ACCUMULATION OF APIGENIN IN CONTROLLED ENVIRONMENT HERBS

by

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(Under the Direction of Leonardo Lombardini)

ABSTRACT

Apigenin is a bioactive compound with antibacterial, anticancer, antifungal, anti-inflammatory, and antioxidant properties. It is biosynthesized in many fruits, vegetables, and herbs as a secondary metabolite and may be isolated from plant tissue as a useful medicinal product. Although apigenin is a valuable compound, the chemical synthesis is challenging and low yielding making it a desirable target for increased production in plants. One of the highest natural producers of apigenin is parsley, and a potential avenue for improving biosynthesis of apigenin is growing parsley in a controlled environment. Controlled environment agriculture (CEA) is characterized by highly specific modifications to the growing conditions, including lighting, temperature, humidity, and CO_2 control, allowing for optimal crop production. The present research investigates the use of CEA to produce biopharmaceutical herbs. Apigenin was successfully accumulated in chamomile and parsley grown in a vertical hydroponic system in the absence of UV-B light. Furthermore, the addition of UV-B to the supplemental lighting greatly increased apigenin accumulation. Finally, this research successfully generated polyploid tissue in parsley with the aim of increasing apigenin biosynthesis; however, the polyploid plants were not stable and reverted to diploid tissue.

INDEX WORDS: Apigenin, biopharmaceuticals, chamomile, controlled environment agriculture, high performance liquid chromatography, induced polyploidy, oryzalin, parsley, hydroponics, ultraviolet B, vertical farming

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DEDICATION

To my wonderful husband Ryan who always believed in me.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1. Medicinal Value of Plants

Many plants produce biologically active compounds which can treat human disease. The oldest known record of plants used for their medicinal benefit is a collection of cuneiform tablets from the Library of Ashurbanipal in ancient Assyria from around 460-370 BC (Simko, 2021). The tablets describe prescriptive uses of many plant genera, including chamomile (*Matricaria*), fenugreek (*Trigonella*), mint (*Mentha*), poppy (*Papaver*), and safflower (*Carthamus*) (Thompson, 1926). Other ancient texts also record the medicinal value of plants, such as the Ebers Papyrus from Egypt, describing herbal treatments for various diseases (Von Klein, 1905) and the Shen Nong Ben Cao Jing, describing 365 herbs used for medicine in China (Nugent-Head, 2014).

Whereas the beneficial nature of medicinal plants was previously unknown, significant advancements in analytical techniques have allowed for the identification of plant-derived compounds that can contribute to human health. The compounds can be isolated from the plant tissue through solvent extraction followed by column chromatography or high performance liquid chromatography. Subsequently, the compounds can be identified with spectroscopy and tested for their bioactivity (Feng et al., 2019). Because of these modern analytical methods, researchers can characterize target compounds and develop preventative or prescriptive medicines.

Today, natural products isolated from plants still contribute to the development of modern drugs, including the pain reliever, codeine (extracted from poppy, (*Papaver somniferum*) (Zarin et al., 2023), digoxin, which is used for the treatment of atrial fibrillation and is extracted from woolly foxglove (*Digitalis lanata*) (de Swiet, 2023), and etoposide, the antitumor agent used in chemotherapy, derived from mayapples (*Podophyllum peltatum*) (Kluska and Woźniak, 2021).

Although medicinal plants have historically been used whole as a dietary supplement, there can be negative side effects associated with this practice. The medicinal benefit of plants is primarily attributed to the biosynthetic production of secondary metabolites, including alkaloids, phenolic compounds, and terpenoids (Kabera et al., 2014). However, plants biosynthesize hundreds of compounds, some of which are beneficial and others which are toxic (George, 2011). For example, *Ephedra* has been used to treat asthma, but alkaloids in the plant can have a negative effect by elevating blood pressure or causing an irregular heartbeat (Tang et al., 2023). Therefore, isolating the few beneficial compounds from medicinal plants is safer than risking the adverse side effects of taking them whole.

In modern drug development, selected secondary metabolites are extracted from the plant tissue. The resulting products, known as biopharmaceuticals, are used to treat various medical conditions (Chen Y.C. and Yeh, 2018). By harnessing the benefit of these naturally occurring medicinal compounds, researchers can contribute to advancements in healthcare and medicine.

2. Increasing Accumulation of Plant Secondary Metabolites

Through domestication, cultivation, and breeding, many medicinal plants have an increased growth rate, higher concentration of the desirable compounds, and greater biomass of

the target tissues (Faehnrich et al., 2021). For example, domestication led to an increased accumulation of phenylpropanoids and terpenoids in jujube (*Ziziphus jujuba*) (Zhang et al., 2022) and alkaloids in yellow lupin (*Lupinus luteus*) (Osorio et al., 2018). Genetically diverse plant populations have differing levels of secondary metabolites (Kessler and Kalske, 2018; Yusnawan, 2016) and should be intentionally selected for increased accumulation. For example, artmisinin was increased in *Artemisia annua* by selecting and cross-pollinating the highest-yielding varieties over four generations (Paul et al., 2014).

Beyond classical breeding and selection, there are other techniques to increase the abundance of secondary metabolites, such as the use of plant hormones. For example, phenolics and flavonoids increased in cat's whiskers (*Orthosiphon stamineus*) with foliar application of abscisic acid (Ibrahim and Jaafar, 2013). Auxin increased the production of linalool in cultured basil (*Ocimum basilicum*) (Monfort et al., 2018) and carvacrol in *Lippia origanoides* (Castilho et al., 2019). Alpine skullcap (*Scutellaria alpina*) treated with cytokinin had an increase in polyphenolic compounds (Grzegorczyk-Karolak et al., 2015). Ethylene treatment increased alkaloids in Madagascar periwinkle (*Catharanthus roseus*) seedlings (Chen Q. et al., 2017) and anthocyanins, flavonoids, and polyphenols in tea (*Camellia sinensis*) seedlings (Ke et al., 2018). Gibberellic acid increased flavonoids in pea (*Pisum sativum*) seedlings (Ahmad et al., 2021) and polyphenols in purple coneflower (*Echinacea purpurea*) roots (Abbasi et al., 2012). Methyl jasmonate increased glucosinolates in broccoli florets (Ku et al., 2013), and salicylic acid increased polyphenols in *Fagonia indica* callus culture (Khan et al., 2019).

In addition to applying plant hormones, the biosynthesis of secondary metabolites can also be modified in response to changing environmental factors such as carbon dioxide, drought, light intensity, ozone, temperature, and UV-B radiation (Pant et al., 2021). For example,

flavonoids and polyphenols increased in wild rosemary (*Eriocephalus africanus*) in response to drought stress (Khalil et al., 2022). In shoot cultures of lemon balm (*Melissa officinalis*), phenolic compounds increased after a brief addition of ozone (Tonelli et al., 2015). High temperatures increased secondary metabolite accumulation in St. John's wort (*Hypericum perforatum*) (Zobayed et al., 2005); however, both high and low temperatures decreased phenolics and flavonoids in Siberian ginseng (*Eleutherococcus senticosus*) (Shohael et al., 2006). Because environmental factors can increase or decrease secondary metabolite accumulation, understanding their impact is important to improve production practices.

3. Apigenin as a Target Secondary Metabolite

One class of secondary metabolites, polyphenols, have several functions in plants including contribution to flavor, color, and defense against biotic and abiotic stressors (Righini et al., 2019; Soto-Vaca et al., 2012). Flavonoids are the largest group of polyphenols, with at least 2,000 compounds found widely in plants that have natural benefits to the plant and humans (Soto-Vaca et al., 2012). The flavonoid apigenin is found in many fruits, vegetables, and herbs (Singh A. et al., 2024) and protects plants against UV-B radiation damage (Righini et al., 2019). Apigenin also contributes to the pale-yellow pigmentation in some flowers (Iwashina, 2015) and impacts herbivory and pollinator visitation. For example, alfalfa (*Medicago sativa*) with higher concentrations of apigenin experienced less pest damage by the pea aphid *(Acyrthosiphon pisum*) (Goławska et al., 2010), and apigenin abundance increased oviposition of the swallowtail butterfly (*Papilio xunthus*) (Harborne and Grayer, 1994)

Apigenin is synthesized in plants on the cytoplasmic surface of the endoplasmic reticulum through the flavonoid biosynthetic pathway (Agati et al., 2012). Within the flavonoid

pathway, apigenin biosynthesis diverges with naringenin, which can be converted to apigenin through the flavone synthase enzyme, dihydrokaempferol through flavanone 3β-hydrozylase enzyme, or eriodictyol through the flavonoid 3'-hydrozylase enzyme (Gebhardt et al., 2005). However, targeted overexpression of the *AgFNS* gene increased apigenin accumulation in celery (*Apium graveolens*) while decreasing other downstream products of naringenin (Tan et al., 2017).

As with other secondary metabolites, apigenin responds to plant stress, and environmental manipulation can increase accumulation. For example, higher temperatures and light intensity increased apigenin accumulation in St. John's wort (Odabas et al., 2010), and drought-stressed olive trees (*Olea europaea*) accumulated more apigenin than the well-watered trees (Mechri et al., 2020). Apigenin concentration increased under a CO_2-enriched environment in *Scutellaria barbata* and *S. lateriflora* (Stutte et al., 2008) and an ozone-enrichment environment in *Salvia officinalis* (Marchica et al., 2021). Furthermore, the application of methyl jasmonate also increased apigenin accumulation in pigeon pea (*Cajunus cajan*) (Du et al., 2021).

Beyond protecting plants from biotic and abiotic stressors, apigenin is also a useful medicinal compound. Apigenin is anticancer and has been used to treat bladder (Zhu et al., 2013), breast (Pham et al., 2021), cervical (Chen Y.-H. et al., 2022), colorectal (Cheng et al., 2021), and prostate (Costea et al., 2020) cancers. Apigenin effectively suppresses cancer cell growth and induces apoptosis (Imran et al., 2020). Despite the cytotoxic activity against cancer cells, apigenin is nutritionally safe with low toxicity against normal cells (Patel et al., 2007). Furthermore, apigenin is antibacterial (Kim et al., 2020), antifungal (Singh G. et al., 2014), anti-inflammatory (Wang J. et al., 2014), and antioxidant (Tian et al., 2021). Therefore, apigenin may be a desirable target for biopharmaceutical production.

Although apigenin is a medicinally useful compound, it has low natural abundance (Wang Y. et al., 2018). One viable strategy for generating apigenin for pharmaceutical use is through chemical synthesis; however, this process is low yielding (55%) across four-steps (Wang Q. et al., 2015). Other research has used genetic transformation of genes in the biosynthetic pathway of apigenin to increase accumulation in *Astragalus trigonus* (Elarabi et al., 2021). Another option is increasing production in plants through breeding and environmental manipulation for biopharmaceutical production in a controlled environment.

4. Use of Controlled Environment Agriculture

Controlled environment agriculture (CEA) is an increasingly popular production method (Gómez et al., 2019). CEA farming within the United States was valued at $3.99 Billion in 2023 and is projected to continue increasing (Grand View Research, 2024). Because CEA is not geographically bound, crop production can be implemented in almost any location, and growers can avoid unfavorable environmental conditions with temperature and irrigation control (Benke and Tomkins, 2017). Furthermore, the ability to produce crops year-round can increase revenue for growers (Benke and Tomkins, 2017). The popularization of CEA is primarily due to a demand for fresh produce with localized food distribution, eliminating the need for long-distance transportation (Benke and Tomkins, 2017; Folta, 2019). However, a new avenue for CEA may be the production of crops used for biopharmaceuticals.

CEA is a broad categorization ranging from greenhouse production to highly regulated, completely indoor systems. Arguably, the most suitable system for biopharmaceutical production would be indoor vertical farms with the ability to control factors including canopy airflow, CO_2 abundance, humidity, light intensity, light quality, and temperature. Furthermore, through their

unconventional use of space, vertical farms can produce a higher yield per acre compared to greenhouses. Although indoor systems can result in higher yields than conventional agricultural practices, high energy costs can be prohibitive (Folta, 2019). One reason for the increased cost is the reliance on artificial lighting to grow the crop instead of using solar radiation. Eaves and Eaves (2018) created a simulation to compare the profitability of lettuce produced in a vertical farm to a greenhouse with equivalent growing space considering energy needs. The researchers found that the cost of starting a vertical farm is higher, but the long-term operation costs are lower than a greenhouse due to differences in heating and cooling the growing space. Nevertheless, the energy efficiency of vertical farms could be improved through the implementation of renewable energy resources and reuse of energy heat waste (O'Sullivan et al., 2020).

A challenge of vertical farming is the limited number of crops suitable for these systems (Benke and Tomkins, 2017). The primary crops currently produced in vertical farms are lettuce, tomatoes, and strawberries (Al-Kodmany, 2018). In general, desirable characteristics for crops grown in vertical farms include short production time, compact structure, and ease of harvest. For field production, crops are bred to perform well under diverse environmental conditions with considerations for disease resistance, abiotic pressures, and postharvest quality. However, controlled environments offer the ability to select for additional factors including novel flavor and enhanced nutrition (Folta, 2019). Therefore, high-value, biopharmaceutical crops may be suitable alternatives for vertical farming. With this goal in mind, the present research aimed to determine 1) if parsley and chamomile grown under controlled environments could biosynthesis apigenin, 2) if UV-B and high-intensity LED light would be effective stressors for increasing accumulation, and 3) if ploidy manipulation could further enhance apigenin biosynthesis.

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CHAPTER 2

APIGENIN ACCUMULATION IN *MATRICARIA CHAMOMILLA* AND *PETROSELINUM CRISPUM* PRODUCED IN A VERTICAL HYDROPONIC SYSTEM

Maynard, R.C.I., S.O. Ogundipe, R.S. Ferrarezi, J.H. Suh, and L. Lombardini. 2024. HortScience 60:17-22. doi: 10.21273/hortsci18240-24.
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Abstract

Apigenin, an anticancer secondary metabolite, is produced in selected organs of a few plant taxa, including chamomile (*Matricaria chamomilla*) flowers and parsley (*Petroselinum crispum*) leaves. In this study, two cultivars of chamomile (Bodegold and Zloty Lan) and three cultivars of parsley (Darki, Giant of Italy, and Wega) were included in an indoor vertical farm trial to determine apigenin accumulation and biomass production. Vertical farming was selected for its ability to produce a quality crop with a tightly controlled growing environment. The plants were started from seed in a growth chamber and transferred to the vertical farm when they reached two sets of true leaves. The plants were maintained solely under light-emitting diodes with daily light integrals of 19 and 17 $mol \cdot m^{-2} \cdot d^{-1}$ for parsley and chamomile, respectively. The photoperiod was set to 16 hours for both species to induce flowering in the chamomile. After 15 weeks, mature parsley leaves and unopened chamomile inflorescences were harvested for analysis. All plants matured quickly during the growing period; however, only 63% of the 'Zloty Lan' chamomile plants produced flowers. At harvest, the total dry mass of each plant was also recorded. The Giant of Italy cultivar produced significantly more usable biomass compared with that of any other cultivar of parsley or chamomile, with 49.3 g usable tissue per plant. Apigenin was extracted from lyophilized samples and quantified using high-performance liquid chromatography–ultraviolet detection. The results showed that total apigenin accumulation was significantly higher in the 'Bodegold' chamomile compared to any parsley cultivar, with 0.70 $mg \cdot g^{-1}$ dried tissue. Additionally, 'Bodegold' generated significantly more usable biomass, suggesting that this cultivar shows potential for producing apigenin in a controlled environment.

Keywords: anticancer, biopharmaceutical, controlled environment

1. Introduction

Plants have long been used for their medicinal purposes, and natural products isolated from many genera still contribute to modern drug development (Jamshidi-Kia et al., 2018). Through domestication, cultivation, and breeding, medicinal plants have an increased growth rate, higher concentration of the desirable compounds, and greater biomass of the target tissues (Faehnrich et al., 2021). The medicinal benefit of plants are the result of secondary metabolite production and accumulation of alkaloids, phenolic compounds, and terpenoids (Kabera et al., 2014). For example, the alkaloid colchicine is an antitumor agent derived from autumn crocus (*Colchicum autumnale*), the furocoumarin khellin is a bronchodilator isolated from toothpick weed (*Ammi visnaga*), and the glycoside acetyldigoxin is a cardiotonic derived from wooly foxglove (*Digitalis lantana*) (Fabricant and Farnsworth, 2001).

Phenolic compounds are one of the most prevalent secondary metabolites and have several functions in plants including contribution to flavor, color, and defense against biotic and abiotic stressors (Righini et al., 2019; Soto-Vaca et al., 2012). Flavonoids are the largest group of phenolics with at least 2000 compounds found widely in plants (Soto-Vaca et al., 2012). Apigenin, an important flavonoid, is produced in several fruits, vegetables, and herbs including celery (*Apium graveolens*) (Yan et al., 2014), chamomile (*Matricaria chamomilla*) (Letchamo, 1996), citrus (*Citrus* spp.) (Abad-García et al., 2014), oregano (*Origanum* spp.) (Mueller et al., 2008) and parsley (*Petroselinum crispum*) (Poureini et al., 2022). Apigenin is one of the most cytotoxically active flavones against many cancers including bladder (Zhu et al., 2013), breast (Pham et al., 2021), cervical (Chen Y.-H. et al., 2022), colorectal (Cheng et al., 2021), and prostate (Costea et al., 2020) cancers. It has also been approved by the Food and Drug Administration for use as a combination cancer therapy to reduce resistance to traditional cancer

treatments (Nozhat et al., 2021). Other medicinal applications of apigenin include antibacterial (Kim et al., 2020), antifungal (Singh et al., 2014), anti-inflammatory (Wang J. et al., 2014), and antioxidant properties (Tian et al., 2021). Beyond prescriptive use, the dietary intake of apigenin is considered nutritionally safe, with no signs of toxicity up to 5 g kg^{-1} in mice (Nozhat et al., 2021).

Apigenin accumulation differs across plant tissues. For example, in physiologically mature celery, younger leaves have the lowest concentration of apigenin, with concentrations increasing in the more developed leaves (Yan et al., 2014). Additionally, apigenin accumulation differs in the flowers, leaves, petioles, and seeds of celery, with the greatest concentration occurring in the leaves (Yan et al., 2014). Although apigenin can be produced in different plant organs, it is typically isolated from the leaves of parsley (Poureini et al., 2022) and oregano (*Origanum* spp.) (Mueller et al., 2008). However, in chamomile, apigenin accumulates primarily in the flowers, where the highest concentration occurs in the newly opened buds, with apigenin levels steadily declining as the flower head matures (Letchamo, 1996).

Although apigenin can be chemically synthesized, the process requires a four-step, multi-day synthesis with a low (55%) yield (Wang Q. et al., 2015). Because of its low natural abundance, apigenin is costly, thus making it a desirable target for enhanced production as a biopharmaceutical (Wang Y. et al., 2018). Other research increased apigenin production in *Astragalus trigonus* through agrobacterium-mediated transformation with the *Chalcone isomerase A* (*chiA*) gene from petunia (*Petunia hybrida*). Production increased from 0.95 mg g^{-1} in the control cells to 19.81 mg g^{-1} in the transformed cells (Elarabi et al., 2021). Although genetic transformation is a viable method for increasing apigenin production, many consumers

and markets are resistant to accepting products derived from transgenic food crops (Teferra, 2021).

