliar spray. After harvest, the packaged microgreens samples were either placed into dark storage or exposed to either 0.09 or 0.18 Wh/m² UV-B light for 2 hours, before being stored in the dark at 4° C for 21 days. Visual quality, off-odor, gas composition of packaging, and tissue electrolyte leakage were measured throughout the experiment. Freeze dried tissue samples were used for analysis of GLS. Results were compared to that of mature broccoli florets and leaves (Lu et al., 2018).

The results indicated that all samples treated with 10 mM Ca provided the best post-harvest quality after day seven with regards to off-odor and visual quality, with light treatment having no effect. The concentration of total GLS (predominantly glucoerucin) was 20-fold higher in microgreens than mature plant tissues, which further confirms previous claims that seedling vegetables are higher in certain phytonutrients than mature forms of the plants. The application of 10 mM Ca increased total GLS in microgreens and was determined to have more of an effect on all measured factors than the application of UV-B lighting (Lu et al., 2018).

Each of these studies have indicated that there are many factors that can influence shelf life and post-harvest quality of fresh cut microgreens, including packaging material, light exposure, sanitizer sources, and pre-harvest Ca foliar sprays. Furthermore, Ca sprays have also been proven to have significant effects on growth, yields and concentrations of certain important nutrients in microgreens. There is a continued need to study factors that affect microgreen shelf life, especially washing and drying procedures, and how different species and varieties respond. Additionally, there is a lack of knowledge about how CaCl₂ concentration could affect yields

and mineral content of microgreens when applied directly to the roots, as opposed to a foliar spray.

Calcium in Microgreens Production

Research in microgreens involving Ca applications focused on shelf life and post-harvest quality. However, the experiments involving these treatments also saw significant effects on yield when Ca was applied as a foliar spray to plants, particularly a foliar spray of 10 mM CaCl₂ concentration. This Ca treatment has been shown to significantly improve shelf life, storage quality, nutrient content, and yields (Kou et al., 2014).

Kou et al. (2014) applied Ca as a foliar spray to broccoli microgreens to evaluate their ability to delay senescence and promote longer shelf life. The results of this experiment not only revealed that 10 mM Ca can increase shelf life, but it also increased cotyledon surface area and hypocotyl length, resulting in 50% greater fresh weight and 25% increased dry weight to 0 mM Ca. The 1 mM Ca foliar spray did not affect growth, while 20 mM Ca was toxic with yellowing cotyledons. Increasing Ca concentration in the spray also resulted in an increase in shoot Ca content after harvest, with 10 mM Ca increasing shoot Ca content almost 160% over the water-treated plants. Due to this response of microgreen yield and Ca content to foliar Ca applications by Kou et al. (2014), the current research evaluated the impacts of Ca availability to roots of hydroponically grown microgreens.

Role of Calcium in Plant Growth and Development

The crucial role of calcium in plant growth and development is diverse and well documented. Calcium is involved in a host of cellular functions and contributes to structural

integrity of cell walls and membranes. It binds with pectate in the middle lamella of cell walls, to provide structural support and strength, and to prevent build-up of compounds that promote cell wall degradation (Marschner, 2012). It functions as an intracellular signaling mechanism that triggers physiological responses to stress and developmental cues such as germination and root hair elongation (Anil and Rao, 2001; Marschner, 2012; White and Broadley, 2003).

Some of the stimuli for changes in cytosolic free Ca²⁺ include aspects of light exposure, temperature changes, osmotic stress, cell division and apoptosis, germination, senescence of tissues, and much more. These changes in cytosolic Ca serve as intracellular messages, triggering physiological responses to the various stimuli and stress factors that plants may experience, and each response is unique in its duration, periodicity, or amplitude (White and Broadley, 2003).

There are three different physiotypes that plants are categorized into according to their specific Ca needs. Calcitrophs belong to plant families that have higher soluble Ca in their tissues. Calcium accumulation in the shoots of calcitrophs can be directly related to the amount of Ca available in the soil solution or growing media. Plants in the Brassicaceae family belong to the calcitroph physiotype and are popular plant families used in microgreens production (White and Broadley, 2003).

Research Objective

The objective of this research was to identify the optimal fertilization rate of CaCl₂ for growth, yield, and nutrient content of hydroponically growth daikon radish (*Raphanus sativus*) microgreens. Radish is a member of the Brassicaceae family and is popular in microgreens production for its fast growth rates and ease of production. The use of radish for this study allowed for multiple experiments to be completed within a relatively short amount of time.

Utilizing a hydroponic growing environment allows nutrients to be made available at precise and continuous concentrations via the nutrient solution. Calcium has been shown to increase yields and shoot Ca concentration when applied to microgreens as a foliar spray (Kou et al., 2014), but to my knowledge no one has evaluated the effects of root-applied Ca to hydroponically grown microgreens to determine optimal Ca fertilization rates for production.

MATERIALS AND METHODS

Experiment One: Broad Range Ca Application

Daikon radish (*Raphanus sativus*) microgreens seeds purchased from Johnny's Selected Seeds (Winslow, ME, USA) were grown in a hydroponic wicking system, using Rubbermaid® TakeAlongs 669 ml food storage containers (Newell Brands Inc. Atlanta, Georgia, USA). Slits were cut on opposite sides of each lid and a 2.5 x 33 cm strip of Capmat II capillary matting (Phytotronics, Inc.® Earth City, MO, USA) was inserted through the slits, allowing it to hang down into the container and wick solution up from inside the container to the seedbed on top of the lid (Figure 1A). A 14 x 14 cm square of matting was placed on top of the lid and the strip to act as the seed bed (Figure 1B). Seeds were dispersed evenly on top of the lids and matting at a rate of 3.3 g per container, equaling about 250 seeds per container (Figure 2). Seeding rate was estimated based on 100 seed count average weight and on seeding recommendations of Johnny's Selected Seeds for 8 to 10 seeds per 2.5 cm² (Johnnys Selected Seeds, 2017).

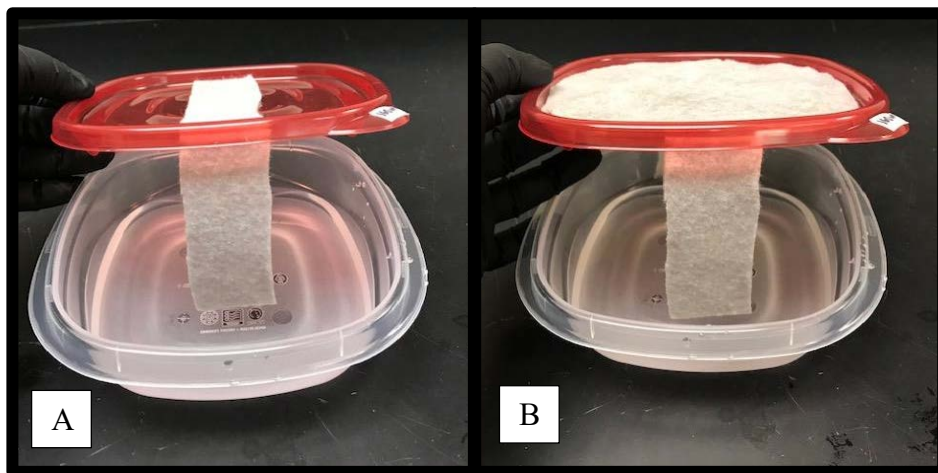


Figure 1. Hydroponic setup for Experiments One through Four with (A) wicking strip and (B) square of capillary matting for seed bed.



Figure 2. Radish seeds distributed on capillary matting.

Treatment solutions of 0, 5, 10, 20, 40, 80, 160 mM Ca were made from calcium chloride (CaCl_2) anhydrous powder (ACROS Organics-Thermo Fisher Scientific, Waltham, MA, USA) and DI water filtered with a Barnstead E-Pure™ filtration system (Thermo Fisher Scientific, Waltham, MA, USA). Each container held 300 ml of treatment solution. The seeds were placed on the capillary mat seed bed with the wicks reaching the treatment solutions, and the containers were then placed in a Percival model LED-41L2 growth chamber (Percival Scientific, Inc. Perry, IA, USA) where temperature and lighting parameters could be controlled. Three replications of each treatment were completely randomized on the two shelves of the growth chamber. Temperature was maintained at 21.5 °C. Lights were turned on when hypocotyls began to elongate, and cotyledons had fully emerged. This stage of growth occurred around three days after planting. Light levels were maintained near $350 \mu\text{mol m}^{-2} \text{s}^{-1}$ at plant level using 12 fixed

cool white (90% power) and red (20% power) dimmable LED lights, for a 12 hour on and off cycle. The microgreens were monitored and photographed every day.

The radish microgreens were ready for harvest when the cotyledons were fully expanded, and the first true leaves were beginning to emerge (Kyriacou et al., 2016). At seven DAP, the plants were at harvest stage. To harvest, each shoot was cut at the base, where the hypocotyl meets the radicle. Fresh weight per container was measured, then each sample was submerged six times each in a series of three DI water rinses. The number of shoots that reached the first true leaf stage, along with the number of seeds that did not germinate, and the number of seeds that stopped growing after radicle emergence were counted and used to determine the percent germination, and the percent of shoots that made it to first true leaf stage.

A subsample of 20 to 30 plants (depending on the number of harvestable shoots present) was taken from each treatment for hypocotyl and cotyledon analysis. Subsample size (n) was recorded to calculate per-plant measurements. Cotyledons and hypocotyls of each plant in the subsample were separated and laid flat on a plexiglass tray (Figure 3). A penny was used as a standard in each scan, to make sure analyses remained consistent across experiments. The tray was placed into a Regent Scanner (Regent Instruments, Quebec, Canada) that captured color and black-and-white images. Winrhizo software (Regent Instruments, Quebec, Canada) was used to analyze the images to determine average hypocotyl length and cotyledon surface area per plant. The plants from the subsample were then added back to the main sample, and the tissues were dried in a forced air oven at 46 °C and dry weight was measured. Tissue samples were ground to a fine powder using a modified coffee grinder and placed into labeled 20 oz Whirl-Pack® bags (Nasco, Fort Atkinson, WI) for storage.