An alternative method for increasing productivity of apigenin is through production in controlled environments, namely indoor vertical farms. Through their unconventional use of space, vertical farms can yield greater biomass per acre compared with that of greenhouses or field production and can optimize growth through their tightly controlled growth conditions. Although advancements in lighting, irrigation, and automation allow for more efficient production of crops compared to conventional agriculture, energy usage is still a common concern for indoor systems (Folta, 2019). Additionally, the high cost of starting a new operation caused more than half of controlled environment farms to be unprofitable in 2017 (O'Sullivan et al., 2019). Therefore, selecting a high-value biopharmaceutical crop may be necessary to make vertical farming more cost-effective (Chen Y.C. and Yeh, 2018). Chamomile and parsley, which naturally produce apigenin, are both suitable crops for an indoor vertical farm because they have a short production time and compact structure. We hypothesize that chamomile and parsley grown in an indoor vertical farm will biosynthesize apigenin in harvestable quantities. The aim of this research was to determine biomass production and apigenin accumulation in selected cultivars of chamomile and parsley as potential biopharmaceutical crops for indoor vertical farm production.

2. Materials and Methods

2.1. Crop Production

Two cultivars of chamomile (*Matricaria chamomilla* L.), Bodegold and Zloty Lan (Jelitto Perennial Seeds, Louisville, KY) and three cultivars of parsley (*Petroselinum crispum*

Mill. Nyman ex A.W. Hill.), Darki, Giant of Italy, and Wega, (Johnny's Selected Seeds, Winslow, ME) were selected for trial in a hydroponic, indoor vertical farm located at the University of Georgia (College of Agricultural and Environmental Sciences, Department of Horticulture, CEA Crop Physiology and Production Laboratory) in Athens, GA, USA in June 2023. Sixteen seeds of each cultivar were directly sown onto 3.5- × 5.5-cm plugs of soilless substrate (Preforma; Jiffy Growing Solutions, Lorain, OH, USA) and germinated in a growth chamber. The light-emitting diode (LED) light intensity was set to 250 μmol m^{-2} s^{-1} for a 16-h photoperiod with setpoint values of 25 °C, 70% humidity, and 800 mg L^{-1} CO_2. The medium was kept consistently moist through subirrigation. The seedlings were ready to transplant 3 weeks after sowing for chamomile and 5 weeks after sowing for parsley. When two sets of true leaves appeared, the seedlings were transferred to a deep-water culture system and placed in net pots spaced evenly on foam rafts in square 60- × 60- × 10-cm containers.

The vertical farm was designed with two 1.2- × 0.6- × 2.0-m sections separating the chamomile and parsley trials. Both sections were subdivided into four vertically stacked shelves with two deep-water culture containers per shelf (Fig. 2.1). For parsley, each hydroponic container held a single replicate of the three trialed cultivars, which were uniformly spaced and randomized in their placement. Similarly, each chamomile hydroponic container held two uniformly spaced plants (one of each cultivar). The plants were grown under an array of three LEDs (RAY Physiospec Spectrum; Fluence, Austin, TX, USA) with a spectral output of 360 to 780 nm and a 30.5-cm spacing between the media surface and the light source. Based on the recommended light intensities, the chamomile and parsley were grown with daily light integrals of 17 and 19 mol m^{-2} d^{-1}, respectively (Litvin-Zabal, 2019; Otto et al., 2017). Because chamomile is a long-day flowering species, both herbs were grown under a photoperiod of 16 h

to induce flowering (Otto et al., 2017). Vertical reflectors were installed on both sides of the shelves to help distribute light across the canopy. The daytime and nighttime temperatures were set to 24 and 20 °C, respectively, with 800 mg L^{-1} of supplemental CO_2 during the day, and a humidity range of 50% to 75%. Air was circulated around the plant canopy through convection tubing at a rate of 1.2 m s^{-1} with two blower fans per shelf (SEAFLO, South Bend, IN, USA).

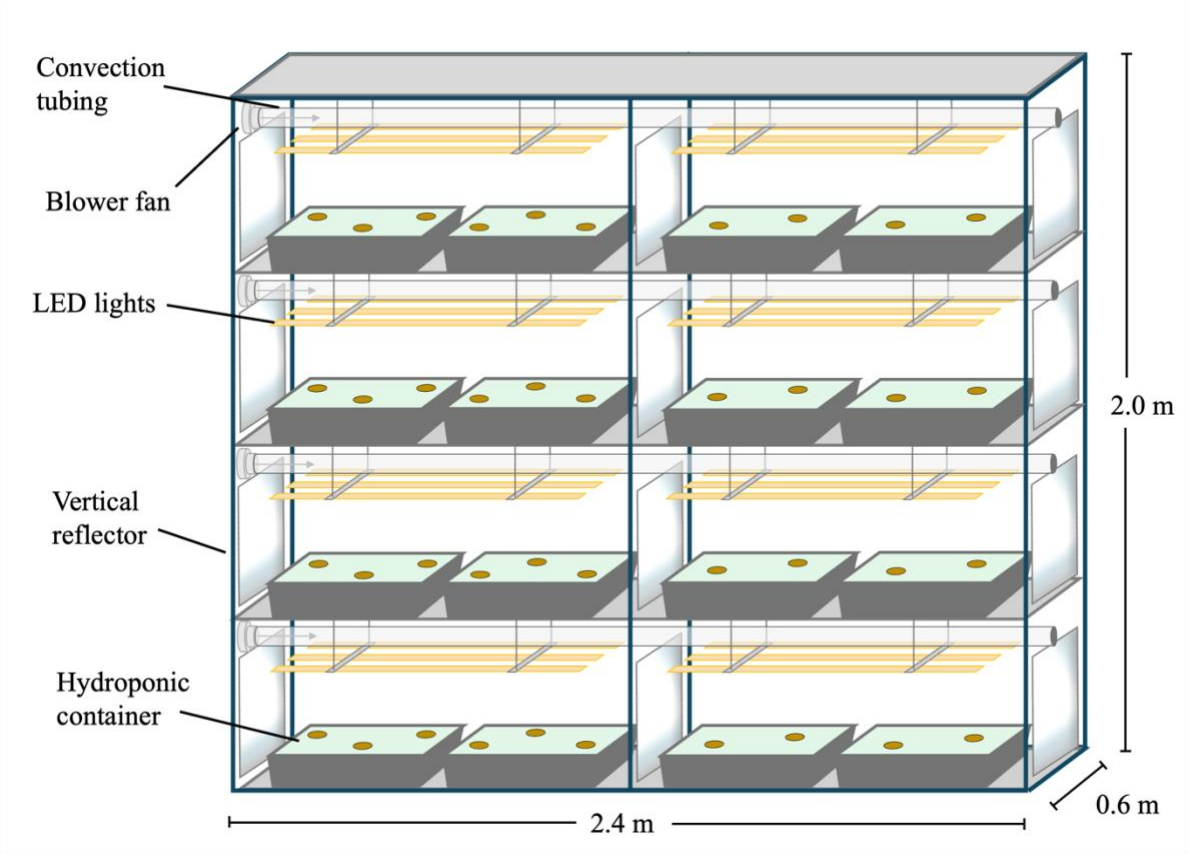

Fig. 2.1: Schematic of vertical hydroponic system divided into two 1.2- × 0.6- × 2.0-m sections to separate the parsley and chamomile trials. Blower fans forced air through convection tubing above the plant canopy to prevent humid pockets. The lighting was supplied by light-emitting diodes (LEDs) with vertical reflectors to distribute the light. The hydroponic containers held one plant from each cultivar (three for the parsley and two for the chamomile).

The net pots were held by a foam raft that allowed the plant roots to be continuously submerged in a fertilizer solution. The fertilizer solution was oxygenated with an air pump and

air stone (Fig. 2.2), and it was adjusted biweekly with a stock solution of 16N–1.8P–14.3K (Jack's Hydro FeED, JR Peters, Inc. Allentown, PA, USA). Because the seedlings were not fertilized in the growth chamber, the electrical conductivity was gradually increased from 0.75 to 1.5 dS m^{-1} over a period of 4 weeks in the vertical farm to prevent shock. The pH was adjusted with KOH or H_3PO_4 to maintain a range of 6.0 to 6.5, and $CaCO_3$ was added to each container to buffer the solution.

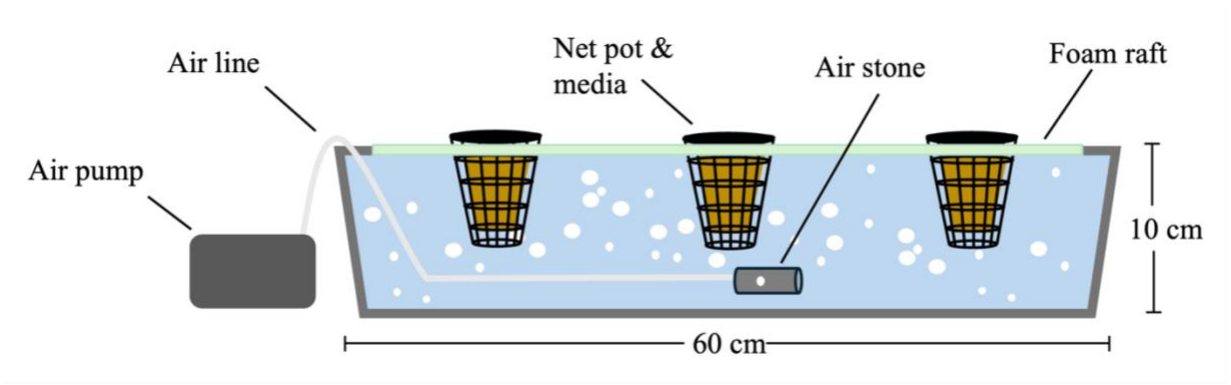

Fig. 2.2: Schematic of the deep-water culture hydroponic containers where seedlings and media were held in net pots and spaced on a floating foam raft. The roots were submerged in an aerated nutrient solution.

2.2. Harvest

Based on known apigenin accumulation, the leaves of parsley and unopened inflorescences of chamomile were considered the usable tissues. When the chamomile plants began flowering, unopened (i.e., prior to dehiscence of the disc florets) buds were harvested from individual plants twice per week. The flower buds were collected in screw-top tubes (Falcon; Corning Inc., Corning, NY, USA) and immediately placed in liquid nitrogen. Then, the samples were transferred to a lyophilizer (Labconco; Marshall Scientific, Hampton, NH, USA) and dried at -84 °C and 0.133 mbar for 24 h. When the samples were fully desiccated, they were stored in

the dark until the chemical analysis was performed. At 14 weeks after transplant, samples of the fully expanded parsley leaves were harvested from each plant and lyophilized as described. The vegetative tissue of the chamomile plants and the remaining leaves of the parsley plants were separated and dried at 80 °C for 48 h in a forced air oven (Shel Lab; Stellar Scientific, Baltimore, MD). After the drying period, the dry weight of the isolates was recorded.

The combined dried mass of the leaves and stems was calculated as the total biomass production for parsley. The combined dry mass of the inflorescences, leaves, and stems was calculated as the total biomass for chamomile. RStudio (version 2023.12.0+369) was used for the data analysis.

2.3. Sample Preparation

The stock solution of apigenin was prepared at a concentration of 1 mg mL^{-1} by dissolving the apigenin standard (95% purity) in high-performance liquid chromatography (HPLC) grade methanol/DMSO (90/10, v/v) (Sigma-Aldrich, St. Louis, MO, USA). The working standard solutions (e.g., calibration standards) were prepared daily by diluting the stock solution with methanol before use. The freeze-dried tissue samples were pulverized using a mortar and pestle. Powdered samples (100 mg for parsley and 50 mg for chamomile) were placed in 2-mL plastic tubes and mixed with 1 mL of 50% methanol. The samples were vortexed for 5 min and centrifuged at 13,500 *g* for 10 min. The supernatants were collected, passed through 0.2-µm membrane filters, and injected into the HPLC system.

2.4. HPLC Analysis

A chemical analysis was performed using HPLC (Shimadzu Nexera; Shimadzu Corp., Tokyo, Japan) equipped with a photodiode array detector. Apigenin was separated on a 4.6- × 150-mm analytical column with a particle size of 5 μm equipped with a guard column (ZORBAX Eclipse XDB-C18; Agilent Technologies, Santa Clara, CA, USA). The column temperature was set to 40 °C. The mobile phase was composed of water/acetonitrile (65/35, v/v, %) containing 0.1% formic acid (Oakwood Products Inc., Estill, SC, USA). The flow rate was 1 mL min^{-1} with an injection volume of 10 μL. After chromatographic separation, apigenin was detected at an ultraviolet wavelength of 336 nm and identified by comparing the retention times and ultraviolet spectra with the apigenin standard. Apigenin was quantified using calibration curves. LabSolutions software (version 5.124) was used for the HPLC data interpretation and analysis.

3. Results

3.1. Biomass Production

Because apigenin accumulates in the highest concentrations in the leaves of parsley (Poureini et al., 2022) and the flowers of chamomile (Letchamo, 1996), these were considered the usable tissues for biosynthesis of apigenin. The Bodegold chamomile cultivar generated 18.5 ± 20.8 g of flowers per plant, accounting for 9% of the total biomass (Fig. 2.3 and Table 2.1).

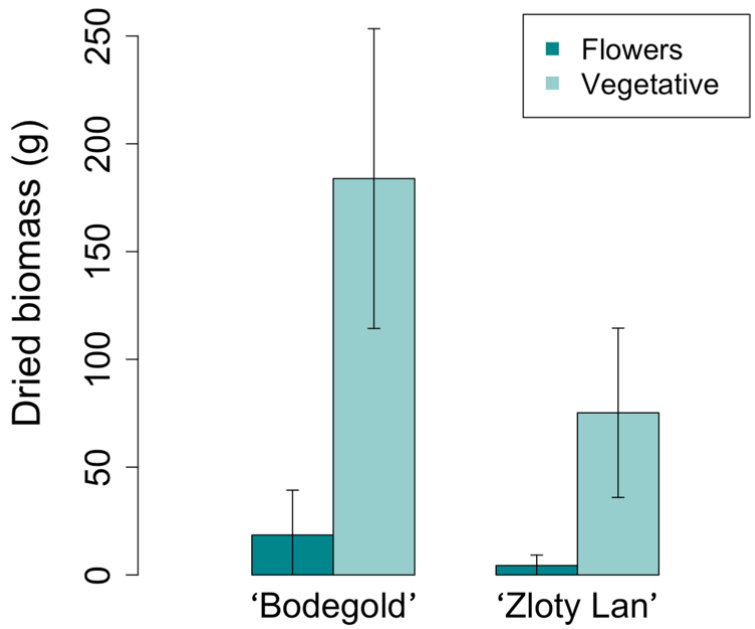

Fig. 2.3: The Bodegold and Zloty Lan chamomile (*Matricaria chamomilla*) cultivars produced 18.5 ± 20.8 g and 4.3 ± 4.8 g of flowers per plant, respectively. No statistically significant difference in flower production was found between the two cultivars. Because apigenin accumulates in the flowers of chamomile, the vegetative tissue was considered unusable.

The large standard deviation for ‘Bodegold’ was observed because the flowering of three plants was much more prolific than the other five plants. The Zloty Lan cultivar produced 4.3 ± 4.8 g of flowers per plant, accounting for 5% of the total biomass. Again, the large deviation was attributed to two of the ‘Zloty Lan’ plants that did not produce flowers. A *t* test indicated that there was no statistically significant difference in biomass of flowers of the two cultivars.

Table 2.1: Dried usable biomass, unusable biomass, and apigenin accumulation in cultivars of chamomile (*Matricaria recutita*) and parsley (*Petroselinum crispum*) produced in an indoor, deep-water culture system. Inflorescences were the usable biomass for chamomile, with all vegetative tissue counted as unusable biomass. For parsley, the leaves were considered usable and the stems were considered unusable biomass. Values are reported as the mean ± standard deviation.

Cultivar	Usable biomass (g)	Unusable biomass (g)	Apigenin concn (mg g^{-1} dried sample)	Total apigenin (mg/plant)
M. recutita Bodegold	18.53 ± 20.79	183.87 ± 69.52	0.7036 ± 0.1726	15.32 ± 20.32
M. recutita Zloty Lan	4.39 ± 4.83	75.22 ± 39.25	0.7334 ± 0.1544	5.49 ± 4.11
P. crispum Darki	21.39 ± 5.11	8.04 ± 1.74	0.0250 ± 0.0463	0.58 ± 1.13
P. crispum Giant of Italy	49.30 ± 15.43	34.96 ± 15.88	0.0032 ± 0.0013	0.15 ± 0.07
P. crispum Wega	32.82 ± 11.38	14.12 ± 6.17	0.0050 ± 0.0050	0.16 ± 0.15

Regarding parsley, the Darki, Giant of Italy, and Wega cultivars produced 21.4 ± 5.1 g, 49.3 ± 15.4 g, and 32.8 ± 11.4 g of leaf tissue per plant, representing 73%, 59%, and 70% of the total plant biomass, respectively. Although the Darki parsley cultivar had the highest percentage of usable biomass relative to total biomass production, the Giant of Italy cultivar was the most productive overall. An analysis of variance (ANOVA) for parsley showed that the dried biomass of the leaves was significantly different among the cultivars [$F(2) = 12$; $p = 0.0003$]. Tukey's honestly significant difference test indicated that the Giant of Italy cultivar generated significantly more usable biomass than that of cultivars Darki or Wega (Table 2.1 and Fig. 2.4). Compared with chamomile, all parsley cultivars produced more usable biomass, which was expected because the leaves of parsley account for most of the plant biomass. Because apigenin accumulates in the chamomile flowers, none of the vegetative tissue was used for extraction.

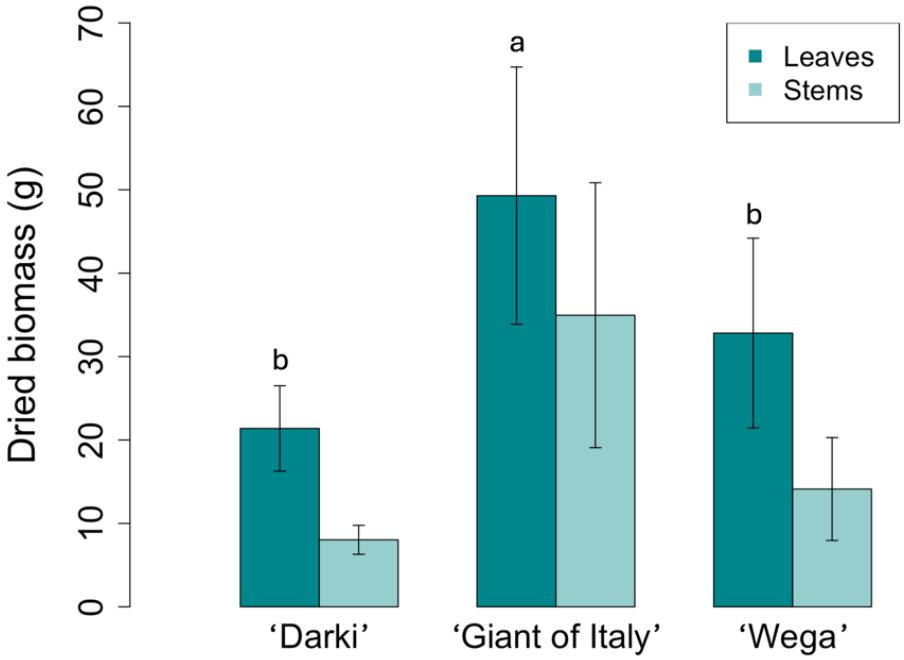

Fig. 2.4: Darki, Giant of Italy, and Wega parsley (*Petroselinum crispum*) cultivars produced 21.4 ± 5.1 g, 49.3 ± 15.4 g, and 32.8 ± 11.4 g of leaf tissue per plant, respectively. The Giant of Italy cultivar produced significantly more leaf tissue than the other cultivars [F(2) = 12; p = 0.0003]. Because apigenin accumulates in the leaves of parsley, the stem tissue was considered unusable.