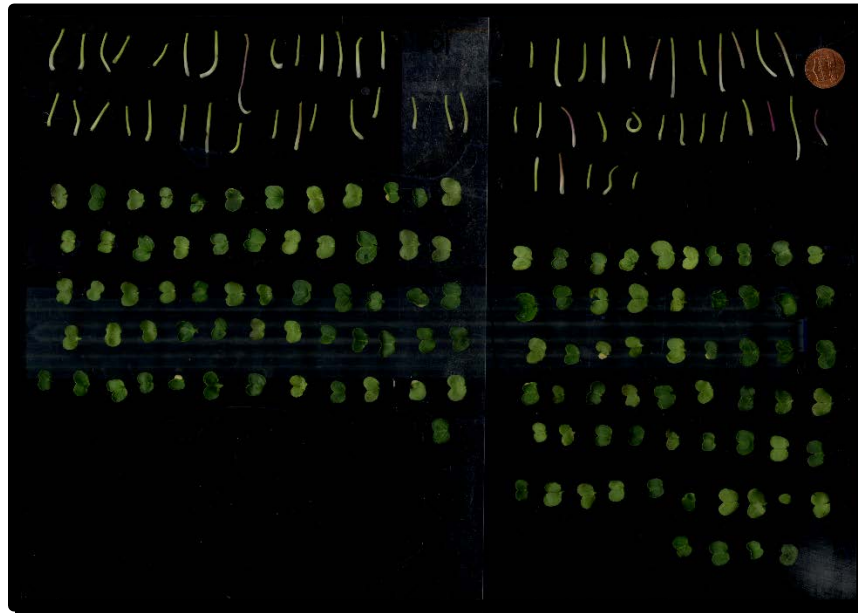


Figure 3. Hypocotyls and cotyledons arranged on the scan tray for analysis.

Experiment Two: Narrow Range Ca Application

For Experiment Two, the hydroponic system was the same, except the solution range was narrowed to include 0, 2.5, 5, 7.5, and 10 mM Ca, with 250 ml of solution added to each container on the day of planting. Each treatment was replicated four times, and the containers were arranged randomly with two replications per shelf in the growth chamber. The microgreens were monitored and photographed daily and harvested seven days after planting. One tray from each treatment was randomly selected to measure solution pH and electrical conductivity (EC) at the beginning and end of the experiments using an Ohaus Starter 300 pH meter (Ohaus Corp., Parsippany, NJ) and a Bluelab conductivity pen (Bluelab Corp. Lmt., New Zealand).

Experiment Three: Delayed Narrow Range Ca Application

Experiment Three was a repeat of Experiment Two, except that the seeds were treated with acid scarification prior to planting. For acid scarification, 3.3 g of radish seeds for each

container were placed into individual 50 ml centrifuge tubes (Fisher Scientific Co LLC, Hanover Park, IL, USA) with small holes in the bottom for drainage. Each tube was submerged in ACS grade concentrated sulfuric acid (Fisher Science Education, Nazareth, PA, USA) for two minutes, followed by three rinses in a DI water. The tubes were then submerged three times in a saturated sodium bicarbonate solution, followed by a continuous flow DI water rinse for two minutes. The seeds were air dried overnight, before being placed onto the growing media.

Another difference from Experiment Two was that the Ca treatments were delayed. At the time of sowing, 300 ml DI water was added to each tray. After three days, at the stage the radicles had elongated and the cotyledons had begun to emerge, the DI water was replaced with the Ca solutions of 0, 2.5, 5, 7.5, and 10 mM. Solutions were added at a rate of 250 ml per container.

Experiments Four A and B: Delayed Ca Application with Subplots

In Experiment Four, each container was divided into four quadrants (four subplots) by drawing lines on the capillary matting. The methods, treatments and number of containers remained the same as Experiment Three. The containers were arranged in a randomized complete block design with blocks one and three on the top shelf of the growth chamber and blocks two and four on the bottom shelf. The same factors were measured and recorded, but they were measured per subplot, rather than per container. Solution pH and EC container were measured at the beginning and end of the experiments as in Experiment Two. This experiment was repeated two times (Experiments Four A and Four B).

Experiment Five: Delayed Narrow Range Ca Application with Vermiculite

In Experiment Five, vermiculite was added as a growing medium. In this hydroponic system, 2.5 cm-wide capillary matting strips were fed through two 2.5 cm slits in the bottom of a 669 ml Rubbermaid® TakeAlongs container. The 669 ml container was nested into a larger, 1.2 L Rubbermaid® TakeAlongs container that held the treatment solutions. Another 14 x 14 cm square of capillary matting was placed in the bottom of the smaller container, and 2.5 cm depth of Sunshine® premium grade, medium-textured vermiculite (Sun Gro, Inc. Agawam, MA, USA) was added on top of the matting (Figure 4A). The 2.5 cm capillary matting strips wicked the solution to the capillary mat square, and then throughout the vermiculite. Strips of plastic were cut to three cm widths and placed into the containers to divide them into four subplots (Figure 4B). Use of vermiculite as a growing media eliminated the need to pre-treat seeds with acid scarification. Non-scarified seeds were evenly distributed at a rate of 3.3 g per container and covered with a light layer of vermiculite (enough to cover the seeds). At the time of sowing, 300 ml DI water was added to each tray. Three DAP, the DI water was removed, and Ca solutions of 0, 2.5, 5, 7.5, and 10 mM were applied at 250 ml per container. Four replications of each treatment were arranged in a randomized complete block design in the growth chamber. The microgreens were monitored and photographed daily and harvested six DAP. The same factors as Experiments One through Four were measured and recorded.

Experiment Six: Delayed Broad Range Ca Application with Vermiculite

The same hydroponic system setup from Experiment Five was applied. Microgreens were provided with only DI water for the first two days. Three DAP, treatment solutions of 0, 5, 10, 15, 20, and 25 mM Ca were added. For this experiment, there were three replications of each

treatment, arranged in a randomized complete block design, with blocks one and two placed on the top shelf of the growth chamber, and block three on the bottom shelf. Each container was divided into four subplots. The microgreens were monitored and photographed daily and harvested six days after planting. The same measurements were collected as in the previous experiments in each subplot. Solution pH and EC were measured at the beginning and end of the experiments as in Experiments Two and Four.

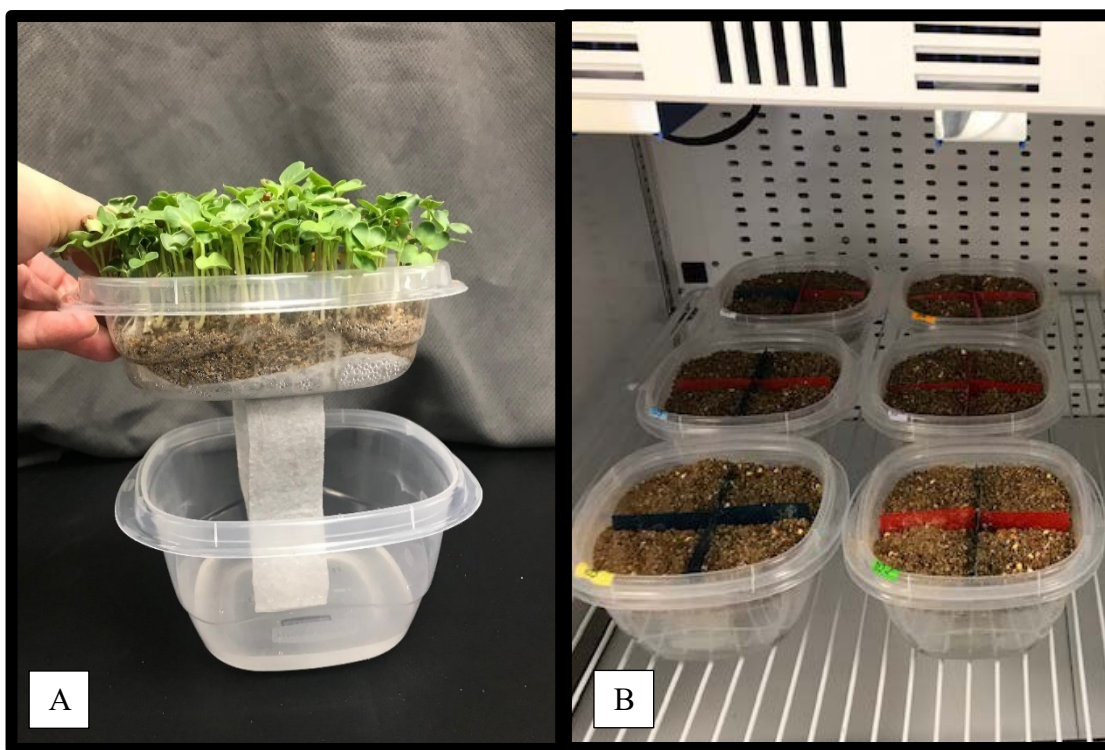


Figure 4. Hydroponic setup for Experiments Five and Six using vermiculite media.

Mineral Nutrient Analysis and Statistical Analysis

Finely ground, dried tissue samples of $0.2500\text{g} \pm 0.005\text{g}$ were digested in 5ml trace grade nitric acid (Thermo Fisher Scientific, Waltham, MA, USA) using a MARS 6 Microwave Accelerated Reaction System (CEM Corp., Matthews, NC, USA). This system consisted of a 25-

minute ramping stage to 200 °C. The temperature is maintained for 10 minutes. Samples were then cooled to 70 °C, before the system was ventilated. After cooling for another one to two hours, the samples were brought to 25 ml volume with DI water, and filtered using Q2 fine, slow flowing, 11cm filter paper (Thermo Fisher Scientific, Waltham, Massachusetts, USA) into 25 ml polypropylene scintillation vials. Digested samples were used to analyze mineral nutrient concentrations on a percent by weight basis.