3.2. HPLC Method Evaluation

Fig. 2.5 shows the chromatograms of apigenin in a standard solution and a sample. Apigenin was clearly separated from matrices, and its retention time was 5.2 min. No matrix effect was observed around the retention time of apigenin, confirming the good selectivity of the method. Linearity (quantification capacity) was achieved by plotting calibration curves of apigenin within the ranges of 6.25 to 200 µg mL^{-1} for chamomile and 0.078 to 2.5 µg mL^{-1} for parsley. For apigenin in chamomile, the calibration data showed that the linear range was 6.25 to 200 µg mL^{-1} and could be described by the linear regression equation $y = 5171\ x - 50$ (r^2 = 0.999). For apigenin in parsley, the linear range extended from 0.078 to 2.5 µg mL^{-1} with a regression equation of $y = 4610x - 832.07$ (r^2 = 0.997).

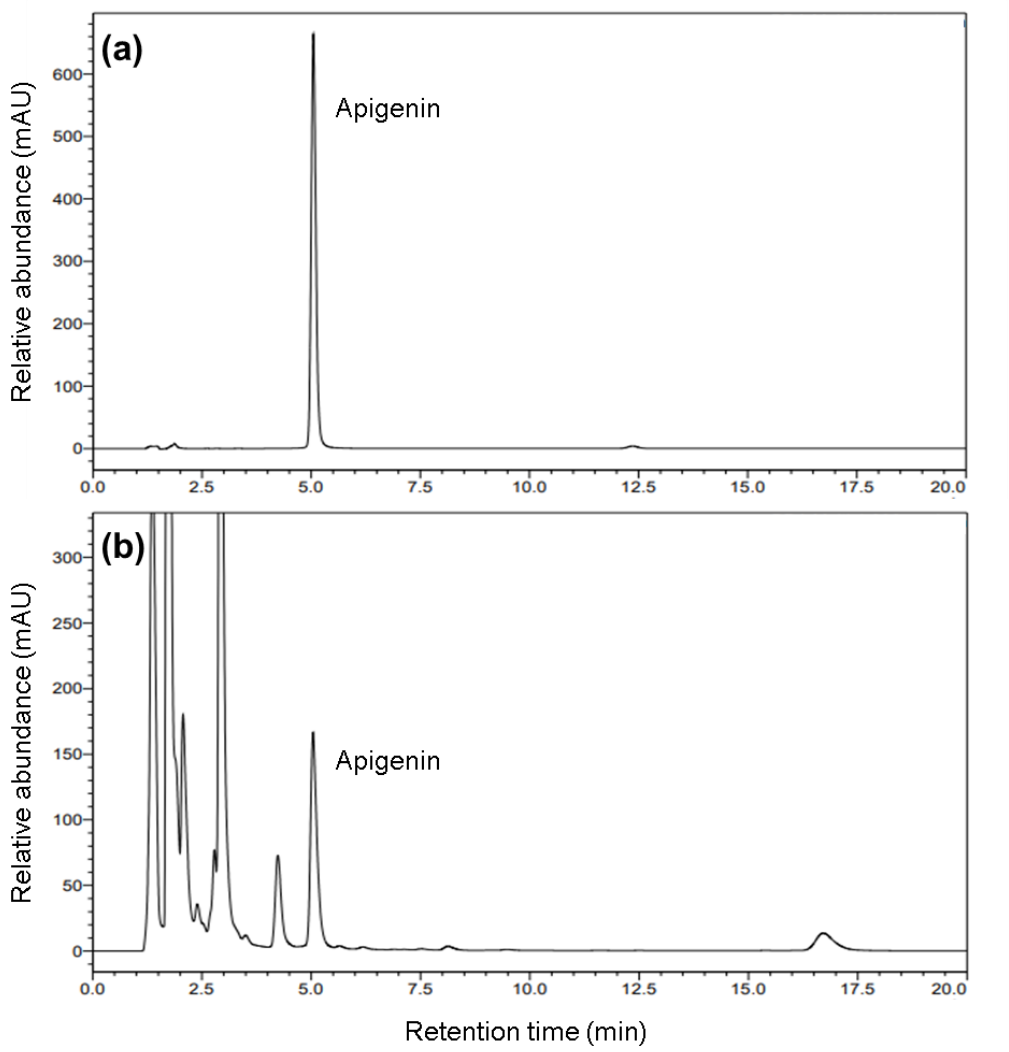

Fig. 2.5: High-performance liquid chromatography (HPLC) chromatograms of apigenin from the standard (a) and a sample from chamomile (b).

3.3. Apigenin Accumulation

The Bodegold and Zloty Lan chamomile cultivars produced 0.704 ± 0.173 and 0.733 ± 0.154 mg apigenin/g dried tissue, respectively (Table 2.1 and Fig. 2.6). A *t* test showed no significant difference in apigenin concentrations of the two cultivars.

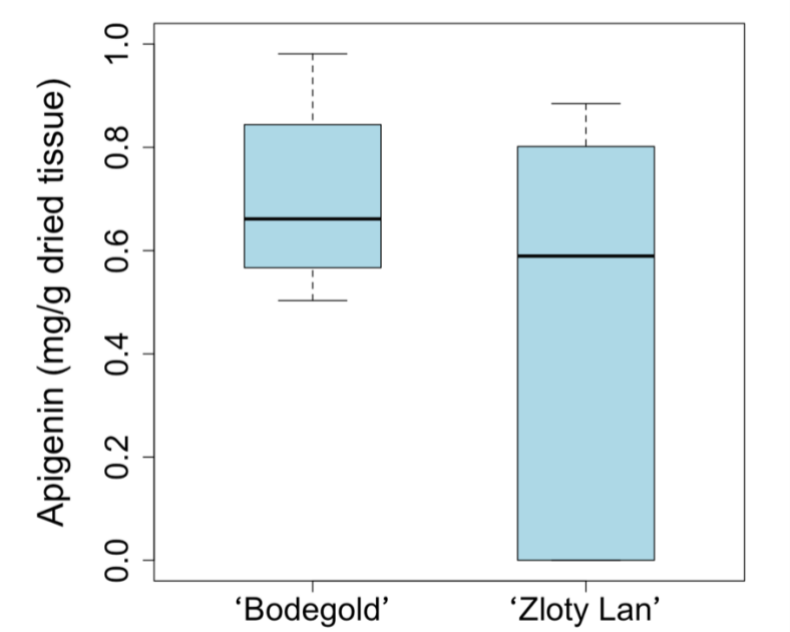

Fig. 2.6: In the inflorescences, the Bodegold and Zloty Lan chamomile (*Matricaria chamomilla*) cultivars produced 0.704 ± 0.173 and 0.733 ± 0.154 mg apigenin/g dried tissue, respectively, with no significant difference in apigenin concentration between the two cultivars.

The parsley cultivars accumulated lower concentrations of apigenin compared with that of chamomile, with 0.025 ± 0.046, 0.003 ± 0.001, and 0.005 ± 0.005 mg apigenin/g dried tissue in the Darki, Giant of Italy, and Wega cultivars, respectively (Table 2.1 and Fig. 2.7). However, one plant from the Darki parsley cultivar was an outlier, with 0.139 mg apigenin/g dried tissue, which slightly skewed the average for this cultivar.

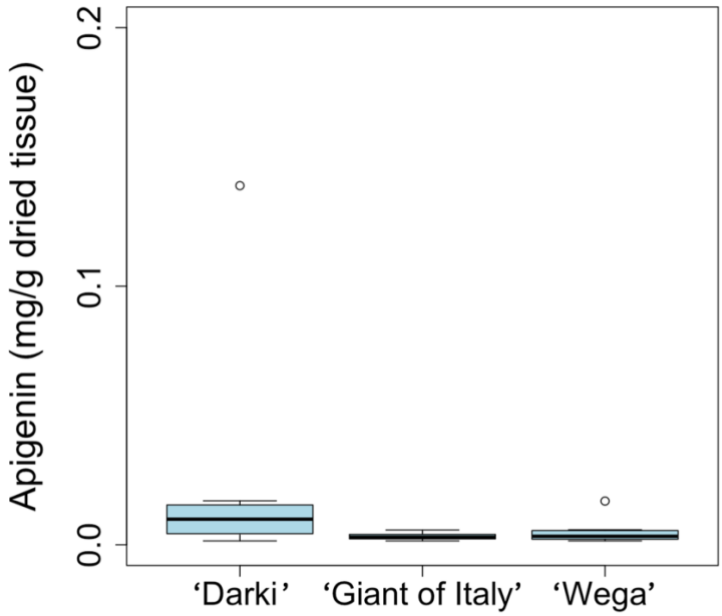

Fig. 2.7: The Darki, Giant of Italy, and Wega parsley (*Petroselinum crispum*) cultivars accumulated 0.025 ± 0.046, 0.003 ± 0.001, and 0.005 ± 0.005 mg apigenin/g dried leaf tissue, respectively, with no significant difference in apigenin accumulation among the cultivars.

An ANOVA also showed no significant difference in the apigenin concentrations among the parsley cultivars, which was unexpected. Previous research of celery and chrysanthemum (*Chrysanthemum ×morifolium*) indicated that apigenin production is cultivar-dependent (Wang Y. et al., 2018; Yan et al., 2014). Although the present research did not find a cultivar dependence for the trialed chamomile or parsley, apigenin levels may differ in other cultivars.

The concentrations of apigenin in the chamomile flowers and parsley leaves were also unexpected. Previous research isolated 7.01 ± 0.07 mg apigenin/g of dried chamomile flowers (Miguel et al., 2015) and 9.48 ± 0.11 mg apigenin/g of dried parsley leaves (Poureini et al., 2022). The present study found considerably lower levels of apigenin accumulation in both herbs. One possible explanation is that the indoor vertical farm with a spectral output of 360 to 780 nm did not have ultraviolet-B light. Previous studies have found that ultraviolet irradiation increases production of flavonoids through activity of the chalcone synthase enzyme, which

catalyzes the first step of the flavonoid biosynthetic pathway (Schmelzer et al., 1988). In plants, apigenin is a pigment that contributes to the color of white and pale-yellow flowers (Iwashina, 2015) and protects against damage by ultraviolet-B radiation (Righini et al., 2019). Therefore, the lack of ultraviolet light may have reduced apigenin biosynthesis. Future research should investigate whether the addition of ultraviolet-B light to indoor production increases accumulation of apigenin.

Because the parsley cultivars generated more usable biomass and the chamomile cultivars accumulated more apigenin in the usable tissue, the overall productivity of each cultivar was ascertained by considering apigenin production on a whole-plant basis. The Bodegold and Zloty Lan chamomile cultivars produced 15.32 ± 20.32 and 5.49 ± 4.11 mg apigenin per plant (Table 2.1 and Fig. 2.8).

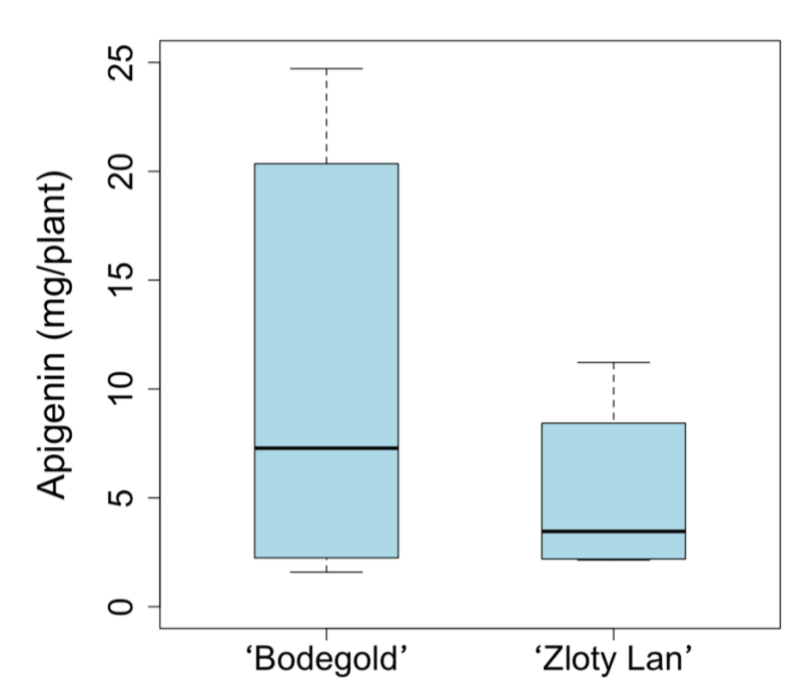

Fig. 2.8: Total apigenin production per plant from chamomile (*Matricaria chamomilla*) inflorescences. The Bodegold and Zloty Lan cultivars produced 15.32 ± 20.32 and 5.49 ± 4.11 mg apigenin per plant, respectively. No significant difference in apigenin production was found between cultivars.

In parsley, the Darki, Giant of Italy, and Wega cultivars produced 0.58 ± 1.13, 0.15 ± 0.07, and 0.16 ± 0.15 mg apigenin per plant (Table 2.1 and Fig. 2.9). When considering the total usable biomass and concentration of apigenin in the dried tissue, there was no significant difference in overall apigenin production between the chamomile cultivars or among the parsley cultivars. However, because of the higher concentration of apigenin in chamomile flowers compared with that in parsley leaves, the chamomile cultivars generated more apigenin than that generated by the parsley cultivars on a whole-plant basis.

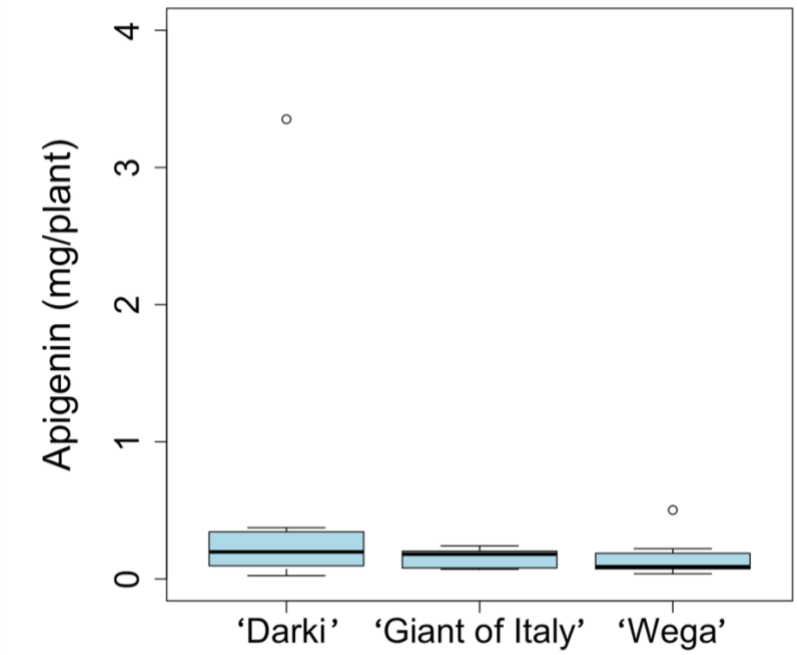

Fig. 2.9: Total apigenin production per plant from parsley (*Petroselinum crispum*) leaves. The Darki, Giant of Italy, and Wega cultivars produced 0.58 ± 1.13, 0.15 ± 0.07, and 0.16 ± 0.15 mg apigenin per plant, respectively. No significant difference in apigenin production was found among cultivars.

4. Discussion

Although the chamomile cultivars produced more apigenin per plant compared to that of parsley, other important considerations are the time to maturity and labor of harvest. Both

parsley and chamomile can be harvested multiple times throughout the growing period. Under field conditions, parsley can be harvested up to eight times, with dry matter content increasing with successive harvests (Alan et al., 2017). Field-grown chamomile is frequently harvested up to four times. However, the efficiency of harvest is variable because the continually blooming plants have inflorescences at different developmental stages (Ghareeb et al., 2022). Previous research of 'Bodegold' chamomile grown under field conditions isolated 70 to 137 g m^{-2} of dried flowers in an 8-month growing season depending on the planting density (Rahmati et al., 2011). Based on spacing used in the present study, 24 'Bodegold' chamomile plants could be grown in a 1-m^2 footprint with 2 m of vertical growing space. Because 'Bodegold' yielded an average of 18.5 g of dried flowers per plant, the expected yield would be approximately 445 g m^{-2} of dried flowers in an indoor vertical farm. Furthermore, the time from transplant to final harvest was 80 d meaning it would be easily possible to generate four crop cycles in a calendar year in a controlled environment yielding approximately 1.78 kg m^{-2} y^{-1} of dried flowers. Therefore, indoor vertical farming of 'Bodegold' chamomile is expected to produce significantly greater yields compared with that of field production because vertical farming uses space more efficiently and indoor systems are not season dependent.

The Bodegold chamomile cultivar yielded the greatest amount of apigenin per plant, with an average of 15.3 mg, meaning it could be possible to isolate approximately 1.5 g m^{-2} y^{-1} of apigenin based on the plant spacing used in this study. It is challenging to estimate the profitability of growing chamomile as a biopharmaceutical because the economic value of apigenin is dependent on the source and purity of the commercial product. Furthermore, it would be important to consider factors such as the cost of inputs, labor, and isolating apigenin to determine the economic viability of selecting chamomile as a biopharmaceutical.

In this study, chamomile was harvested twice per week to collect inflorescences before the disc florets dehisced. It is essential to harvest chamomile at the correct developmental stage because fully opened buds have a lower concentration of apigenin (Letchamo, 1996). In this study, the number of flowers increased over time as the plants matured, but the time to collect the flowers also increased. For commercial production of chamomile, the labor associated with frequent hand-harvesting would likely result in a significant profit reduction compared with that associated with the labor of harvesting parsley. One possibility would be mechanizing the harvesting of the flowers, which is a common method for field production. However, mechanical harvesters have not yet been fully developed for indoor systems, and they would likely need to be modified with lower tine spacing to collect the unopened inflorescences. Future research should also investigate whether apigenin accumulation changes throughout multiple harvests to determine when the plants should be replaced in the vertical farm.

Despite the higher yields of ‘Bodegold’, one challenge identified in this study was the high proportion of wasted biomass. A potential solution would be to harvest and repurpose the vegetative tissue at the end of the growing period. Previous research found that chamomile is a useful filler for rubber biocomposites. Chamomile biomass added to natural rubber as 20% of the constituent material resulted in a biocomposite with higher strength than that of the base polymer and reduced the use of synthetic materials (Masłowski et al., 2021). Therefore, the vegetative byproduct of chamomile, which does not accumulate high levels of apigenin, may be useful for polymer technology.

5. Conclusion

Chamomile and parsley may be effective crops for biopharmaceutical production of the anticancer compound apigenin. Bother herbs grow readily in a controlled environment with compact growth well-suited for year-round indoor vertical farming. In this study, both chamomile and parsley accumulated apigenin, but the chamomile cultivars produced more apigenin than that of any parsley cultivar, yielding 0.704 ± 0.173 and 0.733 ± 0.154 mg apigenin/g dried flowers in the Bodegold and Zloty Lan cultivars, respectively. However, the observed yields were lower than expected. Future research may investigate the impact of adding ultraviolet-B light to the indoor system on overall apigenin accumulation. Despite the greater yield of apigenin in chamomile compared with that in parsley, regularly harvesting the unopened inflorescences is more labor-intensive than harvesting mature parsley leaves, which may reduce the profitability of selecting chamomile as a biopharmaceutical crop. Therefore, future research should also investigate the profitability of isolating apigenin from chamomile and consider novel applications of the unharvestable biomass.

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Author Contributions

All authors listed have made substantial direct and intellectual contribution to the work and approved it for inclusion on this dissertation. Specifically, R.C.I. Maynard contributed to the

experimental design, produced the research crops, collected plant samples for HPLC analysis, analyzed the data, and summarized the findings in this report. S.O. Ogundipe prepared and analyzed all HPLC samples. R.S. Ferrarezi advised the research and provided access to the vertical farm. J.H. Suh advised the HPLC analysis of plant samples and provided access to the HPLC. L. Lombardini advised the research and funded the experiment.

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CHAPTER 3

UV-B IRRADIATION INCREASES APIGENIN ACCUMULATION IN PARSLEY UNDER CONTROLLED ENVIRONMENTS

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Abstract

Parsley (*Petroselinum crispum*) is one of the highest natural producers of apigenin, a medicinally valuable secondary metabolite. This study evaluated the impact of light-emitting diode (LED) light intensity and UV-B exposure on apigenin accumulation in the Giant of Italy parsley cultivar in environment-controlled growth chambers. The plants were maintained under LED lights with a 16-h photoperiod and a daily light integral (DLI) of 19.0, 23.8, or 28.5 mol m^{-2} d^{-1}. After eight weeks, mature parsley leaves of the control plants were harvested for analysis. The remaining plants were supplemented with 475 mW cm^{-2} UV-B light to act as a stressor for inducing apigenin biosynthesis during the full 16-h photoperiod for 1, 2, 4, or 8 days. Apigenin was quantified in lyophilized leaf samples with high-performance liquid chromatography–ultraviolet (HPLC–UV) detection. Results showed that apigenin accumulation was not impacted by LED light intensity but significantly increased with UV-B exposure.

Keywords: anticancer, growth chamber, light intensity, ultraviolet-B

1. Introduction

Plants are naturally exposed to a broad range of solar radiation, including UV-B (290-320 nm), UV-A (320-400 nm), visible (400-750 nm), and infrared (>750 nm) light (Ulm, 2006). The pigments primarily involved in light capture for photosynthesis, namely chlorophyll *a*, *b*, and carotenoids, absorb light in the 400-700 nm range (Simkin et al., 2022). However, infrared light has been found to increase the efficiency of photosynthesis (Zhen et al., 2019). Approximately 5% of the solar radiation transmitted through Earth's atmosphere is in the ultraviolet (UV) range, and due to the high absorption by ozone, only 0.25% is UV-B (IARC, 2012). Despite the low

proportion of UV-B in solar radiation, UV-B can damage plant DNA, cause organelle dysfunction, and ultimately lead to cell death (Nawkar et al., 2013). Plants detect UV-B light with the UVR8 protein (Christie et al., 2012) and exhibit protective responses such as reducing cell expansion (Fasano et al., 2014), suppressing hypocotyl growth (Cloix et al., 2012), and synthesizing flavonoids to absorb the UV radiation (Koes et al., 1994).

UV-B light is not considered essential for normal plant growth and development because plants, such as chrysanthemum (*Chrysanthemum × morifolium*) (Kim et al., 2004), lettuce (*Lactuca sativa*) (Chen X.-l. et al., 2019), and strawberry (*Fragaria × ananassa*) (Samuolienė et al., 2010), can be grown in controlled environments using blue and red light-emitting diodes (LEDs) without UV light. Nevertheless, the addition of UV-B light to controlled environments is a useful method of increasing the biosynthetic production of flavonoids. For example, treatment with UV-B light increased quercetin and luteolin in European privet (*Ligustrum vulgare*) (Agati et al., 2011), kaempferol in *Petunia* (Ryan et al., 2002), and isorhamnetin in ginkgo (*Ginkgo biloba*) (Zhao et al., 2020).

One important flavonoid, apigenin, is biosynthesized in plants to protect against UV-B damage (Righini et al., 2019) by strongly absorbing in the UV range (Amat et al., 2009). Apigenin is medicinally significant with effectiveness against bladder (Zhu et al., 2013), breast (Pham et al., 2021), cervical (Chen Y.-H. et al., 2022), colorectal (Cheng et al., 2021), and prostate (Costea et al., 2020) cancers. It is also useful as an antifungal (Singh et al., 2014), anti-inflammatory (Wang J. et al., 2014), and antioxidant agent (Tian et al., 2021). Apigenin can be chemically synthesized for medicinal use, but the process is multi-day and low-yielding (Poureini et al., 2022). One of the highest natural producers of apigenin is parsley (*Petroselinum crispum*), but, as with many secondary metabolites, accumulation of apigenin is relatively small,

with 9.48 ± 0.11 mg g^{-1} in the dried leaves (Wang Q. et al., 2015). Because apigenin is medicinally valuable, it is an interesting target for enhanced biosynthetic production to be extracted for pharmaceutical use.

One possible method to increase apigenin accumulation is to expose plants to higher levels of UV-B radiation. Elevated apigenin levels in celery (*Apium graveolens*) was correlated with increased transcription of the chalcone synthase (CHS), chalcone isomerase (CHI), and flavone synthase (FSI) enzymes (Yan et al., 2014), which are directly involved in the biosynthetic pathway of apigenin (Marín et al., 2017). Furthermore, CHS activity increased in response to supplemental UV irradiation in parsley (Schmelzer et al., 1988). Based on these findings, we hypothesize that apigenin will accumulate at higher levels in plants through exposure to UV-B light. Therefore, the aim of this study was to determine the impact of increasing LED intensity and UV-B light on apigenin production in parsley.

2. Materials and Methods

2.1. Plant Material and Environmental Conditions

Parsley ‘Giant of Italy’ seeds (Johnny’s Selected Seeds, Winslow, ME) were individually sown on substrate (PRO-MIX HP; Premier Tech Horticulture, Quakertown, PA) in 96-well trays in three identical 1.8 × 0.8 m growth chambers (CMP 3244; Conviron, Pembina, ND) located at the University of Georgia (College of Agricultural and Environmental Sciences, Department of Horticulture, CEA Crop Physiology and Production Laboratory). The temperature in each growth chamber was maintained at 24 °C during the day and 20 °C at night with a 16-h photoperiod, and the trays were manually irrigated overhead to maintain a consistently moist substrate.

2.2. Treatment and Experimental Design

The daily light integral (DLI) was set differently in the three growth chambers to determine the impact of supplemental light-emitting diode (LED) light intensity on apigenin accumulation. One growth chamber received 19.0 mol m^{-2} d^{-1} of light as recommended by Litvin-Zabal (2019), and the other two growth chambers had a 25% and 50% increase in the recommended DLI with 23.8 and 28.5 m^{-2} d^{-1}, respectively. After five weeks, the seedlings were transplanted into 5-cm pots, and after two more weeks, the seedlings were transplanted into 10-cm pots. The plants were arranged in a randomized complete block design based on their assigned light treatment. Each growth chamber had eight plants that would receive 0, 1, 2, 4, or 8 days of exposure to ultraviolet (UV) light.

2.3. Pre-Treatment Harvesting and UV-B Light Application

The control plants were harvested eight weeks after sowing at the end of the 8-h dark period. Due to the size of the plants at the time of harvest, two plants were combined for each replicate to yield four replicates of each treatment per growth chamber. The fully expanded parsley leaves were collected into screw-top tubes (Falcon; Corning Inc., Corning, NY) and transferred to a dewar containing liquid nitrogen. Each sample was dried at -84 °C and 0.133 mbar for 24 h in a lyophilizer (Labconco; Marshall Scientific, Hampton, NH). Then, the freeze-dried samples were ground into a fine powder using a mortar and pestle and stored at -80 °C.

For the remaining plants, the LED light was supplemented with UV light provided by 18W fluorescent tubes (MIGRO UVB 310; MIGRO Grow Lights, Dublin, Ireland) consisting of 25% UV-A and 75% UV-B light with the peak spectral output at 310 nm. Three light bars were spaced 0.6 m apart in the growth chambers. The average 310 nm light intensity, measured at the

height of the pots with a light meter (Light Meter UVA/B; Sper Scientific Direct, Scottsdale, AZ), was 475 mW cm^{-2}. The UV-B light supplemented the LED light for the entire 16-h photoperiod each day after the control plants were harvested. The UV-treated parsley plants were left for an 8-h dark period before the leaves were harvested following the procedure described above. The method of harvesting after a dark period was based on a previous study of parsley which found that mRNA levels of the CHS enzyme, which is part of the apigenin biosynthesis pathway, increased during a 16-h photoperiod of UV-containing light and continued to increase through an 8-h dark period following the treatment (Schmelzer et al., 1988).

2.4. Apigenin Quantification

The dried leaf samples were analyzed using high-performance liquid chromatography–ultraviolet (HPLC–UV) detection to quantify apigenin accumulation in the tissue. A stock solution of apigenin was prepared at a concentration of 1 mg mL^{-1} by dissolving the apigenin standard (95% purity) in HPLC grade methanol/DMSO (90/10, v/v) (Sigma-Aldrich, St. Louis, MO). The working standard solutions (e.g., calibration standards) were prepared daily by diluting the stock solution with methanol prior to use. The lyophilized parsley leaves (100 mg) were mixed with 1 mL of 50% methanol in 2 mL plastic tubes. The samples were vortexed for 10 min and centrifuged at 13,500 × g for 10 min. The supernatants were collected, passed through 0.2 µm membrane filters, and injected into the HPLC system.

The HPLC (Shimadzu Nexera; Shimadzu Corp., Tokyo, Japan) used for apigenin quantification was equipped with a photodiode array detector. Apigenin was separated on a 4.6 × 150 mm analytical column with a 5 µm particle size equipped with a guard column (ZORBAX Eclipse XDB-C18; Agilent Technologies, Santa Clara, CA). The column temperature was set to

35 °C. The mobile phase was composed of water/acetonitrile (65/35, v/v, %) containing 0.1% formic acid (Oakwood Products Inc., Estill, SC). The flow rate was 1 mL min^{-1} with an injection volume of 5 μL. After chromatographic separation, apigenin was detected at a UV wavelength of 336 nm and identified by comparing the retention times and UV spectra with the apigenin standard. Apigenin was quantified using calibration curves.

2.5. Experimental Design and Statistical Analysis

The experiment was designed as a two-way factorial. The first factor, with three levels, was LED light intensity. The second factor, with five levels, was days of UV-B exposure. Each treatment group contained eight parsley plants. LabSolutions software (Ver. 5.124) was used for the HPLC data interpretation. RStudio (version 2023.12.0+369) was used for statistical analysis.

3. Results and Discussion

3.1. HPLC Method Evaluation

Apigenin was clearly separated from matrices (Fig. 3.1), with a retention time of 5.3 min. No matrix effect was observed around the retention time of apigenin, confirming the good selectivity of this method. Linearity was achieved by plotting calibration curves of apigenin within the range of 0.1 to 10.0 μg mL^{-1}. For apigenin in parsley, the linear range can be described by the regression equation $y = 5887x + 33$ ($r^2 = 0.9999$). Therefore, HPLC analysis was considered an appropriate method for isolating and quantifying apigenin abundance.

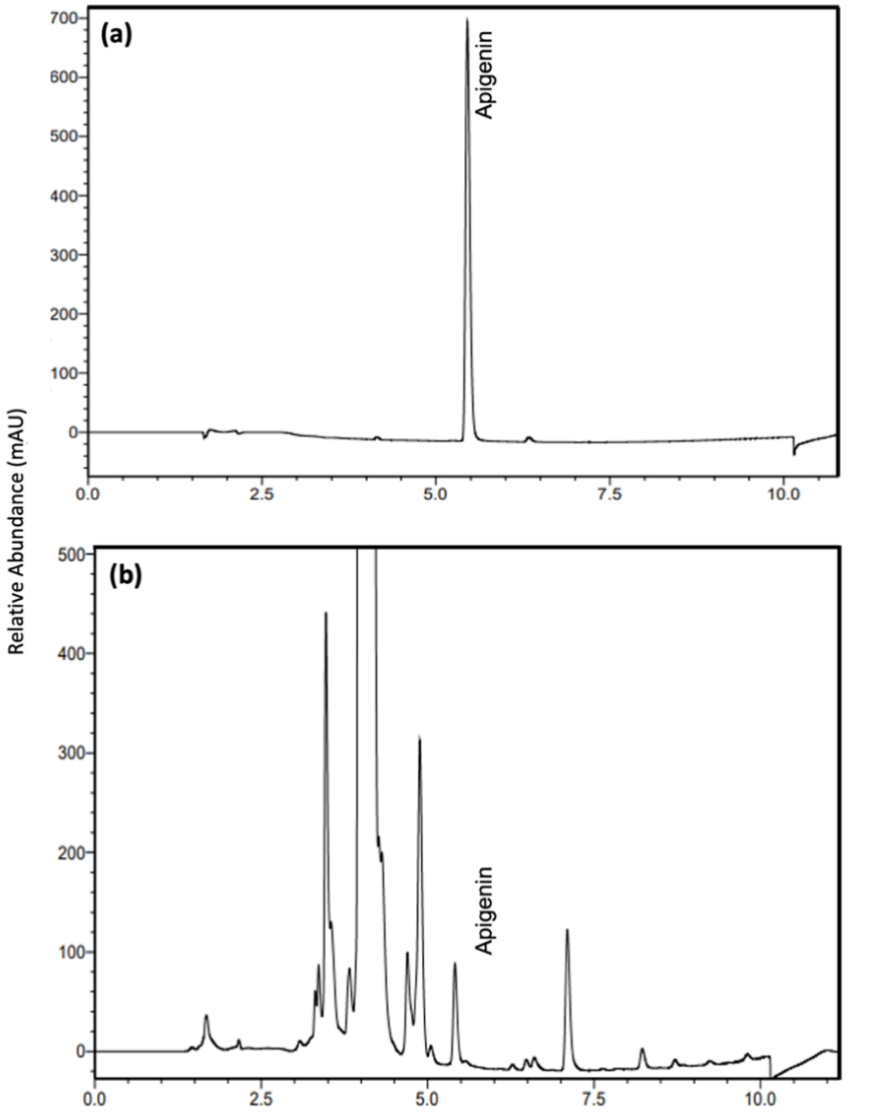

Fig. 3.1: HPLC chromatograms of apigenin from the standard (a) and a sample of lyophilized parsley leaves (b).

3.2. Apigenin Accumulation in Response to LED Intensity

One objective of this study was to investigate the impact of high-intensity light under the normal photosynthetically active range on apigenin accumulation in parsley. Across the treatment groups, apigenin was detected in the range of 0.61-16.52 mg kg^{-1} (Fig. 3.2).

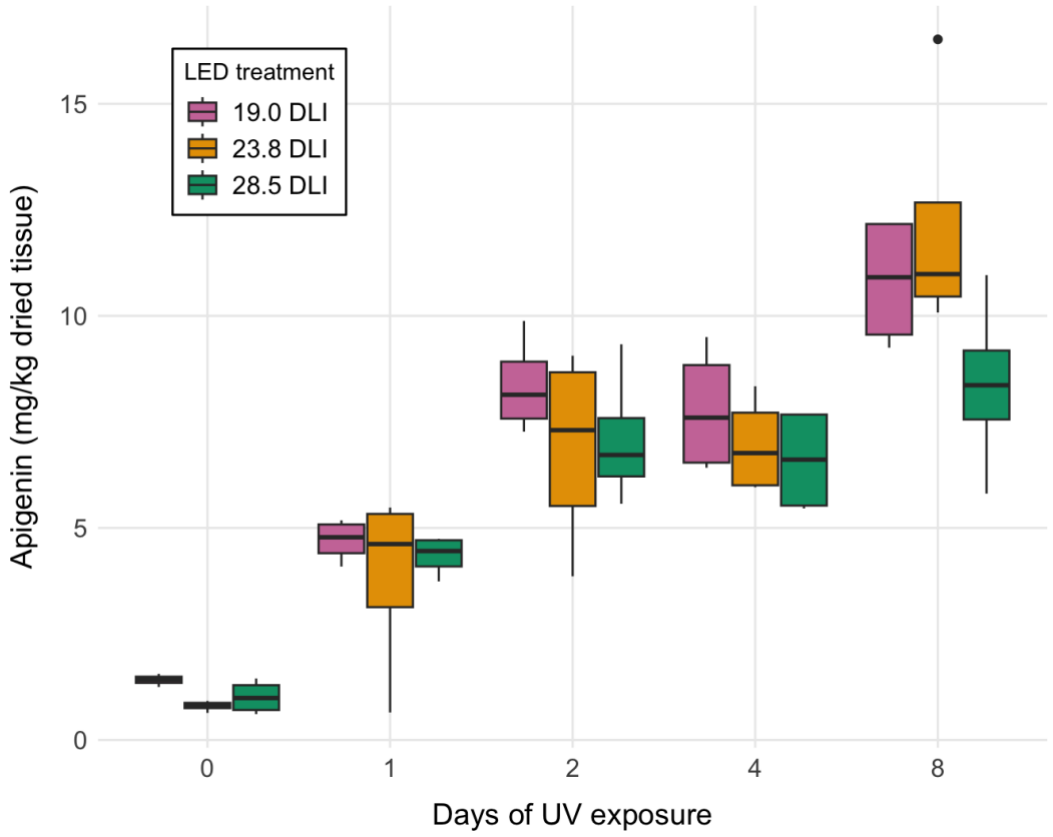

Fig. 3.2: Apigenin accumulation was quantified in dried parsley leaves following different light treatments. The parsley plants were exposed to 19.0, 23.8, or 28.5 DLI of LED light and up to eight days of UV-B light.

The impact of UV-B light on apigenin accumulation was statistically significant ($F(4) = 62.11$, $p < 0.001$), with apigenin accumulation increasing with more prolonged UV-B exposure. Although there appeared to be a trend of lower apigenin accumulation at higher LED intensities, there was no statistically significant difference across LED light levels ($F(2) = 2.67$, $p = 0.081$), indicating that the LED intensities used in this study did not impact apigenin accumulation. Furthermore, there was no significant interaction between LED light intensity and UV-B exposure on apigenin accumulation in parsley ($F(8) = 1.38$, $p = 0.233$). Therefore, UV-B light alone had a strong positive impact on apigenin accumulation. Because the apigenin levels were

not significantly different in parsley under different LED light intensities, the data collected across the three growth chambers was averaged (Table 3.1).