Shoot Ca, Mg, and K concentrations of the digested samples were determined using Atomic Absorption/ Flame Emission Spectrophotometry (Agilent Technologies, 200 Series AA, Santa Clara, CA, USA). The analytical wavelengths were 422.7 nm for Ca, 285.2 nm for Mg, and 766.5 nm for K. Digested samples were diluted at a 1:20 ratio with 0.105% lanthanum oxide (La). Agilent Technologies Ca, Mg, and K standards prepared with 0.105% La and 1% trace grade nitric acid background were used to set absorbance curves. Ca standard concentrations were 1.00, 2.00, 3.00 and 4.00 ppm; Mg 0.25, 0.50, 1.00, 1.50, and 2.00 ppm; K 0.25, 0.50, 1.00, and 2.50 ppm.

Phosphorus concentrations were determined colorimetrically using a GENESYS™ 10S UV-Vis spectrophotometer, a Masterflex sipping system, and VISIONlite software (Thermo Fisher Scientific, Waltham, MA, USA). Absorption was set at 660 nm. Samples were diluted at 1:20 or 1:40 ratios with DI H₂O and then four ml ascorbic acid working solution was added (Murphy and Riley, 1962). The samples were then vortexed and allowed to incubate for 20 minutes before analysis. Phosphorus standards from Ricca Chemical Company, LLC (Arlington, TX, USA) were used to make standard solutions of 0.00, 0.50, 1.00, 2.50, and 5.00 ppm concentrations in DI H₂O. These were used to set the standard curve for P analysis.

Data collected in these experiments were analyzed for significant effects and interactions using a general linear model procedure in SAS version 9.4 (SAS Institute, 2017). Calcium treatments were fixed factors across all experiments. Block was an additional fixed factor in experiments Four through Six. When P value was significant ($\alpha=0.05$) for main effects, Tukey's pairwise comparison was used to compare treatment means. When there was a significant interaction ($\alpha=0.05$) between treatment and block, treatment main effects could not be used for pairwise comparison. Presence of significant interactions are indicated in the results reported.

RESULTS

pH and Electrical Conductivity of Ca Solutions

Electrical conductivity and pH were measured at the beginning and end of experiments Two, Four (A and B), and Six (Tables 1, 2, 3, and 4). pH decreased from the beginning to the end of each experiment, except in Experiment Four A. Regardless of the changes in pH, it generally remained near a desirable level (about 5.5), except in a few treatments where it was slightly lower. Electrical conductivity was also variable, but typically increased slightly over time.

Experiment One: Broad Range Ca Application

Average percent germination (%G) was 100% when seeds were grown in 0 mM Ca, with a significant decline 10 mM Ca and again at 160 mM Ca, where it was as low as 10% (Table 5). There were no significant differences in fresh weight per plant (FW) in the range of 0 to 40 mM Ca (Table 5). At concentrations lower than 40 mM Ca, differences in dry weight (DW) were variable between treatments with no clear growth curve (Table 5). No shoots were developed to a harvestable stage in the 80 and 160 mM Ca treatments (Table 5). Average hypocotyl length per plant (HL) increased in increments of close to 0.5 cm between 0, 5, and 10 mM Ca treatments, before beginning to decline at 20 mM Ca (Table 5). Results of average cotyledon surface area per plant (CSA) were similar to HL with an increase of 18% (0.2 cm²) from 0 to 10 mM Ca, and a slight decrease at higher Ca concentrations (Table 5).

Table 1. pH and EC measured at the beginning and end of Experiment Two.

Treatment mM Ca	0	2.5	5	7.5	10
Starting pH	8.68	5.55	5.57	5.59	5.62
Ending pH	6.46	5.44	5.20	5.21	5.29
Starting EC	0	280	430	620	1010
Ending EC	0.	290	430	830	1150

EC measurements expressed as $S \cdot m^2 \text{ mol}^{-1}$

Table 2. pH and EC measured at the beginning and end of Experiment Four A.

Treatment mM Ca	0	2.5	5	7.5	10
Starting pH	6.46	5.85	5.68	5.60	5.56
Ending pH	7.65	5.91	5.77	6.06	6.01
Starting EC	0	230	550	720	1160
Ending EC	0	280	570	870	1210

EC measurements expressed as $S \cdot m^2 \text{ mol}^{-1}$

Table 3. pH and EC measured at the beginning and end of Experiment Four B.

Treatment mM Ca	0	2.5	5	7.5	10
Starting pH	8.94	5.43	5.68	5.61	5.98
Ending pH	8.51	5.27	5.58	6.51	6.51
Starting EC	0	270	540	750	1220
Ending EC	0	190	480	780	1150

EC measurements expressed as $S \cdot m^2 \text{ mol}^{-1}$

Table 4. pH and EC measured at the beginning and end of Experiment Six.

Treatment mM Ca	0	5	10	15	20	25
Starting pH	8.94	5.43	5.40	5.43	5.53	5.59
Ending pH	8.51	5.27	5.20	5.18	5.13	5.11
Starting EC	0	580	800	1750	1980	2750
Ending EC	0	570	1210	1790	2380	2800

EC measurements expressed as $S \cdot m^2 \text{ mol}^{-1}$

Mineral nutrient analysis for Experiment One revealed incremental increases in Ca content of plant tissues as Ca levels increased in treatment solutions (Figure 5). Compared to 0 mM Ca, 5 mM Ca resulted in a 3.4-fold increase in tissue Ca content and increased an additional 27% to 10 mM Ca and another 18% to 20 mM Ca. In general, added Ca from 5, 10, and 20 mM Ca treatments increased percent Mg in plant tissues compared to 0 mM Ca, but there were no differences in K or P (Figure 5).

Experiment Two: Narrow Range Ca Application

Percent germination followed a similar trend to Experiment One, where germination decreased as Ca increased (Table 6). Germination remained at about 90% or greater from 0 to 5 mM Ca treatments but decreased to 75% in 7.5 mM Ca and 61% in 10 mM Ca treatments. Increases in %H were not significant across treatments (Table 6). Furthermore, no treatment yielded higher than 60% harvestable shoots. There were no differences in FW, except between the 5 and 7.5 mM Ca treatments (Table 6). Calcium treatment did not affect DW, HL, CSA, or the percent of shoots that had a first true leaf (%TL) (Table 6).

Table 5. Experiment One: germination and growth of radish microgreens on Capmat II media after seven days of growth in 0 to 160 mM Ca treatments. ANOVA F statistic and p values.

Treatment mM Ca	% Germination	Fresh wt. (g/plant)	Dry wt. (g/plant)	% Harvestable	Hypocotyl Length (cm/plant)	Cotyledon Surface Area (cm ² /plant)
0	100.0±0.0a	0.164±0.024a	0.025±0.006a	23.8±6.49bc	2.367±0.240bc	1.097±0.025bc
5	88.5±11.5ab	0.117±0.016a	0.015±0.008abc	48.7±7.07a	2.853±0.049ab	1.198±0.019ab
10	72.1±2.5b	0.113±0.005a	0.016±0.009abc	35.6±3.34a	3.331±0.119a	1.294±0.036a
20	70.0±1.4b	0.118±0.007a	0.009±0.003bc	21.0±6.95bcd	2.429±0.164bc	1.082±0.033bc
40	62.4±6.9b	0.112±0.012a	0.019±0.004ab	2.9±0.26cd	2.253±0.065c	0.992±0.034c
80	64.4±1.9b	0.000±0.000b	0.000±0.000c	0.00±0.00d	0.000±0.000d	0.000±0.000d
160	9.7±5.7c	0.000±0.000b	0.000±0.000c	0.00±0.00d	0.000±0.000d	0.000±0.000d
ANOVA F Statistic (and p value)						
df	25.59	27.12	8.74	9.19	119.72	485.54
6	(<0.0001)	(<0.0001)	(0.0004)	(0.0003)	(<0.0001)	(<0.0001)

Column values are means ± standard error. Means within each column followed by different letters are significantly different from one another (p<0.05) based on Tukey's pairwise comparison.

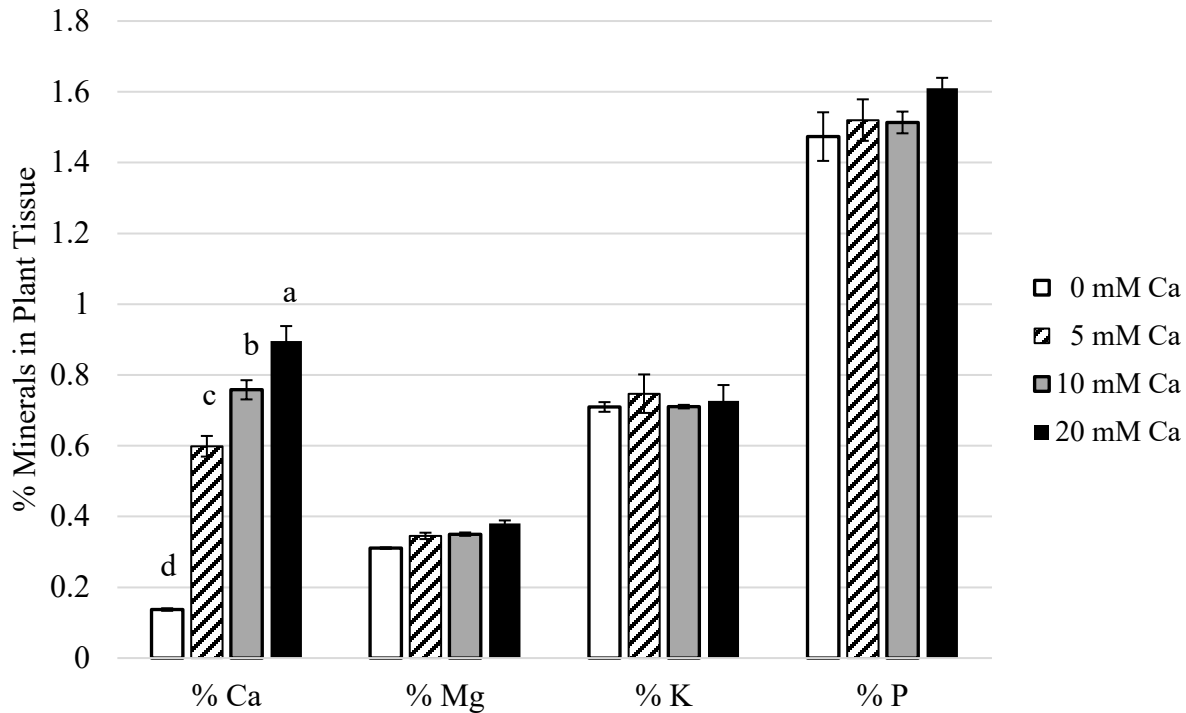


Figure 5. Experiment One: Mineral content in harvested plant tissues Values are means \pm standard error (n=3). Within mineral, values not followed by the same letter are significantly different ($p < 0.05$) based on Tukey's pairwise comparison.