Table 3.1: Mean concentration of apigenin in dried parsley leaves under different LED and UV light treatments.

UV-B (d)	**LED (mol m^{-2} d^{-1})**	**Apigenin (mg kg^{-1} dried tissue)**
0	19	1.42 ± 0.13
	23.8	0.80 ± 0.12
	28.5	1.01 ± 0.40
	Mean	**1.08 ± 0.31**
1	19	4.71 ± 0.50
	23.8	3.84 ± 2.23
	28.5	4.35 ± 0.47
	Mean	**4.30 ± 0.43**
2	19	8.36 ± 1.16
	23.8	6.88 ± 2.40
	28.5	7.09 ± 1.61
	Mean	**7.44 ± 0.63**
4	19	7.78 ± 1.52
	23.8	6.96 ± 1.17
	28.5	6.59 ± 1.25
	Mean	**7.11 ± 0.61**
8	19	10.81 ± 1.58
	23.8	12.14 ± 2.97
	28.5	8.38 ± 2.11
	Mean	**10.44 ± 1.91**

	p-value
UV-B	<0.001
LED	0.081
UV-B × LED	0.233

In previous research, sweet basil (*Ocimum basilicum*) grown below the recommended DLI resulted in lower total flavonoid content (Dou et al., 2018). Furthermore, a study of

strawberry (*Fragaria ×ananassa*) found that flavonoid accumulation decreased at elevated light levels (Maeda and Ito, 2020). These studies suggest that flavonoid accumulation can be negatively impacted above or below the recommended DLI. The present study exceeded the recommended light intensity for parsley with no positive or negative impact on apigenin accumulation. However, it is possible that DLI beyond the range used in this experiment could impact apigenin accumulation in parsley.

3.3. Apigenin Accumulation in Response to UV-B Exposure

Although the impact of LED light intensity was not statistically significant, the slight variation in apigenin accumulation across the three LED treatment groups may be due to the proportion of UV-B in the overall light source. Because the UV bulbs used in this study consisted of 25% UV-A and 75% UV-B light, the addition of 475 mW cm^{-2} of UV light resulted in 7.65 mmol s^{-1} m^{-2} of UV-B reaching the plant canopy. The parsley plants grown at the recommended DLI received a light intensity of 330 mmol s^{-1} m^{-2} from the LED lights, with the higher LED light treatments receiving 412 and 495 mmol s^{-1} m^{-2}, respectively. The intensity of UV-B light was constant across the three growth chambers, meaning that at higher LED intensities, a lower percentage of the overall light was UV-B. For plants grown under the highest LED intensity, 1.5% of the light was UV-B, whereas 2.3% of the light was UV-B in the lowest LED intensity treatment. Even though the percentage of UV-B light varied across growth chambers, the treatment groups still experienced a six to nine-fold increase in UV-B intensity compared to the 0.25% in normal daylight (IARC, 2012). Therefore, the plants in this study experienced higher levels of UV-B light than they would in field conditions. Despite the elevated

UV-B levels, there was no negative impact on the plant's appearance based on visual observation.

Because the experiment was conducted in growth chambers with no UV output from the LED lights, the control plants did not receive any UV light prior to harvest. However, apigenin was detected in the control parsley, albeit at very low levels. Many studies report the positive influence of UV irradiation on flavonoid accumulation (Agati et al., 2011; Ryan et al., 2002; Zhao et al., 2020), but our findings show that UV light is not essential for the biosynthesis of apigenin. Although apigenin accumulated without UV light, the concentration of apigenin significantly increased with greater UV-B exposure in the treated groups ($F(4) = 62.11$, $p < 0.001$).

The greatest apigenin accumulation occurred after 8 d of UV-B exposure with 10.44 ± 1.91 mg kg^{-1} per dried parsley leaves. Compared to the control with 1.08 ± 0.31 mg kg^{-1} dried tissue, the 8-d UV-B exposure resulted in an 867% increase in apigenin concentration. In contrast, a study of hairy root cultures of pigeon pea (*Cajanus cajan*) found the greatest concentration of apigenin, approximately 7 mg kg^{-1} dried tissue, accumulated after 4 h of irradiation with UV-B light and gradually decreased with longer exposure (Gai et al., 2022). Although the present study found an increase in apigenin through 8 d of UV-B exposure, it is not known whether apigenin would have a response to reduced or prolonged treatment.

4. Conclusion

Apigenin is an effective biopharmaceutical and may be naturally increased in plants by manipulating the wavelength range and intensity of the light source. The present study determined that UV-B light is not essential for the biosynthesis of apigenin, but accumulation

increased with longer exposure to UV-B. The treatment group with 8 d of UV-B light had the highest apigenin concentration with 10.44 ± 1.91 mg kg^{-1} dried tissue, which was 867% greater than the control group. Because apigenin accumulation was not impacted by exceeding the recommended DLI with LED light, future research should focus on using a higher-intensity UV-B source and prolonging exposure beyond the 8-d period to determine the optimal UV-B light treatment for maximizing apigenin production in parsley.

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Author Contributions

All authors listed have made substantial direct and intellectual contribution to the work and approved it for inclusion on this dissertation. Specifically, R.C.I. Maynard contributed to the experimental design, produced the research crops, collected plant samples for HPLC analysis, analyzed the data, and summarized the findings in this report. S.O. Ogundipe prepared and analyzed all HPLC samples. R.S. Ferrarezi advised the research and provided access to the growth chambers. J.H. Suh advised the HPLC analysis of plant samples and provided access to the HPLC. L. Lombardini advised the research and funded the experiment.

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CHAPTER 4

IMPACT OF POLYPLOID INDUCTION ON ‘GIANT OF ITALY’ PARSLEY (*PETROSELENUM CRISPUM)*

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Abstract

Apigenin is a medicinally useful secondary metabolite with anticancer, antibacterial, antifungal, anti-inflammatory, and antioxidant properties. Although parsley (*Petroselinum crispum*) is one of the highest natural producers of apigenin, polyploid induction may be a viable strategy for increasing apigenin production. To test this hypothesis, an experiment was designed to determine the impact of colchicine on seed germination, ploidy level, and apigenin concentration in 'Giant of Italy' parsley. The diploid parsley seeds were treated with 0.00, 0.02, 0.04, 0.08, or 0.16% colchicine for 48 hours. Results showed that seedling emergence decreased with increasing treatment concentration. The seeds which survived the colchicine treatment were then grown to maturity in a growth chamber. Flow cytometry confirmed four plants from the 0.02% and three from the 0.04% treatment groups to have mixoploid tissue. Although colchicine successfully increased the parsley ploidy, all mixoploid plants reverted to diploid in their mature stage. Because the reverted plants were indistinguishable from the control parsley, HPLC quantification of apigenin accumulation in these previously mixoploid plants was inconclusive.

Keywords: anticancer, colchicine**,** polyploidy, secondary metabolites

1. Introduction

Polyploidy is the generation of additional chromosome sets within the cell nucleus and can occur naturally, leading to the evolution of wild populations, or can be artificially induced as a breeding tool (Sattler et al., 2016). Polyploid plants can be generated through chemical disruption of spindle formation during mitosis, causing a complete duplication of the genome (Dhooghe et al., 2011). Some of the most common antimitotic agents used for polyploid

induction are colchicine, oryzalin, and trifluralin. For example, colchicine treatment generated polyploid ginger (*Zingiber officinale*) (Zhou et al., 2020) and orchid (*Dendrobium wardianum)* (Wang F. et al., 2023). Oryzalin induced polyploidy in onions (*Allium cepa*) (Yun et al., 2021) and watermelon (*Citrullus lanatus*) (Bae et al., 2020), and trifluralin generated polyploid blueberry (*Vaccinium duclouxii*) (Lei et al., 2023) and fenugreek (*Trigonella feonum-graecum*) (Alavi et al., 2022). Colchicine is the most efficient and reliable of these antimitotic agents, but it is also highly toxic to humans (Eng and Ho, 2019).

Artificial polyploidization is a useful breeding technique because elevated ploidy often leads to increased yield or improved plant performance. For example, induced polyploidy resulted in increased fruit size of star fruit (*Averrhoa carambola*) (Hu et al., 2021) and kiwifruit (*Actinidia chinensis*) (Wu et al., 2011). In tetraploid radish (*Raphanus sativus*), the root yield was lower, but the tissue was less pithy, which is a desirable trait (Kim H.L. et al., 2022). Furthermore, drought stress tolerance was improved in polyploid hybrids of atemoyas (*Annona cherimola* × *Annona squamosa*) due to structure alterations of the xylem vessels (Losada et al., 2023).

Polyploid induction is also a viable breeding method to increase the production of secondary metabolites (Madani et al., 2021). For example, tetraploid wormseed (*Artemisia cina*) had greater quercetin and kaempferol content compared to the diploid form (Kasmiyati et al., 2020). Artificial polyploidization led to higher yields of the flavonoid baicalin in Baikal skullcap roots (*Scutellaria baicalensis*) (Gao et al., 2002) and the triterpenoid ginsenoside in ginseng roots (*Panax ginseng*) (Kim Y.-S. et al., 2004). Additionally, in *Salvia leriifolia*, polyploid induction increased the overall quantity of existing secondary metabolites and resulted in the biosynthesis of additional compounds (Estaji et al., 2017). The increased production of

secondary metabolites in higher ploidy species may be due to greater gene activity from more gene copies in the duplicated genomes. For example, several genes in the biosynthetic pathway of artemisinin were upregulated in tetraploid plants of sweet wormwood (*Artemisia annua*) (Lin et al., 2011), and tetraploid opium poppy (*Papaver somniferum*) showed increased activity of genes that biosynthesize morphinanes (Mishra et al., 2010).

Improving secondary metabolite production is an important breeding goal because plant secondary metabolites contribute to modern drug development (Jamshidi-Kia et al., 2018). One example of an important secondary metabolite is apigenin, which is cytotoxically active against bladder (Zhu et al., 2013), breast (Pham et al., 2021), cervical (Chen et al., 2022), colorectal (Cheng et al., 2021), and prostate (Costea et al., 2020) cancers. Apigenin is also used as an antibacterial (Kim S. et al., 2020), antifungal (Singh et al., 2014), anti-inflammatory (Wang J. et al., 2014), and antioxidant (Tian et al., 2021) agent. A previous study reported greater apigenin concentration in the diploid chamomile (*Chamomilla recutita*) cultivar Novbona compared to the tetraploid cultivar Lutea (Švehlíková and Repák, 2000). However, cultivar differences beyond the ploidy level may have impacted apigenin accumulation. Therefore, artificial polyploid induction of a single cultivar may be a better comparison for apigenin accumulation.

Parsley (*Petroselinum crispum*) was selected for this study because it is one of the highest natural sources of apigenin with concentrations of 9.48 mg/g in the leaf tissue (Poureini et al., 2020). Parsley, which has been reported as $2n = 2x = 22$, has successfully been converted to a tetraploid by treating seeds with 0.05% colchicine, yielding larger leaves than the diploid control (Nasirvand et al., 2018). However, the impact of polyploid induction on apigenin concentration has not been studied in parsley. Because parsley has naturally high apigenin levels and can be induced to form polyploid tissue, this crop may be a suitable target for increasing apigenin

production. Therefore, this experiment aimed to increase concentration of apigenin in parsley through induction of polyploidy.

2. Materials and Methods

2.1. Colchicine Treatment

Parsley 'Giant of Italy' seeds (Johnny's Selected Seeds, Winslow, ME) were soaked in a colchicine solution to determine the impact of treatment concentration on germination and polyploid induction. Four replicates of 25 seeds were treated with 0.00, 0.02, 0.04, 0.08, or 0.16% colchicine. Based on seedling emergence and findings in previous research, an additional group of parsley seeds was treated at the 0.04% colchicine rate to generate more polyploid plants. Ten replicates of 100 seeds were treated with a 0.04% colchicine solution and four replicates of 25 seeds were in deionized water alone for 48 h on the rotary shaker. The beakers containing the seeds and colchicine solution were covered in parafilm and placed on a rotary shaker (New Brunswick Scientific, Edison, NJ) at 200 rpm for 48 h. After the treatment period, the solution was decanted from the beakers, and the seeds were rinsed three times with deionized water to remove residual colchicine.

The parsley seeds were then individually sown on substrate (PRO-MIX HP; Premier Tech Horticulture, Quakertown, PA) and arranged in a randomized complete block design inside a growth chamber (CMP 3244; Conviron, Pembina, ND) located at the University of Georgia (College of Agricultural and Environmental Sciences, Department of Horticulture, CEA Crop Physiology and Production Laboratory). The growth chamber temperature was maintained at 24 °C during the day and 20 °C at night with 19 mol m^{-2} d^{-1} of supplemental white LED lighting for

a 16-h photoperiod. The trays were overhead irrigated to keep the substrate moist, and seedling emergence was measured after four weeks.

2.2. Ploidy Determination

The ploidy of all surviving seedlings was determined using flow cytometry with the 4',6-diamidino-2-phenylindole (DAPI) fluorescent stain kit (CyStain UV Precise P; Sysmex America, Inc., Lincolnshire, IL). For each seedling, a 0.5 cm^2 sample of the youngest leaf was placed in a Petri dish with 500 mL of the nuclei extraction buffer. The leaf was then finely chopped using a clean razor blade and left in solution for 60 s. The extracted DNA was passed through a 50 µL filter (CellTrics; Sysmex America, Inc., Lincolnshire, IL) into a test tube and stained with 1.0 mL of the DAPI solution. The sample was then transferred to a dark refrigerator for 20 min before the cells were analyzed.

After the staining period, the samples were analyzed with a flow cytometer (CytoFLEX S; Beckman Coulter, Hialeah, FL) using the CytExpert software. The optical filter was set to select a wavelength range of 405 – 450 nm. The suspended cells were drawn into the cytometer at a medium flow rate of 30 µL s^{-1}, and a minimum of 1,000 events were recorded within the gate of the 2x peak. The number of 4x cells was divided by the number of all measured cells to determine the percentage of tetraploid tissue in each sample.

2.3. Apigenin Quantification

Ten controls and all seedlings with elevated ploidy were transplanted into 1.0-L 10-cm square pots (T.O. Plastics Inc., Clearwater, MN) with the PRO-MIX HP substrate. The seedlings were fertilized once per week with 200 ppm nitrogen from a 15N–2.2P–12.5K stock solution

(Jack's 15-5-15 Ca-Mg; JR Peters, Inc. Allentown, PA). After 13 weeks from sowing, leaf tissue was collected for apigenin quantification. Fully expanded leaves were harvested into screw-top tubes (Falcon; Corning Inc., Corning, NY) and placed in liquid nitrogen. The samples were dried at -84 °C and 0.133 mbar for 24 h in a lyophilizer (Labconco; Marshall Scientific, Hampton, NH). The lyophilized samples were ground into a fine powder using a mortar and pestle and stored in a -80 °C freezer.

Apigenin quantification was achieved using high-performance liquid chromatography–ultraviolet (HPLC–UV) detection (Shimadzu Nexera; Shimadzu Corp., Tokyo, Japan) with a photodiode array detector. A 5-µm particle size, 4.6 × 150 mm analytical column and guard column (ZORBAX Eclipse XDB-C18; Agilent Technologies, Santa Clara, CA) were selected to separate apigenin. The column was set to 35 °C with a mobile phase of water/acetonitrile (65/35, v/v, %) containing 0.1% formic acid (Oakwood Products Inc., Estill, SC). The flow rate was 1 mL min^{-1} with an injection volume of 5 µL.

A 1 mg mL^{-1} stock solution of apigenin was prepared using an apigenin standard (95% purity) dissolved in HPLC grade methanol/DMSO (90/10, v/v) (Sigma-Aldrich, St. Louis, MO). The stock solution was diluted with methanol to prepare working standard solutions (e.g., calibration standards). To extract apigenin from the parsley samples, 100 mg of the powdered leaves was vortexed with 1 mL of 50% methanol for 10 min. The samples were centrifuged at 13,500 × g for 10 min, and the supernatants were passed through a 0.2 µm filter.

The supernatant samples were injected into the HPLC, chromatographically separated, and detected at 336 nm. Apigenin in the samples was identified by comparing the retention times and UV spectra with the apigenin standard, and it was quantified using calibration curves.

LabSolutions software (Ver. 5.124) was used for the HPLC data interpretation, and RStudio (version 2023.12.0+369) was used for statistical analysis.

3. Results and Discussion

3.1. Seedling Emergence

In the first seed treatment, none of the groups had complete seedling emergence. The control group had a mean emergence of 84%, and the 0.02%, 0.04%, 0.08%, and 0.16% colchicine treatment groups had 43%, 27%, 8%, and 2% seedling emergence, respectively (Fig. 4.1). An ANOVA indicated that declining seedling emergence with increasing colchicine concentration was statistically significant ($F(4) = 165.4$, $p < 0.001$). Of the additional 1000 seeds treated with 0.04% colchicine, only 21% emerged (data not shown), indicating a consistent germination rate with the previous treatment.

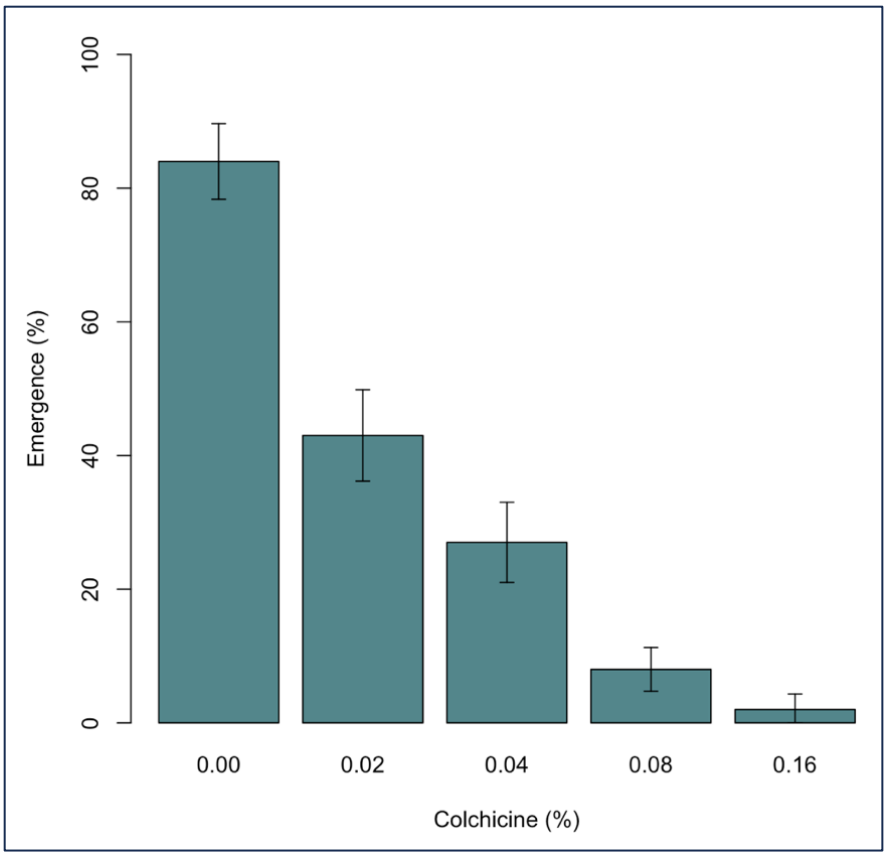

Fig. 4.1: Seedling emergence of parsley (*Petroselinum crispum*) treated with 0%, 0.02%, 0.04%, 0.08%, or 0.16% colchicine for 48 h ($F(4) = 165.4$, $p < 0.001$).