In Experiment Two, Ca content of shoots increased as Ca treatment level increased, similar to Experiment One (Figure 6). Ca content of shoots from 2.5 mM Ca treatments (0.485%) was 2.8-fold greater than the control (0.129%). Ca content increased again at the 7.5 mM Ca, increasing 67% from the 2.5 mM Ca treatment. Treatment did not affect Mg, K, or P content of harvested plant tissues (Figure 6).

Experiment Three: Delayed Narrow Range Ca Application

When Ca was delayed until after germination and acid scarification was used to prepare seeds before planting, %G for all treatments was near 100% (Table 7). There were no differences identified between treatments in any of the growth or yield factors measured. This included %H, FW, DW, %TL, HL, and CSA (Table 7).

Table 6. Experiment Two: germination and growth of radish microgreens on Capmat II media after seven days of growth in 0 to 10 mM Ca treatments. ANOVA F statistic and p values.

Treatment mM Ca	% Germination	Fresh wt. (g/plant)	Dry wt. (g/plant)	% Harvestable	Hypocotyl Length (cm/plant)	Cotyledon Surface Area (cm ² /plant)	% Plants with First True Leaf
0	96.9±1.3a	0.056±0.001ab	0.010±0.0003a	39.8±6.57a	1.923±0.129a	3.973±0.272b	34.2±7.6a
2.5	93.6±1.9a	0.068±0.003ab	0.001±0.0002a	56.3±10.4a	2.412±0.166a	4.636±0.151ab	35.0±4.4a
5	89.4±2.4ab	0.071±0.003a	0.010±0.0001a	55.4±5.23a	2.135±0.084a	4.865±0.135a	28.3±15.2a
7.5	74.9±7.9bc	0.050±0.009b	0.009±0.0019a	39.0±11.6a	2.274±0.105a	4.440±0.269ab	10.0±3.6a
10	61.1±3.3c	0.066±0.001ab	0.010±0.0002a	54.6±4.11a	2.222±0.044a	4.543±0.001ab	15.8±2.5a
ANOVA F Statistic (and p value)							
df	13.10	3.69	0.47	1.18	2.56	2.70	1.92
4	(<0.0001)	(0.0276)	(0.7542)	(0.3606)	(0.0815)	(0.0707)	(0.1588)

Column values are means ± standard error. Means within each column followed by different letters are significantly different from one another (p<0.05) based on Tukey's pairwise comparison.

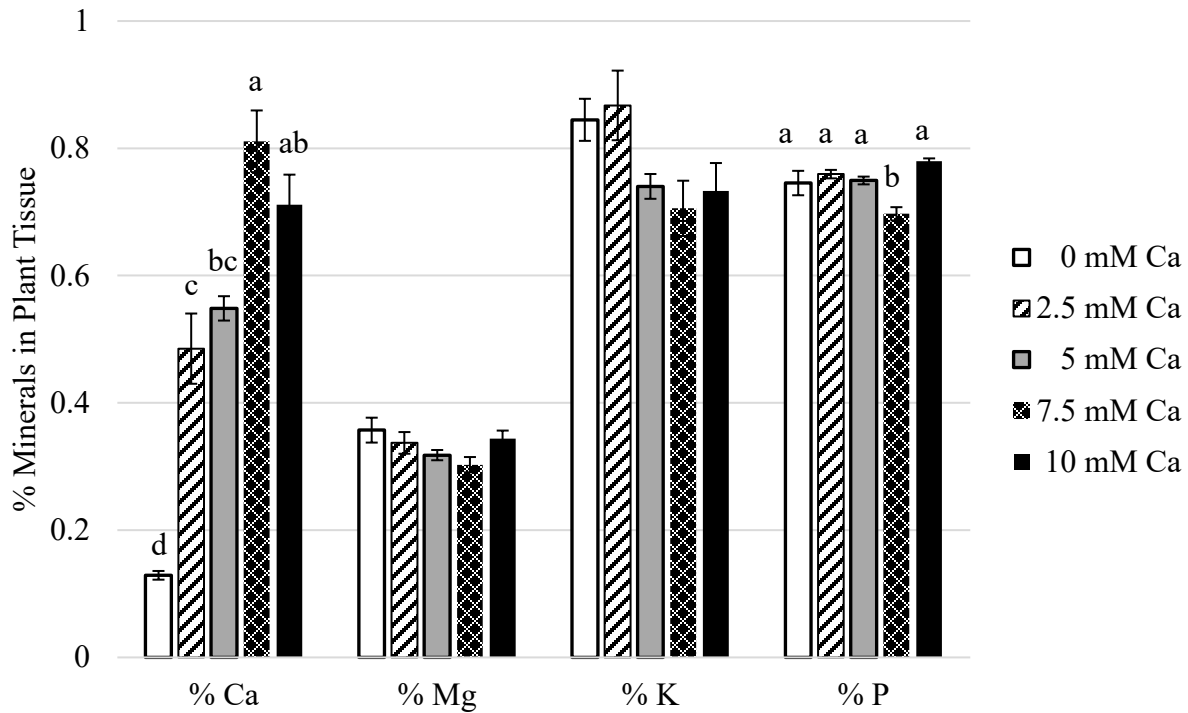


Figure 6. Experiment Two: Mineral content in harvested plant tissues. Values are means \pm standard error (n=4). Within mineral, values not followed by the same letter are significantly different ($p < 0.05$) based on Tukey's pairwise comparison.

In Experiment Three, Ca content increased in plant tissues as Ca treatment concentration increased, though overall Ca content was lower than previous experiments (Figure 7). Percent Ca in plant tissues of 2.5 mM Ca was 2.8 fold greater than the control. It increased an additional 27% in 5 mM Ca treatments, and another 24% in 7.5 mM Ca treatments. The differences in Mg were variable, showing no clear trend in treatment effects (Figure 7). Ca treatment yielded no differences in K and P content of plant tissues (Figure 7).

Table 7. Experiment Three: germination and growth of radish microgreens on Capmat II media after four days of growth in 0 to 10 mM Ca treatments. ANOVA F statistic and p values.

Treatment mM Ca	% Germination	Fresh wt. (g/plant)	Dry wt. (g/plant)	% Harvestable	Hypocotyl Length (cm/plant)	Cotyledon Surface Area (cm ² /plant)	% Plants with First True Leaf
0	97.1±1.1	0.058±0.001	0.0105±0.0004	44.5±2.1	1.837±0.058	4.105±0.141	39.2±9.9
2.5	96.5±1.0	0.056±0.003	0.0103±0.0004	43.0±3.5	1.793±0.058	4.687±0.213	55.8±15.1
5	98.2±0.5	0.063±0.003	0.0103±0.0004	52.2±5.0	2.029±0.050	4.788±0.169	54.2±13.0
7.5	98.1±1.0	0.060±0.001	0.0102±0.0001	45.7±5.4	1.973±0.080	4.643±0.180	30.8±3.4
10	98.3±0.6	0.061±0.002	0.0109±0.0002	46.3±5.6	1.792±0.068	4.243±0.124	46.7±5.3
ANOVA F Statistic (and p value)							
df	0.08	1.42	0.70	0.61	2.99	3.18	1.03
4	(0.5421)	(0.2751)	(0.6019)	(0.6644)	(0.0533)	(0.0446)	(0.4259)

Column values are means ± standard error. Means within each column followed by different letters are significantly different from one another (p<0.05) based on Tukey's pairwise comparison.

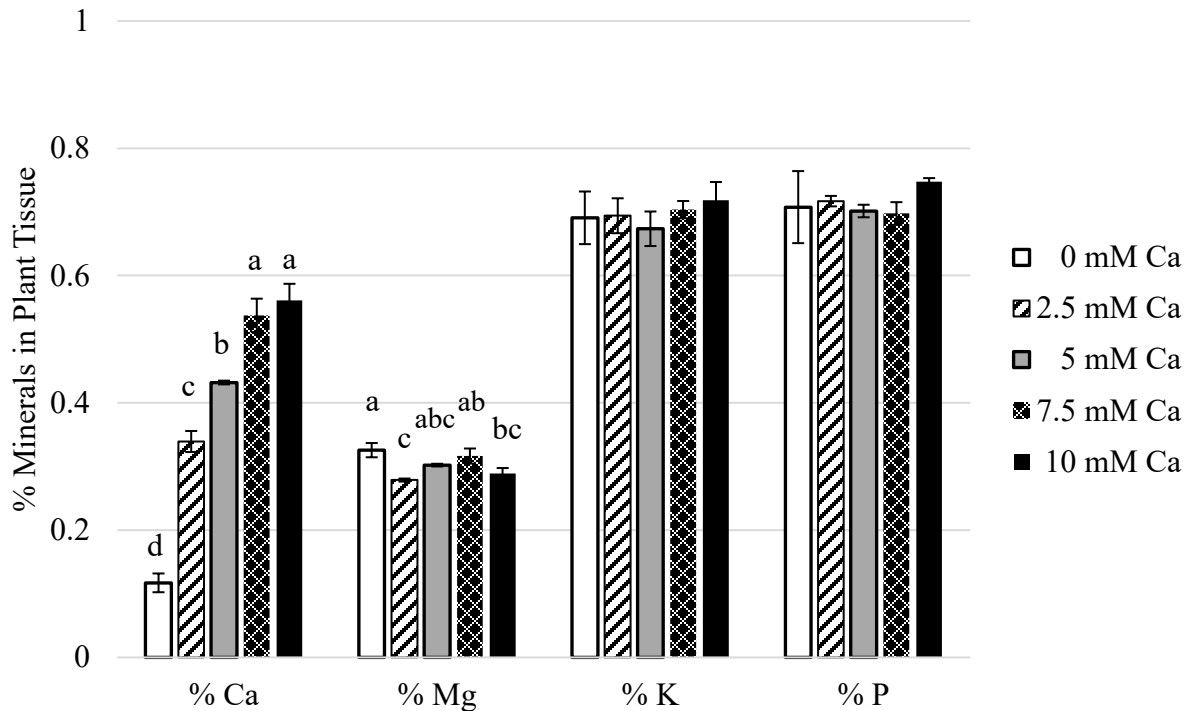


Figure 7. Experiment Three: Mineral content in harvested plant tissues. Values are means \pm standard error (n=4). Within mineral, values not followed by the same letter are significantly different ($p < 0.05$) based on Tukey's pairwise comparison.

Experiment Four A: Delayed Narrow Range Ca Application with Subplots

Germination remained above 90% across treatments (Table 8). There were no identified effects of treatment on %H (Table 8). FW was greatest in the range of 2.5 to 7.5 mM Ca (Table 8). DW was variable, with no clear trend (Table 8). Differences in effects on HL could not be identified (Table 8). Cotyledons from 2.5 mM Ca were 23% larger than 0 mM Ca (Table 8).

Tissue Ca content followed the same trend as in previous experiments. Shoots grown in 2.5 mM Ca were 2.6- fold greater than the control, on average (Figure 8). There was an additional increase of 66% from 2.5 mM Ca to 7.5 mM Ca treatments. There were no differences in Mg, K, or P content of plant tissues in this experiment (Figure 8).

Table 8. Experiment Four A: germination and growth of radish microgreens on Capmat II media after four days of growth in 0 to 10 mM Ca treatments (with subplots). ANOVA F statistic and p values.

Treatment mM Ca	% Germination	Fresh wt. (g/plant)	Dry wt. (g/plant)	% Harvestable	Hypocotyl Length (cm/plant)	Cotyledon Surface Area (cm ² /plant)	% Plants with First True Leaf
0	91.6±5.3	0.072±0.004b	0.013±0.0006a	33.8±5.3	2.016±0.155	1.210±0.069b	40.9±8.5
2.5	96.6±2.2	0.084±0.003a	0.013±0.0007ab	39.6±3.5	2.168±0.171	1.490±0.073a	47.4±6.2
5	94.5±4.0	0.080±0.004a	0.012±0.0006ab	38.3±8.5	2.099±0.207	1.293±0.079b	51.8±6.2
7.5	93.7±3.2	0.079±0.003a	0.013±0.0006a	39.0±6.0	1.929±0.121	1.347±0.068ab	42.3±8.4
10	94.9±3.6	0.072±0.003b	0.011±0.0005b	36.6±5.9	2.005±0.161	1.252±0.083b	41.6±6.0
ANOVA F Statistic (and p value)							
df							
4	1.27* (0.2920)	10.56 (<0.0001)	3.36 (0.0150)	1.01* (0.4071)	1.65* (0.1736)	8.86 (<0.0001)	1.67 (0.1696)

Column values are means ± standard error. Means followed by different letters are significantly different from one another (p<0.05) based on Tukey's pairwise comparison. A * following F statistic values in the ANOVA table indicates a significant interaction (p<0.05) between treatment and block. Tukey's pairwise comparison could not be used to identify significant treatment effects when there was a significant interaction.

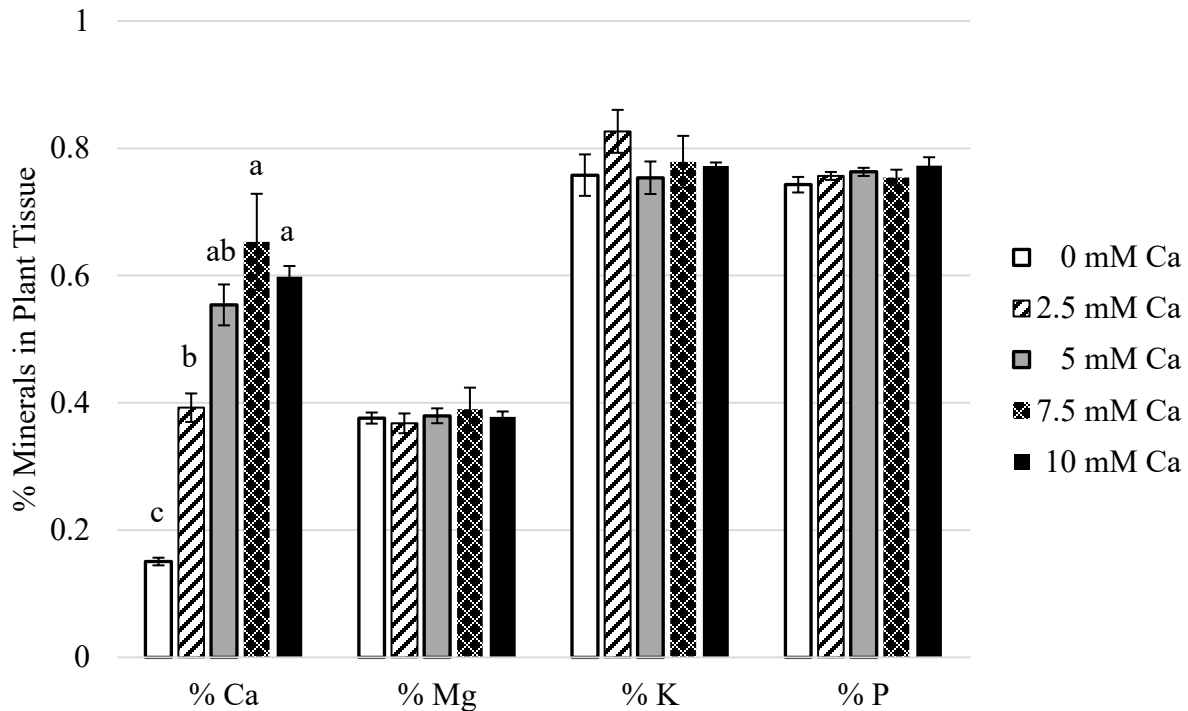


Figure 8. Experiment Four A: Mineral content in harvested plant tissues Values are means \pm standard error (n=4). Within mineral, values not followed by the same letter are significantly different ($p < 0.05$) based on Tukey's pairwise comparison.

Experiment Four B: Delayed Narrow Range Ca Application with Subplots

Germination was greater than 90% across all treatments (Table 9). Results of %H and FW were variable, showing clear trend (Table 9). No difference in DW could be identified (Table 9). All treatments, except 10 mM Ca, resulted in greater %TL than the control (Table 9). Treatments did not affect HL until a level of 10 mM Ca (Table 9). The CSA measurements showed the same trend as HL (Table 9).

Calcium content of tissues was the same as previous experiments. Ca content of shoots in 2.5 mM Ca was 2.1-fold greater than the control (Figure 9). There was an additional increase of 38% from 2.5 to 5 mM Ca treatments, and 30% from 5 to 10 mM Ca treatments. Treatment did not cause differences in Mg in this experiment (Figure 9). Potassium and P were highly variable but did not reveal a clear trend (Figure 9).

Table 9. Experiment Four B: germination and growth of radish microgreens on Capmat II media after four days of growth in 0 to 10 mM Ca treatments (with subplots). ANOVA F statistic and p values.

Treatment mM Ca	% Germination	Fresh wt. (g/plant)	Dry wt. (g/plant)	% Harvestable	Hypocotyl Length (cm/plant)	Cotyledon Surface Area (cm ² /plant)	% Plants with First True Leaf
0	95.1±1.6	0.070±0.002	0.012±0.0002	44.8±3.3	1.954±0.083ab	1.293±0.049ab	26.9±2.7b
2.5	98.1±0.3	0.079±0.002	0.012±0.0002	49.0±2.0	1.985±0.077ab	1.384±0.036ab	44.4±3.1a
5	94.1±1.3	0.072±0.002	0.012±0.0003	41.1±2.1	2.093±0.118ab	1.341±0.072ab	40.2±2.6a
7.5	97.9±0.7	0.074±0.002	0.012±0.0003	53.6±2.2	2.174±0.066a	1.447±0.049a	39.6±2.2a
10	94.6±1.1	0.067±0.002	0.012±0.0002	42.6±3.0	1.844±0.078b	1.244±0.043b	35.4±2.5a b

ANOVA F Statistic (and p value)

df							
	3.52*	8.60*	0.95	5.17*	2.80	2.71	6.74
4	(0.0119)	(<0.0001)	(0.4413)	(0.0012)	(0.0337)	(0.0381)	(0.0002)

Column values are means ± standard error. Means followed by different letters are significantly different from one another (p<0.05) based on Tukey's pairwise comparison. A * following F statistic values in the ANOVA table indicates a significant interaction (p<0.05) between treatment and block. Tukey's pairwise comparison could not be used to identify significant treatment effects when there was a significant interaction.