Reduced seed germination with increased colchicine exposure has been reported in many other genera, including butterfly bush (*Buddleja lindleyana*) (Yan et al., 2022), cowpea (*Vigna unguiculata*) (Essel et al., 2015), and Indian pennywort (*Centella asiatica*) (Surson et al., 2024). Colchicine also reduced seed germination in bamboo (*Dendrocalamus brandisii*) by preventing radicle extension and delaying plumule development. The abnormal behavior of the radicle and plumule was attributed to reduced starch degradation, limiting sugar availability for seedling growth and development (Lv et al., 2021). Therefore, low seedling emergence at the higher treatment rates was expected.

3.2. Ploidy Determination

Flow cytometric analysis of the parsley cells generated a histogram for each sample, quantifying the number of cells fluorescing at different intensities. All the untreated parsley plants had one prominent peak and one smaller peak with twice the fluorescent intensity. The presence of two peaks indicated diploid cells in the G1 and G2 phases of the cell cycle. Within a species, the cell fluorescence intensity of a cell is proportional to the amount of DAPI bound to the nuclear DNA (Doležel et al., 1992). Therefore, cells in the G2 phase, with fully duplicated chromosomes (Sliwinska et al., 2022), fluoresce twice as intensely as the G1 phase cells. Because tetraploid cells also have duplicated chromosomes, they can be challenging to distinguish from diploid cells in the G2 phase. In this experiment, the greatest number of control parsley cells in the G2 phase was 20%. Therefore, for the parsley treated with colchicine, a secondary peak representing more than 20% of the cells was considered an indication of additional 4x tissue.

Although higher rates of colchicine can decrease seedling emergence, it can also increase the polyploid induction rate, as was observed in loquat (*Eriobotrya japonica*) (Blasco et al., 2015) and orchid (*Dendrobium scabrilingue*) (Sarathum et al., 2010). However, none of the surviving seedlings from the 0.08% or 0.16% colchicine treatment groups generated polyploid tissue in this experiment. Only four plants from the 0.02% and three from the 0.04% treatment groups had more than 20% of the cells under the 4x peak. Each of these individuals maintained the 2x peak, indicating they were mixoploid.

One plant from the 0.02% colchicine treatment group had 29% of cells in the 2x peak, 44% in the 4x peak, and 29% in a third peak (Fig. 4.2). Because more than 20% of cells were in the third peak, it likely consisted of 4x cells in the G2 phase and additional 8x cells. Tetraploid

and octoploid cells were also generated in the diploid paintbrush plant (*Haemanthus albiflos*) treated with colchicine (Nakano and Hoshino, 2022). Because colchicine disrupts spindle formation during mitosis (Dhooghe et al., 2011), diploid cells, which had already been converted to tetraploid, could be further doubled to form octoploid cells.

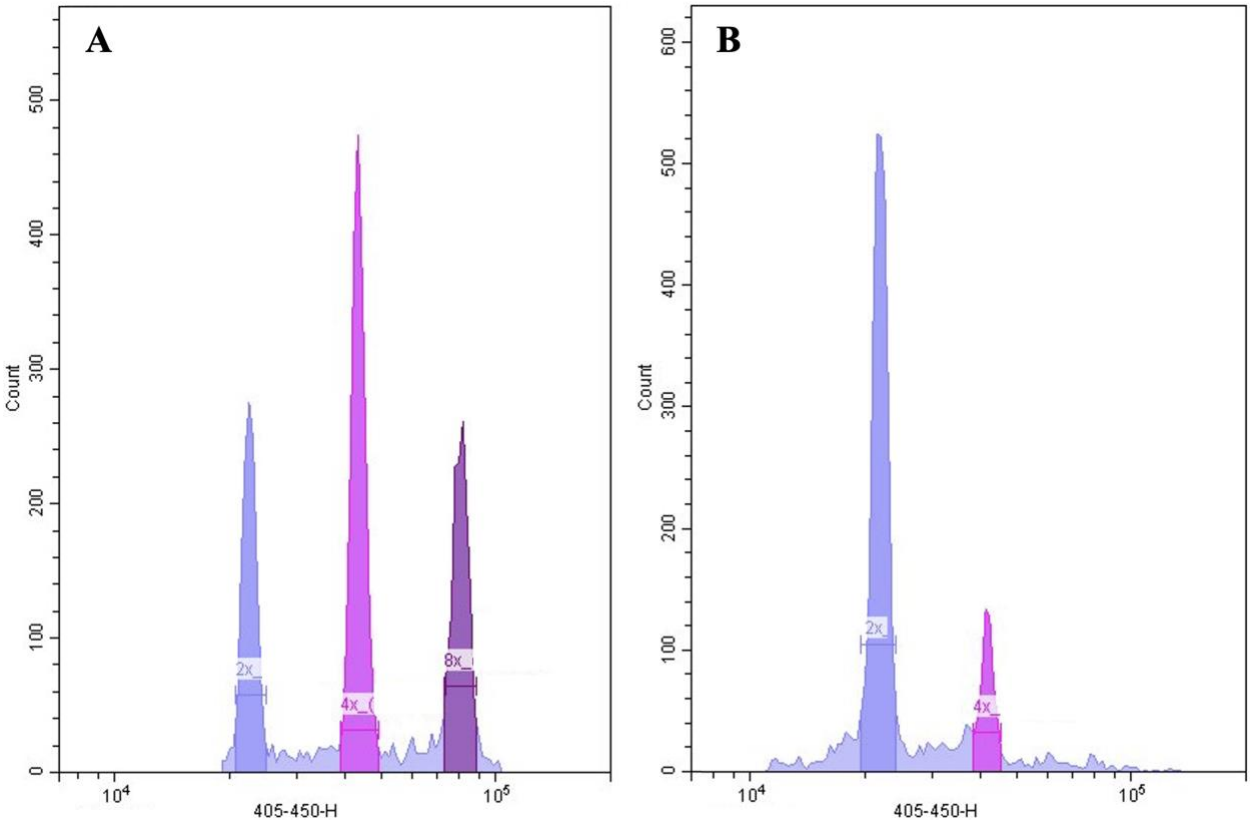

Fig. 4.2: The ploidy level of parsley (*Petroselinum crispum*) treated with colchicine were determined using flow cytometry. (**A**) One sample treated with 0.02% colchicine was mixoploid with diploid, tetraploid, and octoploid cells. (**B**) After 28 days of growth, the mixoploid plant reverted to diploidy and was indistinguishable from the control plants.

Because all parsley with elevated ploidy were mixoploid, they were screened again using flow cytometry after 28 days of growth to determine their ploidy stability. After the 28-day period, every sample, which was previously identified as mixoploid, became indistinguishable from the control parsley, with 20% or fewer cells in the secondary peak and none in a third peak

(Fig. 4.2). The tissue reversion was not surprising because plants treated with colchicine can be reduced from mixoploid to diploid due to genetic instability (Eng and Ho, 2019). Ploidy reversion may be due to the diploid cells dividing more rapidly than the tetraploid cells (Giménez-Martín et al., 1966) and has been observed in induced mixoploids, including Norway maple (*Acer platanoides*) (Lattier et al., 2013) and rose gum (*Eucalyptus grandis*) (Silva et al., 2019).

3.3. Apigenin Quantification

Although none of the mature parsley had elevated ploidy, the apigenin concentration in each surviving plant was still quantified using HPLC (Table 4.1). Only two of the four mixoploids from the 0.02% and all from the 0.04% survived for apigenin analysis. Although the levels of apigenin in treated plants was lower than those in control plants, it was difficult to draw a conclusion from this data since the mixoploid tissue reverted to diploid. To determine the impact of elevated ploidy on apigenin accumulation, future studies should isolate stable tetraploid parsley plants prior to HPLC analysis.

Table 4.1: The concentration of apigenin in parsley (*Petroselinum crispum*) was determined using HPLC. Four control plants and all surviving mixoploid plants, treated with 0.02% or 0.04% colchicine, were analyzed.

Control parsley	**Apigenin (mg/100g dried tissue)** [a]
0.00%	0.75 ± 0.15
0.00%	0.20 ± 0.03
0.00%	1.01 ± 0.03
0.00%	1.58 ± 0.09
Treated parsley	
0.02%	0.23 ± 0.02
0.02%	0.04 ± 0.00
0.04%	0.04 ± 0.01
0.04%	0.09 ± 0.02
0.04%	0.14 ± 0.03

[a] Values listed as mean ± standard deviation, n = 4

4. Conclusion

In this experiment, colchicine was effective in inducing elevated ploidy in parsley seeds. However, the seedling emergence rate significantly declined with increasing concentrations of colchicine, necessitating the treatment of a large population to isolate plants with elevated ploidy. After seed treatment, four parsley plants from the 0.02% and three plants from the 0.04% colchicine treatment groups were determined to have mixoploid tissue. However, all mixoploid seedlings were unstable, reverting to diploid in their maturity. Because the previously mixoploid plants later contained only diploid tissue like the control parsley, the impact of elevated ploidy on apigenin accumulation could not be determined using HPLC.

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Author Contributions

All authors listed have made substantial direct and intellectual contribution to the work and approved it for inclusion on this dissertation. Specifically, R.C.I. Maynard contributed to the experimental design, produced the research crops, collected plant samples for HPLC analysis, conducted ploidy analysis with flow cytometry, analyzed the data, and summarized the findings in this report. S.O. Ogundipe prepared and analyzed all HPLC samples. J.H. Suh advised the HPLC analysis of plant samples and provided access to the HPLC. L. Lombardini advised the research and funded the experiment.

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CHAPTER 5

CONCLUSION

Controlled environment agriculture (CEA) is a growing industry, and a new avenue for production may be biopharmaceutical crops. This research explored the use of parsley and chamomile as potential biopharmaceutical herbs for CEA production which biosynthesize the medicinally useful compound apigenin. The primary objectives of this research were to determine (1) if apigenin could be produced in herbs grown in an indoor vertical farm, (2) if adding UV-B light at the end of production could increase the accumulation of apigenin, and (3) if generating polyploid parsley could lead to an increase in the biosynthesis of apigenin compared to their diploid counterparts.

Parsley and chamomile are known to biosynthesis apigenin, but our research was novel because their performance has not been previously studied in vertical farm production. Although the literature suggested that apigenin biosynthesis is dependent on UV-B light, this research demonstrated that apigenin was accumulated in all trialed chamomile and parsley varieties with no UV-B exposure. To our knowledge, this is the first report of apigenin biosynthesis in the absence of UV-B light. However, the levels of apigenin accumulating in the chamomile inflorescence and parsley leaves were lower than expected. Many factors in a hydroponic vertical farm differ from in-ground production, including controlled light intensity, daytime and nighttime temperatures, humidity, CO_2 level, airflow, and solution pH and EC. Therefore, it is challenging to identify which factor was responsible for the lower-than-expected apigenin accumulation. However, because apigenin is known to act as a protective compound against UV-

B damage in plants, the lack of UV-B light was likely the cause. Future studies should investigate whether apigenin biosynthesis changes in response to other environmental factors.

Based on the findings in the first study, the second experiment was designed to test whether including UV-B into the supplemental lighting would positively impact apigenin accumulation in parsley. In this study a ten-fold increase in apigenin accumulation was measured for plants treated for the longest UV-B exposure (8 days) compared to the untreated control. To our knowledge, this is the first report of using UV-B light as a treatment to increase apigenin accumulation in plants. Although the research was successful, it would be beneficial for future studies to investigate the impact of shorter or longer UV-B exposure times to determine the optimal treatment for maximizing apigenin. Because UV-B light is harmful to humans, the research was conducted inside growth chambers, but future research should also study the feasibility of safely implementing UV-B light into commercial production in a vertical farm.

Although the first two studies focused on the growing environment, the third study focused on the crop, namely inducing polyploidy. Other research has successfully increased secondary metabolite production in polyploid plants, but the impact of induced polyploidy on apigenin accumulation has not previously been studied. Although this research successfully generated tetraploid parsley by treating seeds with oryzalin, the plants reverted to normal growth, with the mature plants having only diploid tissue. Therefore, this study was unable to test the potential of a polyploid to have greater apigenin accumulation, and future studies should develop a method for inducing stable parsley polyploids.

Overall, we believe biopharmaceutical production of apigenin could be a promising avenue for CEA. Our findings show that accumulation of apigenin in parsley and chamomile is achievable in a vertical hydroponics system with the addition of UV-B light improving

productivity. Future research should continue exploring best production methods, further manipulating the environmental conditions and plant material to optimize apigenin accumulation. Because these experiments were conducted on a small scale compared to commercial production, it is challenging to accurately calculate the financial feasibility of selecting parsley and chamomile as biopharmaceuticals in a commercial setting. Therefore, future research should consider the cost of producing, harvesting, extracting, purifying, and marketing apigenin as a biopharmaceutical compound.

In addition to researching apigenin, environmental and ploidy manipulation may positively impact other secondary metabolites. Therefore, future research could investigate accumulation of several secondary metabolites in parsley and chamomile. This research approach would require the identification of additional beneficial compounds in the plant tissue followed by isolation and quantification of their abundance. Overall, future research should aim to maximize productivity of biopharmaceutical herbs for production in controlled environments.

ADDITIONAL DOCTORAL RESEARCH

CHAPTER 6

IDENTIFICAITON OF LOCI RESPONSIBLE FOR PERICARP ELONGATION IN TOMATO

Maynard, R.C.I., L. Zhang, Y. Topcu, and E. van der Knaap. To be submitted to *Horticulturae.*

Abstract

Modern tomato (*Solanum lycopersicum*) cultivars are highly varied in fruit shape and size compared to their wild ancestor (*S. pimpinellifolium*) which have small, rounded fruits. One of the most pronounced differences is the significant fruit elongation, primarily due to the *sun* and *ovate* mutant alleles. However, it is unclear whether fruit size has an influence on fruit shape. The present study generated hybrids of the Long John tomato cultivar, with *sun* and *ovate* mutant alleles, and SA30, with *sun* and *ovate* introgressed into the LA1589 (*S. pimpinellifolium*) background. Although the F_2 progeny were fixed for *sun* and *ovate*, the population had a continuous distribution of the curved fruit shape index (CFSI), measuring the degree of fruit elongation. Furthermore, a significant positive correlation was identified between fruit elongation and overall size ($r = 0.63$, $p < 0.0001$), with larger fruits having greater pericarp elongation. Two significant QTL correlated with fruit elongation were identified on chromosomes 2 and 3. Furthermore, a recombinant F_2 population confirmed the QTL most significantly correlated with the CFSI was located at SL4.0ch03: 59187292, mapping closely to *fw3.2*. Our findings suggest that the fruit weight gene *fw3.2* exaggerates elongation of tomato fruit.

Keywords: fruit shape, KASP, marker selection, QTL seq

1. Introduction

Solanum pimpinellifolium is one of the oldest and most closely related wild ancestors of the cultivated tomato, *S. lycopersicum*, with small, spherical, bright red fruit (Peralta et al., 2008). Through domestication and breeding, tomatoes have undergone many changes beginning

with selections for increased yield, followed by improvements in shelf-life, flavor, nutritional quality, and more recently, resistance to biotic and abiotic stressors (Bai and Lindhout, 2007). The most notable difference between the wild progenitors and cultivated varieties is a significant increase in fruit size, which is controlled by many loci including *fw2.2* (Alpert et al., 1995), *fw3.2* (Chakrabarti et al., 2013), *fw6.3* (Ning et al., 2023), *fw11.3* (Huang and van der Knaap, 2011), *lc* and *fas* (Chu et al., 2019), and many additional fruit weight QTL (Barrantes et al., 2016; Pereira et al., 2021). Furthermore, modern tomato varieties also have highly diverse fruit shapes including ellipsoid, flat, heart, long, obovoid, oxheart, rectangular, and round, controlled by genes and gene mutants of *FAS*, *GLOBE*, *LC*, *OVATE*, *PT*, *SUN*, and *fw8.2* (Gonzalo and van der Knaap, 2008; Rodríguez et al., 2011; Sierra-Orozco et al., 2021; Song et al., 2022).

One trait that is uncommon in commercial tomato varieties is an elongated pericarp. Two loci, *ovate* and *sun*, primarily control elongation; however, in the absence of *ovate* and *sun*, fruit elongation can occur with the *fs8.2* and *tri2.1*/*dblk2.1* loci (Gonzalo and van der Knaap, 2008). Although both *ovate* and *sun* influence pericarp elongation, they have slightly different effects. The *ovate* locus, on chromosome 2, was identified as a loss of function mutation of the wild-type *OVATE* (Liu et al., 2002) contributing to elongated, pear-shaped fruits with pericarp constriction (Ku et al., 1999). Furthermore, other *OVATE*-like genes impact fruit elongation in eggplant (*Solanum melongena*) (Shi et al., 2023) and pepper (*Capsicum annum*) (Tsaballa et al., 2011), grain elongation in rice (*Japonica*) (Xiao Y. et al., 2017), and cell elongation in the hypocotyl and leaf petiole of *Arabidopsis thaliana* (Wang et al., 2007).

Whereas *ovate* is a loss of function mutation, *sun* is a gain of function mutation. The translocation of the *sun* locus caused overexpression of the *SUN* gene, further promoting elongation (Wu et al., 2011; Xiao H. et al., 2008). Of the QTL found to influence fruit shape, the

sun locus has the greatest impact on elongation, explaining 58% of fruit shape variance in elongated tomatoes compared to *S. pimpinellifolium* (van der Knaap and Tanksley, 2001). In a population of 368 tomato accessions, 88% in the long tomato category contained the *sun* mutant allele (Rodríguez et al., 2011). *SUN* impacts fruit shape by increasing longitudinal cell division while decreasing transverse cell division thereby leading to an elongated fruit shape (Wu et al., 2011). *SUN*-related genes also impact fruit elongation in cucumber (*Cucumis sativus*) (Pan et al., 2017) and watermelon (*Citrullus lantanus*) (Legendre et al., 2020).

One tomato with pronounced pericarp elongation is the Long John cultivar. Previous research identified four fruit shape QTL influencing fruit shape in Long John. The QTL *ljfs7,* allelic to *sun,* contributed to elongation whereas *ljfs2*, allelic to *ovate*, *ljfs3*, near *fw3.2*, and *ljfs11*, near *fs11.1*, all contributed to the pear shape (van der Knaap et al., 2002). Although *fw3.2* is known to impact fruit size, the influence of a fruit weight genes on fruit shape is not well understood. Therefore, the aim of this research was to identify the loci or potential gene interactions influencing the exaggerated elongation of the Long John cultivar.

2. Materials and Methods

2.1. Development of the Study Population

Two parental cultivars, SA30 and Long John, were selected to investigate the influence of QTL, beyond *sun* and *ovate*, on fruit elongation. The two cultivars were crossed to form a segregating F_2 population fixed for *sun* and *ovate*. The SA30 tomato has both the *ovate* and *sun* mutant alleles introgressed into the LA1589 (*S. pimpinellifolium*) background and forms small, pear-shaped fruit. The Long John cultivar also contains the *sun* and *ovate* mutant alleles yet the fruits are much larger and more elongated compared with SA30.