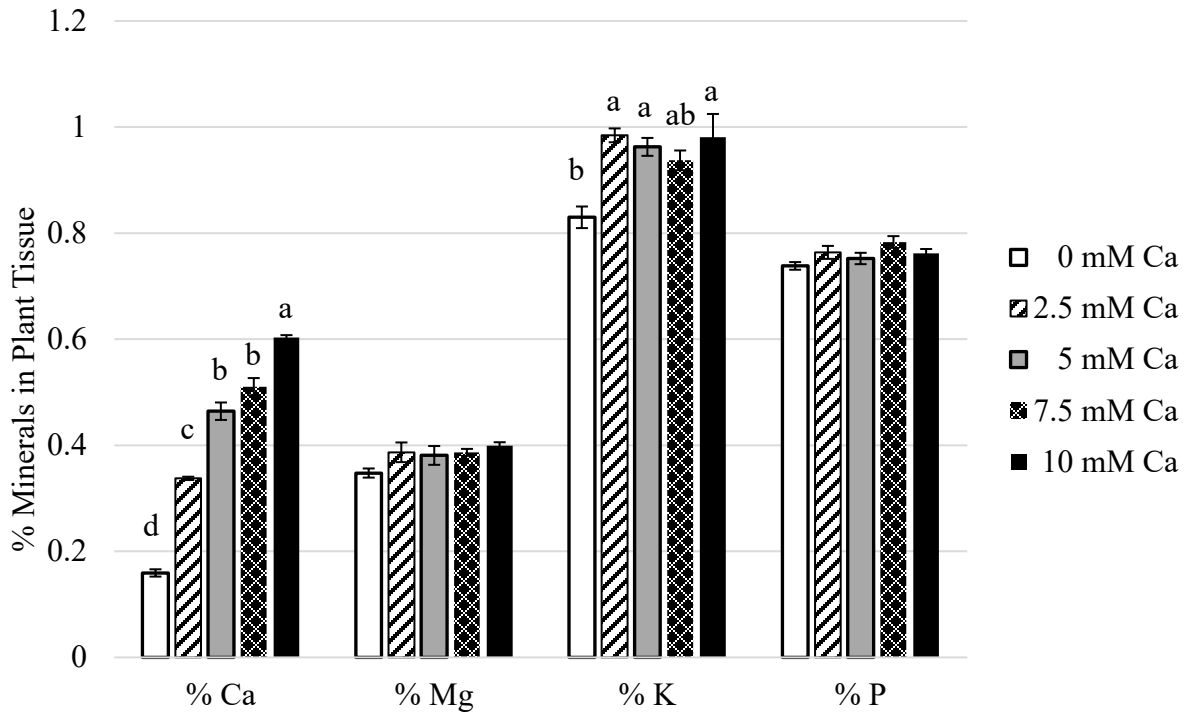


Figure 9. Experiment Four B: Mineral content in harvested plant tissues (\pm standard error) Values are means \pm standard error (n=4). Within mineral, values not followed by the same letter are significantly different ($p < 0.05$) based on Tukey's pairwise comparison.

Experiment Five: Delayed Narrow Range Ca Application with Vermiculite

In this experiment, %G was above 98% across all treatments (Table 10). No treatment effects were identified in %H, FW, or DW (Table 10). Treatments above 2.5 mM Ca increased %TL of 18.6% (5 mM Ca) to 27.3% (10 mM Ca). From 0 mM Ca, CSA increased 15.1% in 10 mM Ca treatments (Table 10).

Nutrient analysis revealed no difference in Ca content until 10 mM Ca, increasing 14% over the control (Figure 10). Magnesium and K increased with Ca levels (Figure 10). Magnesium increased 20.4% from 0 to 5 mM Ca, and an additional 8.4% from 5 to 10 mM Ca. Potassium increased 15.6% from 0 to 2.5 mM Ca, and another 8.2% from 2.5 to 10 mM Ca treatments. Decreases in P anions were not significant (Figure 10).

Table 10. Experiment Five: germination and growth of radish microgreens in vermiculite media after four days of growth in 0 to 10 mM Ca treatments (with subplots). ANOVA F statistic and p values.

Treatment mM Ca	% Germination	Fresh wt. (g/plant)	Dry wt. (g/plant)	% Harvestable	Hypocotyl Length (cm/plant)	Cotyledon Surface Area (cm ² /plant)	% Plants with First True Leaf
0	99.5±0.3	0.085±0.001	0.011±0.0003	91.7±1.7	1.932±0.064	1.611±0.044b	47.9±1.5b
2.5	99.7±0.2	0.091±0.001	0.012±0.0002	91.0±1.5	2.057±0.045	1.714±0.055ab	54.1±2.1ab
5	99.1±0.4	0.096±0.003	0.012±0.0004	91.7±1.4	2.091±0.047	1.653±0.038b	56.8±3.4a
7.5	99.5±0.2	0.090±0.002	0.012±0.0002	85.6±2.3	1.927±0.049	1.682±0.040ab	60.4±2.2a
10	99.6±0.2	0.104±0.003	0.012±0.0002	94.4±0.7	2.229±0.046	1.854±0.042a	61.0±2.3a
ANOVA F Statistic (and p value)							
df	0.73	16.03*	1.24	6.00*	9.82*	4.55	5.93
4	(0.5750)	(<0.0001)	(0.3028)	(0.0004)	(<0.0001)	(0.0028)	(0.0004)

Column values are means ± standard error. Means followed by different letters are significantly different from one another (p<0.05) based on Tukey's pairwise comparison. A * following F statistic values in the ANOVA table indicates a significant interaction (p<0.05) between treatment and block. Tukey's pairwise comparison could not be used to identify significant treatment effects when there was a significant interaction.

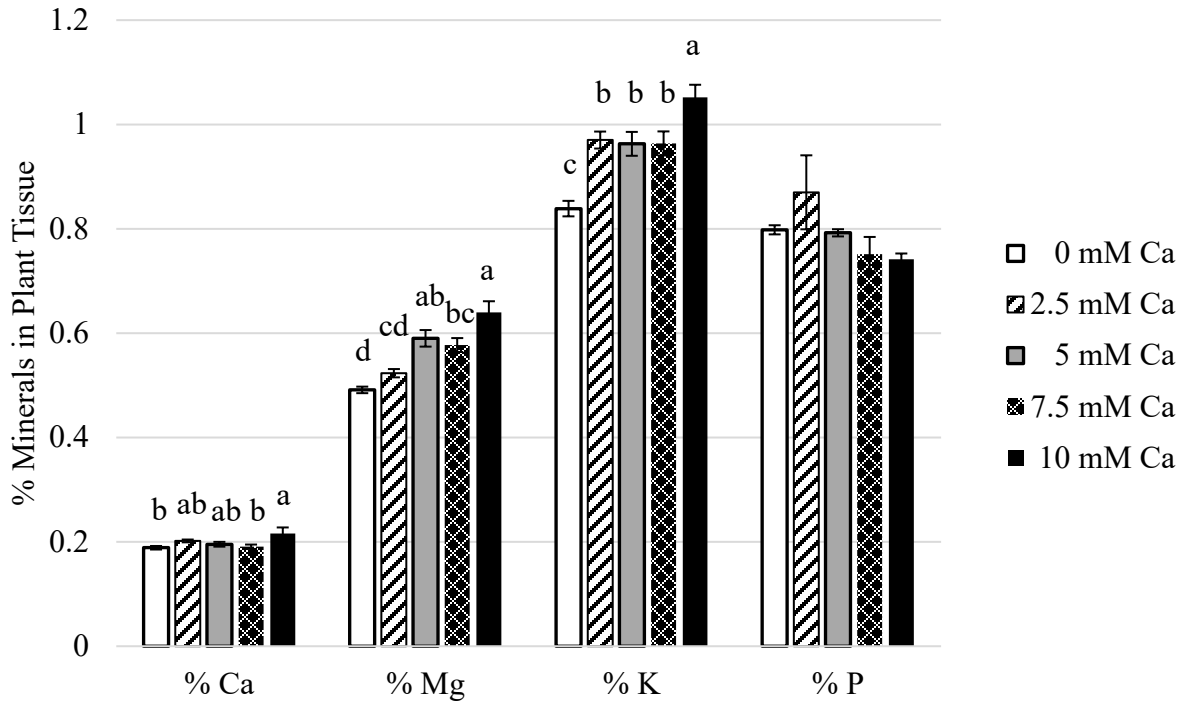


Figure 10. Experiment Five: Mineral content in harvested plant tissues. Values are means \pm standard error (n=4). Within mineral, values not followed by the same letter are significantly different ($p < 0.05$) based on Tukey's pairwise comparison.

Experiment Six: Delayed Broad Range Ca Application with Vermiculite

In this experiment, %G was between 99% and 100% across all treatments (Table 11).

There were no significant effects of Ca treatment on %H (Table 11). Fresh weight increases were 11.5% in the 15 mM Ca treatment, and 14.2% in the 20 mM Ca compared to the control (Table 11). No differences in DW occurred across treatments (Table 11). Gradual increases in %TL occurred as Ca increased (Table 11). From 0 to 25 mM Ca %M increased 50.4% (Table 11).

There were no differences from treatment on HL (Table 11). Plants grown in 20 and 25 mM Ca had greater 20% increase in CSA compared to the control and 5 mM Ca treatments (Table 11).

Ca increased 20.7% from 0 to 5 mM Ca treatments and 79.3% from 0 to 20 mM Ca treatments, however significant differences could not be identified due to a significant interaction ($p < 0.05$) (Figure 11). There were gradual increases in tissue content of Mg and K cations

(Figure 11). Magnesium increased 24.8% from 0 to 15 mM Ca treatments. Potassium increased 16.9% from 0 to 5 mM Ca, and 39.5% from 0 to 15 mM Ca treatments. Phosphorus in plant tissues decreased as Ca concentration increased (Figure 11). Phosphorus decreased 3.8% from 0 to 10 mM Ca treatments, 4.9% from 10 to 15 mM Ca, 8% from 15 to 20 mM Ca, and 6.9% from 20 to 25 mM Ca treatments.

Table 11. Experiment Six: germination and growth of radish microgreens in vermiculite media after four days of growth in 0 to 25 mM Ca treatments (with subplots). ANOVA F statistic and p values.

Treatment mM Ca	% Germination	Fresh wt. (g/plant)	Dry wt. (g/plant)	% Harvestable	Hypocotyl Length (cm/plant)	Cotyledon Surface Area (cm ² /plant)	% Plants with First True Leaf
0	99.9±0.1	0.098±0.003b	0.011±0.0002	97.0±0.7	2.900±0.070	1.385±0.033c	47.3±2.1c
5	99.7±0.2	0.104±0.004ab	0.012±0.0003	97.0±1.0	3.018±0.102	1.373±0.042c	49.4±1.9c
10	99.9±0.1	0.104±0.001ab	0.011±0.0001	96.0±0.6	3.083±0.064	1.485±0.066bc	57.5±3.1bc
15	99.7±0.2	0.109±0.002a	0.011±0.0003	98.1±0.4	2.992±0.060	1.487±0.056bc	52.6±1.6bc
20	100.0±0.0	0.112±0.002a	0.012±0.0003	97.2±0.7	2.883±0.096	1.721±0.039a	62.5±3.6ab
25	99.7±0.2	0.107±0.002ab	0.011±0.0003	96.4±0.9	2.879±0.071	1.638±0.051ab	71.2± 3.1a
df	ANOVA F Statistic (and p value)						
5	0.55 (0.7392)	3.52 (0.0080)	1.01 (0.4207)	0.99 (0.4306)	1.29 (0.2818)	8.76 (0.0001)	11.42 (<0.0001)

Column values are means ± standard error. Means followed by different letters are significantly different from one another (p<0.05) based on Tukey's pairwise comparison.

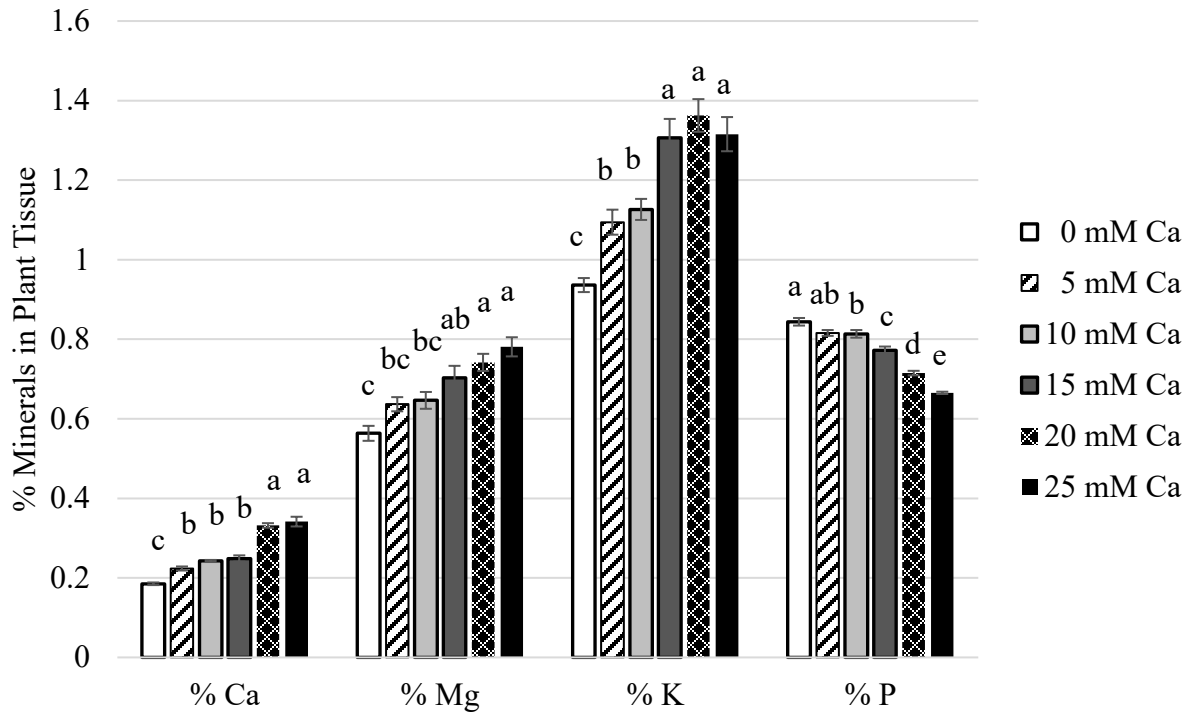


Figure 11. Experiment Six: Mineral content in harvested plant tissues. Values are means \pm standard error (n=4). Within mineral, values not followed by the same letter are significantly different ($p < 0.05$) based on Tukey's pairwise comparison.

DISCUSSION

Germination of Radish Microgreens

In Experiments One and Two, where Ca was applied from the time of planting, there were significant decreases in %G as Ca increased in treatment solutions. When a broad range of solutions, up to 160 mM Ca were applied in Experiment One, treatment solutions greater than 40 mM concentration resulted in toxic effects on germination. Furthermore, these higher concentrations also had a negative impact on growth after germination. There were very few harvestable shoots in 40 mM Ca, and no harvestable shoots in 80 and 160 mM Ca, demonstrating that these concentrations had a toxic effect on growth and development after germination.

In Experiment Two, treatment solutions were narrowed down to a range of 0 to 10 mM, but germination still showed a decreasing trend as Ca increased. This suggested that mineral salts in treatment solutions had a negative impact on seed imbibition. It is also possible that imbibition was slowed in general due to very limited contact with the growing media, since the seeds were only laying on top of the Capmat II felt material. In addition to these limitations, the radish microgreen seed that was purchased for these experiments was found to be a mixture of varieties, therefore not genetically pure. This was evident in the variable color and size of individual radish shoots at the time of harvest and contributed to the high variation seen across all experiments (Figure 12). For subsequent experiments (after Experiment Two), seeds were treated with sulfuric acid scarification prior to planting, and Ca treatments were delayed until after radicles had elongated and cotyledons were beginning to emerge. This greatly improved %G in the Capmat II media, helping it to remain above 90% for all treatments. Still, growth of plants after germination was highly variable, due to the limitations previously noted.

After performing several experiments with the Capmat II media, and getting less than ideal growth and yields, even with consistent germination, it was decided that vermiculite should be added above the Capmat II media. In Experiments Five and Six, where vermiculite was used, acid scarification was not required, because germination remained around 100% for all treatments. This was attributed to better contact of seed surfaces to the growing vermiculite media surrounding the seeds



Figure 12. Variability in size and variety of radish microgreens when harvested seven DAP.

Growth and Yields of Radish Microgreens

Growth and yield measurements (FW, DW, %H, %TL, HL, CSA) were highly variable across all experiments. Differences between treatments were apparent in Experiment One, when a broad range of solutions were applied. Based on research published by Kou et al. (2014), where pre-harvest Ca foliar sprays at a concentration of 10 mM increased fresh weight yields of broccoli microgreens by more than 50%, it was expected that the results of biomass measurements in the current experiments would reflect a similar trend. This was not the case, as variation within treatments resulted in the absence of a clear growth curve in measurements of FW and DW on a per plant basis. However, measurements of %H, HL, and CSA did suggest that yields could be improved with added Ca, because these variables showed an increase up to 10 mM Ca treatments. At 20 mM Ca, these began to decline, revealing the limits of Ca nutrition in the microgreens.

When the solution range was narrowed for Experiments Two through Four, there were some trends revealed in the data, even though differences may not have been statistically significant, or there may have been block effects or interactions between treatment and block (in the case of Experiment Four A and B). In general, added Ca increased FW, %H, CSA, and %TL across the Capmat II experiments, even though a particular rate of Ca could not be identified as the best treatment. Furthermore, when microgreens were grown in vermiculite, growth and germination were better overall, though experiments using different growing medias could not be statistically compared to one another, in this case. When grown in vermiculite, microgreens generally had greater %H and %TL in a shorter amount of time than those grown using Capmat II media. This resulted in more consistent growth and development throughout the vermiculite experiments.

Mineral Content of Radish Microgreens

When radish microgreens were grown using Capmat II media, there were incremental increases in Ca content of plant tissues as Ca increased in treatment solutions. The Ca content of harvested shoots was generally as much as four times that of the control (0 mM Ca) in the higher treatment levels (7.5 and 10 mM Ca). This is similar to the results reported by Kou et al. (2014), when Ca foliar sprays were applied to broccoli microgreens. These results show that similar to foliar spray, Ca applied as a hydroponic solution does increase Ca content of harvested shoots. When radish microgreens were grown in vermiculite, the increase of Ca content in plant tissues was more gradual. There were no differences from the control in the lower concentrations until Ca reached 10 mM in Experiment 5, though Experiment 6 showed differences beginning in the 5 mM Ca treatment but leveling off until another increase at 20 mM Ca. It is possible that vermiculite made some of the Ca unavailable to the plants, or that the plants did not take up as much in this media because they did not require as much Ca under better growing conditions.

In the Experiments One through Four, where Capmat II media was used, there was some variation in Mg, K, or P content of plant tissues after harvest, but for the most part, these levels were not affected by Ca treatment. Where there were differences, there was no clear trend identified, which suggests that this could be due to human errors in collection and/or processing of samples, or the variation of the plants, themselves. Possible experimental errors could include mineral contamination at any point during data collection and processing (harvesting or grinding samples), or during microwave digestion and sample preparation for mineral analysis. Several studies have shown that variations in mineral content of different varieties of microgreens, even within the same species, are common (Di Gioia et al., 2017; Mir et al., 2016; Xiao et al., 2016).

With the mixture of varieties in the seed that was used in these experiments, these differences in mineral content could be attributed to this genetic variation.