2.2. Crop Production

The F_2 tomato seeds were soaked in a 50% bleach solution for 30 min to improve germination, rinsed with water, then directly sown on substrate (Propagation Mix; Sungro, Agawam, MA) in 288-cell trays (288 Square Plug Tray Deep; Landmark, Akron, OH). The trays were placed in a temperature-controlled room (27°C day/21°C night) and overhead irrigated to keep the substrate consistently moist. The seedlings were provided with supplemental lighting from fluorescent bulbs (ProLume T8 Eco-Shield Lamp; Halco Lighting Technologies, Norcross, GA) with a light intensity of 125 μmol m^{-2} s^{-1} for a 16-h photoperiod.

Once the seedlings had several sets of true leaves, they were transplanted into 72-cell trays (L Series 1020 Tray Insert; Landmark, Akron, OH) with commercial substrate (Professional Growing Mix; Sungro, Agawam, MA) and 15 g per tray of a 5:1 ratio slow-release fertilizer blend of 18-6-8 Nutricote Total: Florikin Meg-Iron V Micronutrient Mix. The seedlings were transferred to a greenhouse with temperatures of 27-29°C in the daytime and 21-24°C in the nighttime. The tomatoes were provided with supplemental LED lighting (VYPR 3p Broad R3; Fluence, Arlington, VA) for a 16-h photoperiod to achieve at least 250 μmol m^{-2} s^{-1} in the greenhouse. After 38 d from sowing, 205 of the F_2 plants, named the 23S76 population, and five of each parent were transplanted to the field at the University of Georgia Horticulture Research Farm in Watkinsville, GA in May 2023. After 67 d from transplant, the mature fruits were harvested for phenotypic analysis of the fruit shape.

2.3. Phenotypic Analysis of the 23S76 F_2 Population

Twelve mature, intact fruits were selected from each plant and halved from the stem to blossom end with a sharp razor blade to expose the locules. The cut fruit were arranged

individually on a scanner (Scanjet G4050; HP Inc., Palo Alto, CA), covered with a black box, and imaged with 300 dpi resolution. The fruit morphology was characterized with the Tomato Analyzer (version 4.0) software to measure the curved fruit shape index, obovoid shape, perimeter, and proximal- and distal-ends. The measurements were averaged for each plant, and RStudio (version 2023.12.0+369) was used to for the statistical analysis.

2.4. Bulk Segregant Analysis for QTL Seq

Bulk segregant analysis was used to determine the loci responsible for the phenotypic difference between the compact and elongated fruits. Two groups were formed by bulking 1 mg of lyophilized leaf tissue from each of the 15 most compact fruits and the 15 most elongated fruits from the 205 F_2 plants. The DNA was extracted from the leaf tissue and purified using the GeneJet Plant Genomic DNA Purification Mini Kit (Thermo Fisher Scientific Inc., Waltham, MA). The extracted samples were sequenced by Novogene (Duram, NC) with the Illumina platform. The paired-end short reads from the two bulked populations were aligned to the reference SL4.0 tomato genome using Bowtie2 (Langmead and Salzberg, 2012). The SNPs were extracted with the Genome Analysis Toolkit (Van der Auwera and O'Connor, 2020) and filtered for high quality variants. The difference in SNP frequency between the bulked populations was mapped for each chromosome for a genome-wide QTL seq.

2.5. Marker Development and Genotypic Analysis of the 23S76 F_2 Population

For genotypic analysis, DNA was extracted from the fresh cotyledons of each seedling using the CTAB extraction buffer followed by chloroform purification. Allele-specific KASP primer pairs were developed to discriminate between genetic polymorphisms among the

segregating F_2 population. The Integrative Genomics Viewer (version 2.12.2) was used to visualize SNPs between the bulk populations using the tomato genome version SL4.0 with the ITAG4.0 annotation as a reference. Several forward and reverse primers were developed within the regions of interest on chromosomes 2 (ch2) and chromosome 3 (ch3), based on the QTL seq results. The primers were developed to flank the target polymorphisms with fewer than five repeating nucleotides in a sequence to prevent DNA looping. The viability of the primer pairs was determined with the Primer Probe Test Tool (Primer Express version 3.0.1) to ensure a similar Tm and GC content. The selected genomic sequences were confirmed to be unique using BLAST on the Solanaceae Genomics Network with SL4.0. Each target SNP had one common reverse primer and two allele-specific forward primers labeled with the FAM and HEX fluorophores at the 5' end (Table S.6.1). The DNA oligos were then synthesized by Sigma-Aldrich (St. Louis, MO).

For each DNA sample, a 5 μL PCR reaction mix was prepared with the allele-specific forward primers, common reverse primer, FAM and HEX FRET cassette, Taq polymerase, and buffer (Table 6.1). The samples were prepared in 384-well PCR plates (MicroAmp Optical 384-Well Reaction Plate; Thermo Fisher Scientific Inc., Waltham, MA) and centrifuged for 10 sec at 2100 RPM. The PCR was completed with a thermal cycler (Veriti 384 Well Thermal Cycler; Applied Biosystems, Waltham, MA) with cycles of denaturation and annealing (Table 6.2). Fluorescence of the amplified fragments (FAM at 518 nm and HEX at 554 nm) was measured with a plate reader (Infinite M200PRO; Tecan, Männedorf, Switzerland) and analyzed using the KlusterCaller (version 3.4.1.39) software. Each DNA sample was determined to be heterozygous or homozygous for either parent.

Table 6.1: PCR reaction mix for KASP markers.

Component	**Volume (μL)**
Forward primer allele 1 (10 μM)	1.3×10^{-3}
Forward primer allele 2 (10 μM)	1.3×10^{-3}
Reverse primer (10 μM)	3.3×10^{-3}
KASP 2x enzyme	2.5
dd H_2O	0.5
DNA	2.0
Total	5.0

Table 6.2: PCR amplification program for KASP markers.

PCR Program	**Cycle (no.)**	**Temperature (°C)**	**Time**
stage 1	1	94.0	15:00
stage 2	12	94.0	0:20
		65.0	1:00
stage 3	40	94.0	0:20
		57.0	1:00
stage 4	1	10.0	∞

2.6. Confirmation of QTL Location

Another population of 224 F_2 seeds from the same Long John x SA30 cross, named 24S54, was sown in April 2024 to confirm the QTL location on ch3. The cotyledons were collected for DNA extraction, and the seedlings were screened with additional genetic markers only on ch3 (Table S.6.2). The plants were selected for recombination between 18EP724 (56.2 Mb) and 24EP97 (61.1 Mb) on ch3. These markers were selected for the recombination study because they flank the 19EP261 (59.2 Mb) marker on ch3, which was most significantly correlated with the CFSI in the 23S76 F_2 population. The recombinant plants were cultivated using the same method described previously. Mature fruits were harvested from each plant in the field 118 days from sowing in July 2024, and the fruits were phenotyped as previously described.

3. Results and Discussion

3.1. Phenotypic Analysis of the 23S76 F_2 Population

The 23S76 F_2 population was varied in fruit shape from compact fruit resembling the SA30 parent to elongated fruit like the Long John parent (Fig. 6.1).

Fig. 6.1: Variation in fruit shape between the tomato parents (Long John and SA30) and among selected F_2 hybrids.

Because this study aimed to determine the loci responsible for fruit elongation, the most relevant morphological measurement was the ratio of the fruit length to the fruit width. However, as shown in Fig. 6.1, the parents and F_2 hybrid fruit were curved along the axis from the distal to proximal end. Therefore, the most representative measurement was the curved fruit shape index (CFSI), which measures the ratio of the curved length (along the distal to proximal axis) to the curved width at the midpoint of the fruit. For fruits that were highly elongated, the midpoint was within the pericarp region, but for fruits with minimal elongation, the midpoint was measured through the locules. An important note is that the Long John cultivar had poor seed development,

resulting in underdeveloped locules. However, due to the significant elongation of Long John, the mid-width measurement was in the pericarp region and was not impacted by the width of the locules.

The CFSI of the F_2 hybrids was widely distributed (Fig. 6.2), consistent with a quantitative trait. Although most of the F_2 hybrids had a CFSI between the two parents, five plants (2.4%) were more rounded than SA30, with a smaller CFSI, and 29 plants (14.1%) were more elongated than Long John, with a larger CFSI. Because the CFSI is a ratio of the curved height to width, fruits with a higher CFSI had either a longer or narrower pericarp relative to Long John.

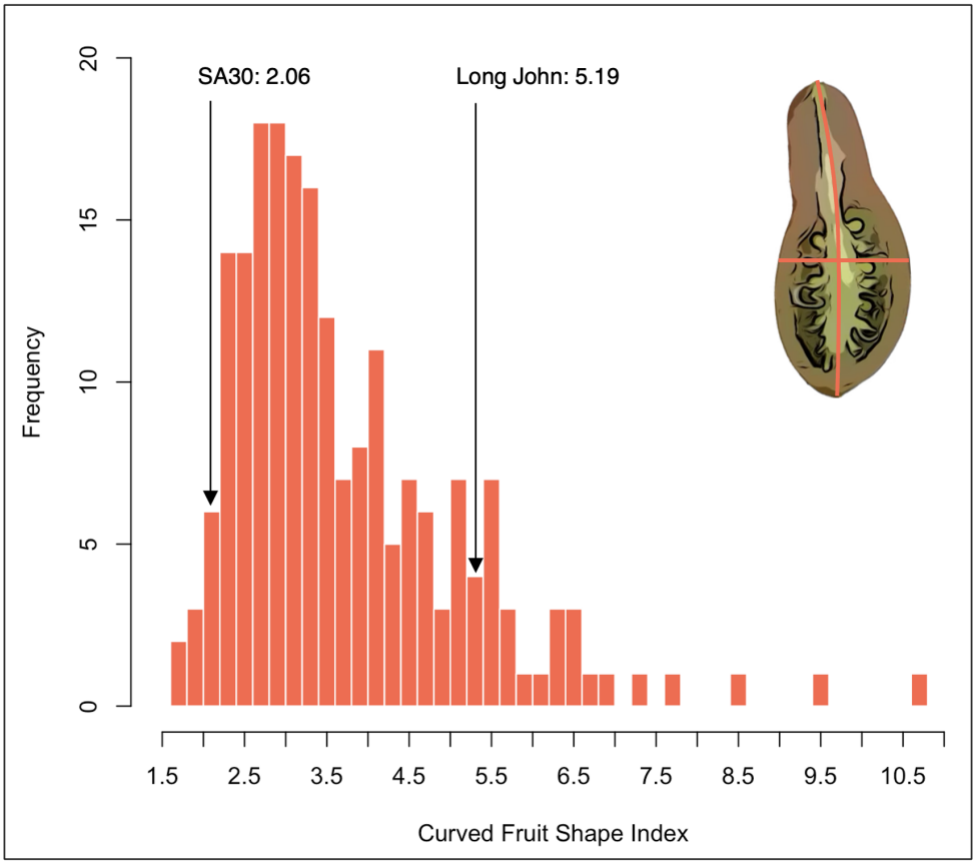


Fig. 6.2: Distribution of the measured curved fruit shape index (CFSI) for the 23S76 F_2 hybrid population of Long John and SA30 tomato (*Solanum lycopersicum*).

The relationship between the CFSI and other measured parameters was compared with a correlation matrix (Fig. 6.3). The strongest positive correlation was identified between the CFSI and the obovoid shape ($r = 0.77$, $p < 0.0001$). The obovoid measurement compared the area of fruit below the midpoint to the area of fruit above the midpoint. A positive obovoid value indicated a greater fruit area below the midpoint. Because CFSI and obovoid were positively correlated, fruit elongation in the F_2 hybrids occurred primarily in the pericarp region. This result was expected as both SA30 and Long John are pear-shaped fruits.

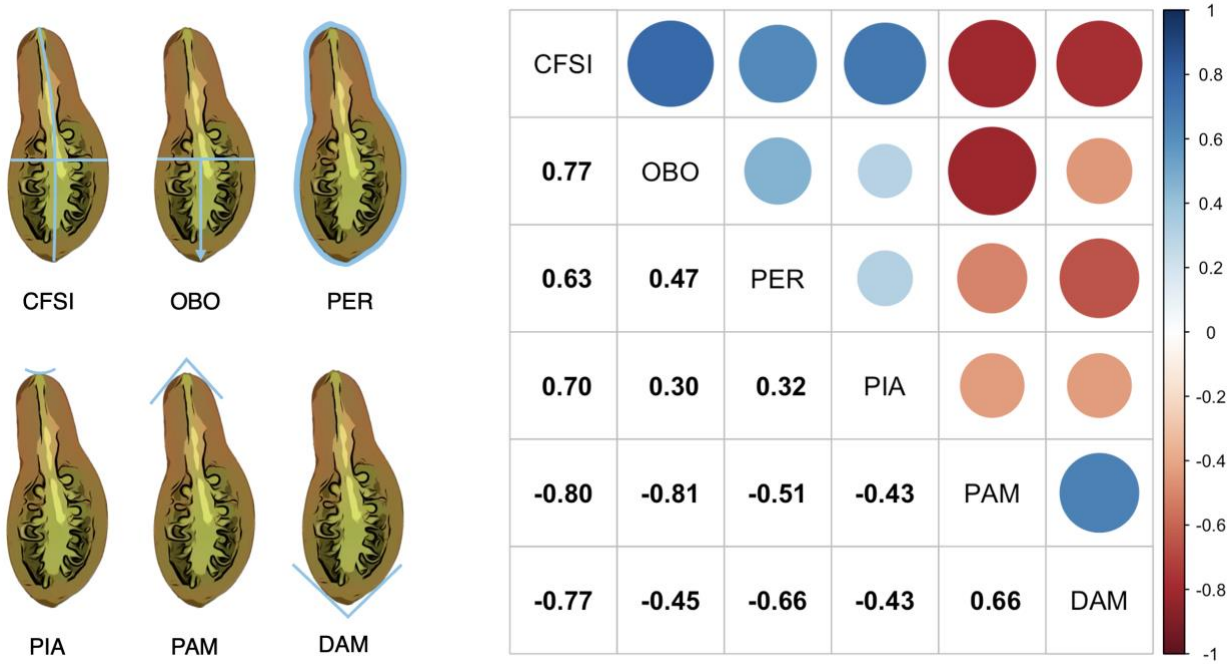


Fig. 6.3: Correlation of phenotypic parameters for the 23S76 F_2 hybrid population of Long John and SA30 tomato (*Solanum lycopersicum*). The measured parameters included the curved fruit shape index (CFSI), obovoid shape (OBO), perimeter (PER), proximal indentation area (PIA), proximal angle macro (PAM), and distal angle macro (DAM).

The CFSI was also positively correlated with the fruit perimeter ($r = 0.63$, $p < 0.0001$), which estimates fruit weight. Although fruit weight and fruit shape have not previously been shown to be related, this positive correlation between CFSI and perimeter indicates that larger

fruits in the F_2 population were were more likely to be elongated than smaller fruits. Therefore, as the size of the fruits increased, the elongation of the fruits also increased. Elongated fruits were also correlated with an indentation on the proximal end (r (PIA) = 0.70, $p < 0.0001$). Whereas SA30 had a proximal end protrusion, Long John was characterized by a proximal end indentation. Therefore, fruit elongation and a pronounced PIA appeared to be inheriting together in the F_2 as observed in the Long John parent. A high CFSI was negatively correlated with a wide angle on the proximal end (r (PAM) = -0.80, $p <0.0001$) and distal end (r (DAM) = -0.77, $p < 0.0001$), both of which were characteristic of the SA30 parent. Therefore, F_2 hybrid fruits with pronounced pericarp elongation shared several phenotypic characteristics with the Long John parent including an obovoid shape, increased fruit size, proximal-end indentation, and narrow proximal- and distal-end angles.

3.2. Genotypic Analysis of the 23S76 F_2 Population

Although several phenotypic measurements distinguished fruits in the F_2 population, the CFSI was the most relevant parameter for understanding genotypic differences between the compact and elongated fruits. The 15 most compact fruits were considered representative of SA30, and the 15 most elongated fruits were selected to represent of Long John. Bulk segregant analysis of the two populations identified two significant fruit shape QTLs. The difference in SNPs between the populations was significantly different on ch2 and ch3, exceeding $\alpha = 0.01$ (Fig. 6.4).

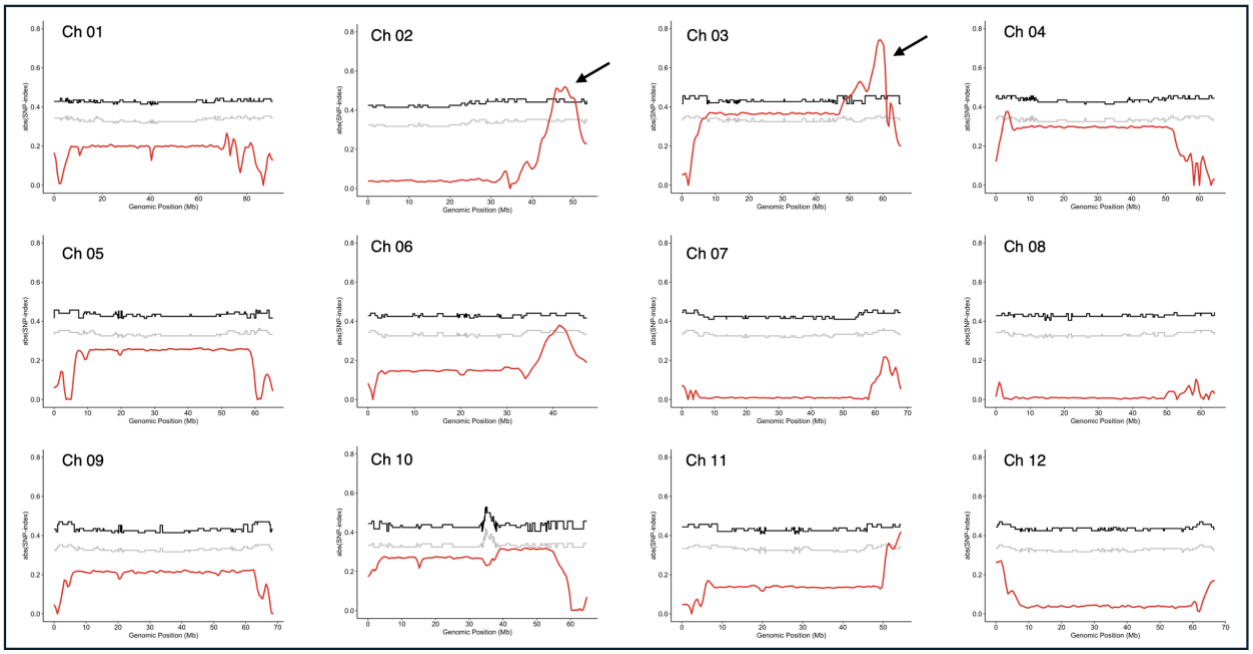


Fig. 6.4: QTL seq results between a bulked population of compact F_2 hybrids and a bulked population of elongated F_2 hybrids. The black line and gray lines represent the threshold for p = 0.01 and p = 0.05, respectively, and the red line indicates the average change in SNPs between the two populations. Significant differences in SNP frequency between the bulked populations was identified for ch2 and ch3.