When microgreens were grown in vermiculite, the results of Mg, K, and P analysis were very different than the previous experiments. The cations, Mg and K, increased incrementally as Ca increased in treatment solutions. In addition to this, P anions decreased as Ca treatment concentration increased. These results are likely attributed to the fact that vermiculite is not an inert growing media. A high cation exchange capacity (CEC) can affect the levels of mineral nutrients that are available to be taken up by plant roots. Vermiculite is a silicate clay that has a relatively high CEC. Ca ions have a higher affinity to cation exchange sites than Mg or K ions, so it is possible that Mg and K ions that were already adsorbed in the vermiculite were exchanged with Ca and released into the solution. This would result in the lower levels of Ca available in solution and increases in available Mg in the solution, resulting in the reported lower tissue Ca and greater tissue Mg. Phosphorus content of plant tissues decreased as Ca treatment levels increased, in Experiment Six. In this experiment, the microgreen shoots grew to a large enough size that it is possible P was diluted within the plant. Another possibility is that P complexed with positively charged Ca ions in the nutrient solution. This tends to happen in field soils at low pH levels (Brady and Weil, 2002).

Limitations of the Study

This series of experiments progressed through trial and error, as limitations were identified. Germination was greatly limited in the Capmat II growing media. Acid scarification in addition to delaying Ca treatments improved germination, but growth and yields were still lower than desired. Vermiculite improved germination growth, and yields, but it is not the ideal

growing media for mineral nutrient studies, as these results suggest that the CEC of vermiculite influenced mineral content of plant tissues in addition to the Ca treatments.

The main limitation across all experiments was a high degree of variation within Ca treatments. This made it difficult and sometimes impossible to identify trends that may have been present in the treatments. Early on, sampling errors may have contributed to some of this variation, but after revising harvest protocol and increasing sample size, variation was still very high. In Experiments Four B and Four B, when plots were divided into subplots to increase sample size, and the experimental design was changed to randomized complete block, the results revealed block affects and interactions between treatment and block that were significant ($p < 0.05$). As mentioned previously, this could likely be due to the variation of the seed causing different seed sizes, and the inadequate conditions provided by the Capmat II growing media. Possible solutions to this would be to find a new seed source that is genetically pure, and to increase sample size by subsampling over time, rather than dividing plots into subplots and harvesting all samples in the same day.

Future Research and Impact of the Study

Microgreens experiments are still being conducted for this project, based on the results of the reported vermiculite experiments. Because the most recent broad range experiment (Experiment Six) did not conclusively identify the upper limit of Ca in microgreens grown in vermiculite, this is the next step to continue this research. In addition, it would be beneficial to evaluate a range of solutions when applied from the time of planting versus after germination to see if there are still detrimental effects on germination and growth. If vermiculite can provide a

better growing environment to allow Ca to be applied from the time of planting, differences in yields may be more apparent.

Despite the limitations of these experiments, they have culminated to a foundation of knowledge and experimental methods that will allow this research to continue. The system that has been developed through these experiments will allow future graduate students to evaluate different microgreens varieties and different mineral nutrients in this system to gain a better perspective on how hydroponic microgreens production can be improved to benefit producers with higher yields. This study has also shown that nutrition of hydroponically grown microgreens can be improved for consumers, as well, through careful application of Ca fertilizer.

REFERENCES

- Anil, V.S. and S.K. Rao. 2001. Calcium-mediated signal transduction in plants: A perspective on the role of Ca²⁺ and CDPKs during early plant development. *J. Plant Physiol.* 158:1237-1256.
- Brady, N.C. and R.R. Weil. 2002. The phosphorus cycle. p. 601-610. In: S. Helba and D. Yarnell (eds.). *The nature and properties of soils.* Pearson Educ. Ltd, Upper Saddle River, NJ.
- Brazaitytė, A., J. Jankauskienė, and A. Novičkovas. 2013. The effects of supplementary short-term red LEDs lighting on nutritional quality of *Perilla frutescens* L. microgreens. *Proc. Intl. Scientific Conf.: Rural Dev.* 6:54-58.
- Bulgari, R., A. Baldi, A. Ferrante, and A. Lenzi. 2017. Yield and quality of basil, swiss chard, and rocket microgreens grown in a hydroponic system. *N.Z. J. Crop Hort. Sci.* 45:119-129.
- Chandra, D., J. G. Kim, and Y.P. Kim. 2012. Changes in microbial population and quality of microgreens treated with different sanitizers and packaging films. *Hort. Environ. Biotechnol.* 53:32-40.
- Di Gioia, F., P. Bellis, C. Mininni, P. Santamaria, and F. Serio. 2017. Physiochemical, agronomical and microbiological evaluation of alternative growing media for the production of rapini (*Brassica rapa* L.) microgreens. *J. Sci. Food Agr.* 97:1212-1219.
- Gerovac, J., J. Craver, J. Boldt, and R. Lopez. 2016. Light intensity and quality from sole-source light-emitting diodes impact growth, morphology, and nutrient content of brassica microgreens. *HortScience.* 51:497-503.
- Hodges, M.D. and P. Toivonen. 2008. Quality of fresh-cut fruits and vegetables as affected by exposure to abiotic stress. *Postharvest Biol. Technol.* 48:155-162.
- Johnnys Selected Seeds. 2017. Microgreens production tech sheet. 1 January 2018. <<http://www.johnnyseeds.com/growers-library/micro-greens-production.html?q=microgreens%20production>>
- Kopsell, D. and C. Sams. 2013. Increases in shoot tissue pigments, glucosinolates, and mineral elements in sprouting broccoli after exposure to short-duration blue light from light emitting diodes. *J. Amer. Soc. Hort. Sci.* 138:31-37.
- Kopsell, D., C. Sams, and T. Barickman. 2014. Sprouting broccoli accumulate higher concentrations of nutritionally important metabolites under narrow-band light-emitting diode lighting. *J. Amer. Soc. Hort. Sci.* 139:469-477.

- Kou, L., T. Yang, Y. Luo, X. Liu, L. Huang, and E. Codling. 2014. Pre-harvest calcium application increases biomass and delays senescence of broccoli microgreens. *Postharvest Biol. Technol.* 87:70-78.
- Kou, L., T. Yang, X. Liu, and Y. Luo. 2015. Effects of pre- and postharvest calcium treatments on shelf life and postharvest qualities on broccoli microgreens. *Hortscience.* 50:1801-1808.
- Kyriacou, M.C., Y. Roupael, F. Di Gioia, A. Kyrtzis, F. Serio, M. Renna, S. De Pascale, and P. Santamaria. 2016. Micro-scale vegetable production and the rise of microgreens. *Trends Food Sci. Technol.* 57:103-115.
- Kyriacou, M.C., S. De Pascale, A. Kyrtzis, and Y. Roupael. 2017. Microgreens as a component of space life support systems: A cornucopia of functional food. *Frontiers Plant Sci.* 8:1587.
- Lee, J.S., W.G. Pill, B.B. Cobb, and M. Olszewski. 2004. Seed treatments to advance greenhouse establishment of beet and chard microgreens. *J. Hort. Sci. Biotechnol.* 79:565-570.
- Lu, Y., W. Dong, J. Alcazar, T. Yang, Y. Luo, and Q. Wang. 2018. Effect of preharvest CaCl_2 spray and postharvest UV-B radiation on storage quality of broccoli microgreens, a richer source of glucosinolates. *J. Food Composition Anal.* 67: 55-62.
- Marschner, M. 2012. Calcium. p. 171-178. In. P. Marschner (ed.). *Mineral nutrition of higher plants.* Elsevier Ltd., Oxford, UK.
- Mir, S.A., M.A. Shah, M.M. Mir. 2016. Microgreens: Production, shelf life, and bioactive components. *Critical Rev. Food Sci. Nutr.* 57:2730-2736.
- Morrow, R.C. 2008 LED lighting in horticulture. *Hortscience.* 43:1947-1950.
- Murphy, C., K.F. Llort, and W. Pill. 2010. Factors affecting the growth of microgreen table beet. *Intl. J. Veg. Sci.* 16:253-266.
- Murphy, C., and W. Pill. 2010. Cultural practices to speed the growth of microgreen arugula. *J. Hort. Sci. Biotechnol.* 85:171-176.
- Murphy, J., and J.P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chimica Acta* 27:31-36.
- Pinto, E., A.A. Almeida, A.A. Aguiar, and I. M. Ferreira. 2015. Comparison between the mineral profile and nitrate content of microgreens and mature lettuces. *J. Food Composition Anal.* 37:38-43.

- Samuolienė, G., A. Brazaitytė, J. Jančiauskienė, A. Viršilė, R. Sirtautas, A. Novičkovas, and P. Duchovskis. 2013. LED irradiance level affects growth and nutritional quality of Brassica microgreens. *Central European J. Biol.* 8:1241-1249.
- Sun, J., L. Kou, P. Geng, H. Huang, T. Yang, Y. Luo, and P. Chen. 2015. Metabolic assessment reveals an elevated level of glucosinolate content in CaCl₂ treated broccoli microgreens. *J. Agr. Food Chem.* 63:1863-1868.
- Watkins, C.B., and J. F. Nock. 2012. Storage of Organic Fruits and Vegetables. NYS Integrated Pest Mgt. Program Publ. 10:6.
- White, P.J., and M.R. Broadley. 2003. Calcium in Plants. *Ann. Bot.* 92:487-511.
- Xiao, Z., E.E. Codling, Y. Luo, X. Nou, G.E. Lester, and Q. Wang. 2016. Microgreens of Brassicaceae: Mineral composition and content of 30 varieties. *J. of Food Composition Anal.* 49:87-93.
- Xiao, Z., G.E. Lester, Y. Luo, and Q. Wang. 2012. Assessment of vitamin and carotenoid content of emerging food products: edible microgreens. *J. Agr. Food Chem.* 60:7644-7651.
- Xiao, Z., G.E. Lester, Y. Luo, Z. Xie, L. Yu, and Q. Wang. 2014. Effect of light exposure on sensorial quality, concentrations of bioactive compounds and antioxidant capacity of radish microgreens during low temperature storage. *Food Chem.* 151:472-479.