The entire F_2 population was genotyped using KASP markers around the QTL on ch2 and ch3. Four markers on ch2 and eight markers on ch3 were significantly correlated with the CFSI genotype (Table S.6.1). The most significantly correlated markers were 24EP52, located at 45.00 Mb on ch2 ($F(2) = 7.511$, $p = 0.007$), and 19EP261, located at 59.19 Mb on ch3 ($F(2) = 62.73$, $p < 2 \times 10^{-16}$). As expected, the markers on both chromosomes correlated a high CFSI with the genotype of the Long John parent (Fig. 6.5). Furthermore, the fruits with a heterozygous genotype had a CFSI intermediate between the Long John and SA30 genotypes. Although both markers significantly correlated phenotype and genotype, an ANOVA indicated that the interaction between the two loci was only minimally significant ($F(4) = 2.619$, $p = 0.0366$).

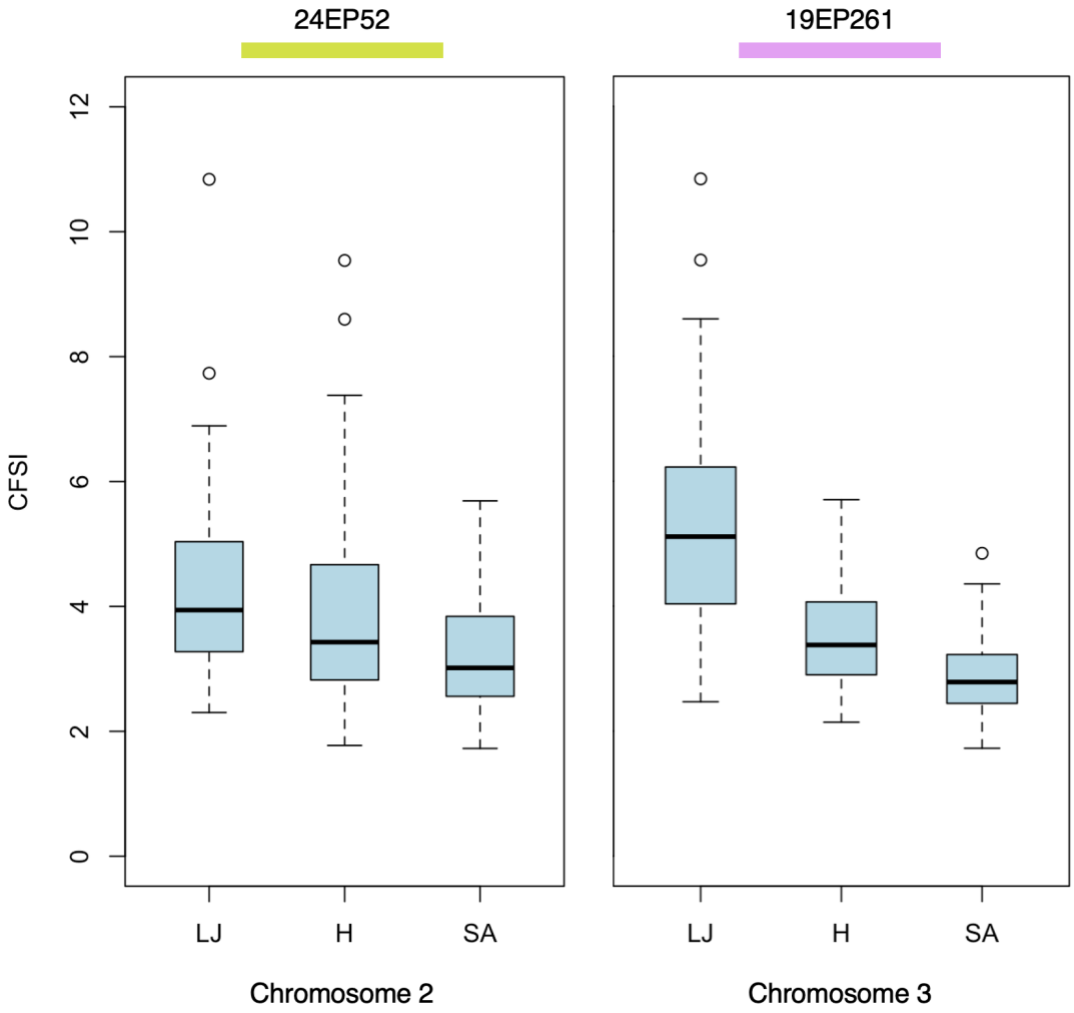


Fig. 6.5: Marker 25EP52 (45.00 Mb) on chromosome 2 and marker 19EP261 (59.19 Mb) on chromosome 3 were most significantly correlated with the CFSI. The genotypes were: homozygous for the Long John parent (LJ), heterozygous (H), or homozygous for the SA30 parent (SA).

A simple interval map (SIM) was created to determine the probability of a single QTL at each genomic position by calculating the logarithm of odds (LOD) for each marker (Fig. 6.6). Again, marker 24EP52 was the most significant on ch2 with an LOD of 2.86, and 19EP261 was the most significant marker on ch3 an LOD of 21.34. In addition to the SIM showing the greatest LOD for ch3, an ANOVA also indicated that 24EP52 only explained 7.5% of the phenotypic variance whereas 19EP261 explained 40.2%. Again, the interaction between the two loci was not

statistically significant (F(4) = 2.012, p = 0.094). Therefore, the locus on ch3 was considered the primary contributor to pericarp elongation for the F_2 population.

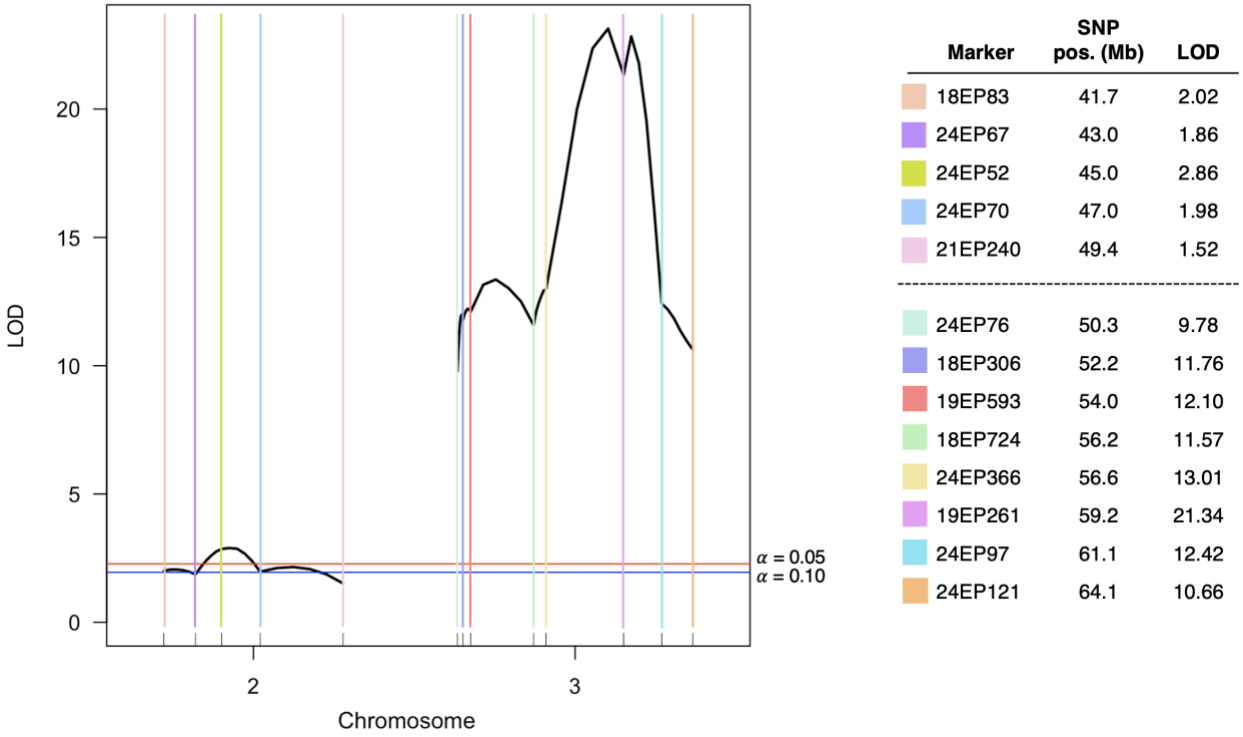
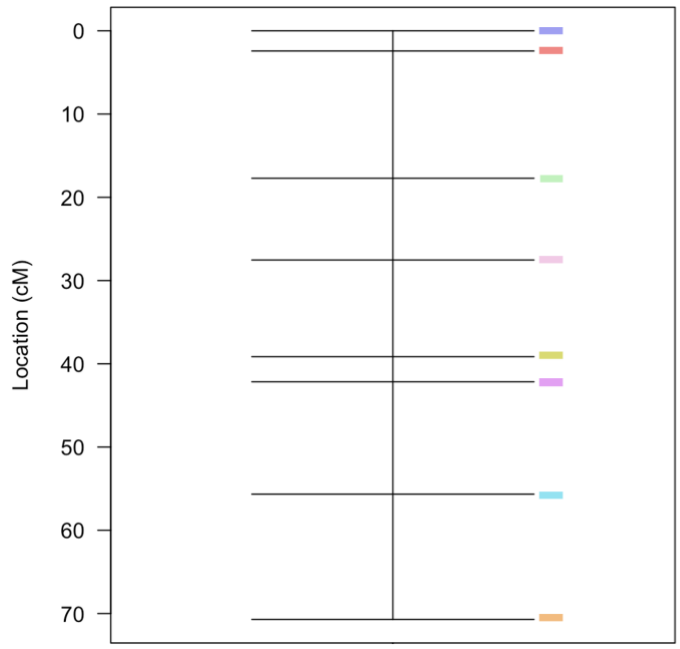


Fig. 6.6: Simple interval map (SIM) showing the logarithm of odds (LOD) of a quantitative trait locus for the curved fruit shape index phenotype for each marker on chromosomes 2 and 3.

3.3. Genetic Recombination in the 24S54 F_2 Population

The 24S54 population, F_2 hybrids of SA30 and Long John, was developed to confirm the location of the most significant QTL on ch3. The F_2 population was initially genotyped with the 18EP724 (56.2 Mb) and 24EP97 (61.1 Mb) markers which flanked 19EP261 (59.2Mb). In the entire population, 81 out of the 224 seedlings (36.2%) were recombinant between the two markers. Furthermore, thirteen plants had a double recombination (Table S.6.3). The recombinant plants were selected to disrupt potential interactions within this genomic region.

The recombination rate of 36% was expected because the genetic distance between the two markers, calculated with the Kosambi mapping function, was 38.0 cM (Fig. 6.7).



Marker	SNP pos. (Mb)	Location (cM)	LOD
18EP306	52.2	0.0	0.69
19EP593	54.0	2.4	0.94
18EP724	56.2	17.7	1.37
24EP147	57.8	24.5	1.00
19EP694	58.7	39.1	2.51
19EP261	59.2	42.2	3.32
24EP97	61.1	55.7	0.51
24EP121	64.1	70.7	0.24

Fig. 6.7: Calculated genetic distance for each genetic marker on chromosomes 3.

3.4. Phenotypic Analysis of the 24S54 F_2 Population

As with the 23S76 population, significant pericarp elongation was observed in the 24S54 F_2 recombinants (Fig. 6.8). Although only the recombinant plants were grown to maturity, phenotypic variation was still observed among the F_2 hybrids.

Fig. 6.8: Selected F_2 hybrids of Long John and SA30 from the 24S54 population with larger CFSI than the Long John parent.

Again, most of the 24S54 recombinant plants had a CFSI between the SA30 and Long John parents. However, none had a CFSI less than SA30. In the 23S76 population, 29 of the 205 F_2 plants (14%) had a greater CFSI than the Long John parent. However, in the 24S54 population, 33 of the 81 recombinant plants (41%) had a greater CFSI than Long John (Fig. 6.9). It is challenging to make a direct comparison between the two populations because only the recombinant 24S54 plants (81 of 224) were phenotyped. However, it is likely that the proportion of plants with a greater CFSI than Long John would be consistent when considering the entire population because the 23S76 and 24S54 populations were formed from the same cross.

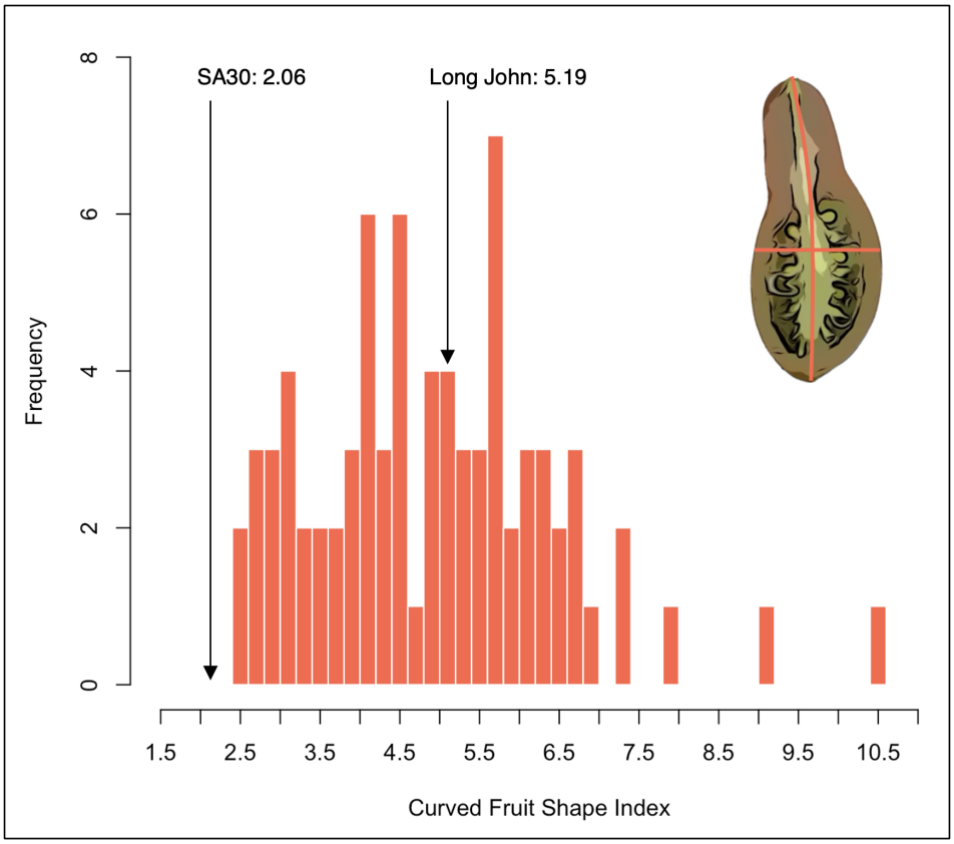


Fig. 6.9: Distribution of the measured curved fruit shape index (CFSI) for the 24S54 recombinant F_2 hybrid population of Long John and SA30 tomato (*Solanum lycopersicum*).

The relationship between the CFSI and other phenotypic measurements in the recombinant 24S54 F_2 population was consistent with 23S76. Again, significantly positive correlations were identified between the CFSI and OBO ($r = 0.77$, $p < 0.0001$), PER ($r = 0.59$, $p < 0.0001$), and PIA ($r = 0.63$, $p < 0.0001$), and CFSI was significantly negatively correlation with PAM ($r = -0.55$, $p < 0.0001$) and DAM ($r = -0.66$, $p < 0.0001$) (Fig. 6.10). However, the strength of the correlations were slightly lower for PER, PIA, PAM, and DAM compared with the 23S65 population.

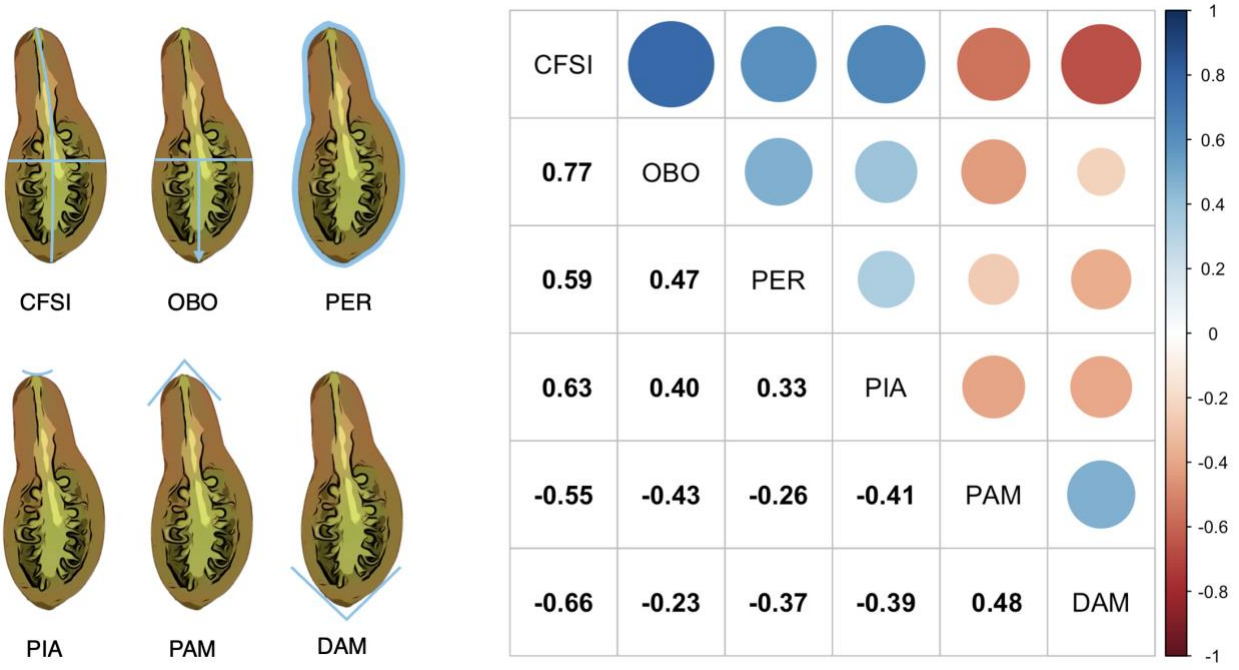


Fig. 6.10: Correlation of phenotypic parameters for the 24S54 F_2 hybrid population of Long John and SA30 tomato (*Solanum lycopersicum*). The measured parameters included the curved fruit shape index (CFSI), obovoid shape (OBO), perimeter (PER), proximal indentation area (PIA), proximal angle macro (PAM), and distal angle macro (DAM).

3.5. Genotypic Analysis of the 24S54 F_2 Population

For the recombinant 24S54 population, the only KASP markers on ch3 significantly correlated with the CFSI phenotype were 19EP694 (58.7 Mb) ($F(2) = 5.28$, $p = 0.007$) and 19EP261 (59.2 Mb) ($F(2) = 8.12$, $p = 0.001$). Again, both correlated a high CFSI with the genotype of the Long John parent. Furthermore, a simple interval map indicated these were the only markers significantly correlated with the QTL on ch3 with LOD of 2.51 and 3.32, respectively (Fig. 6.11).

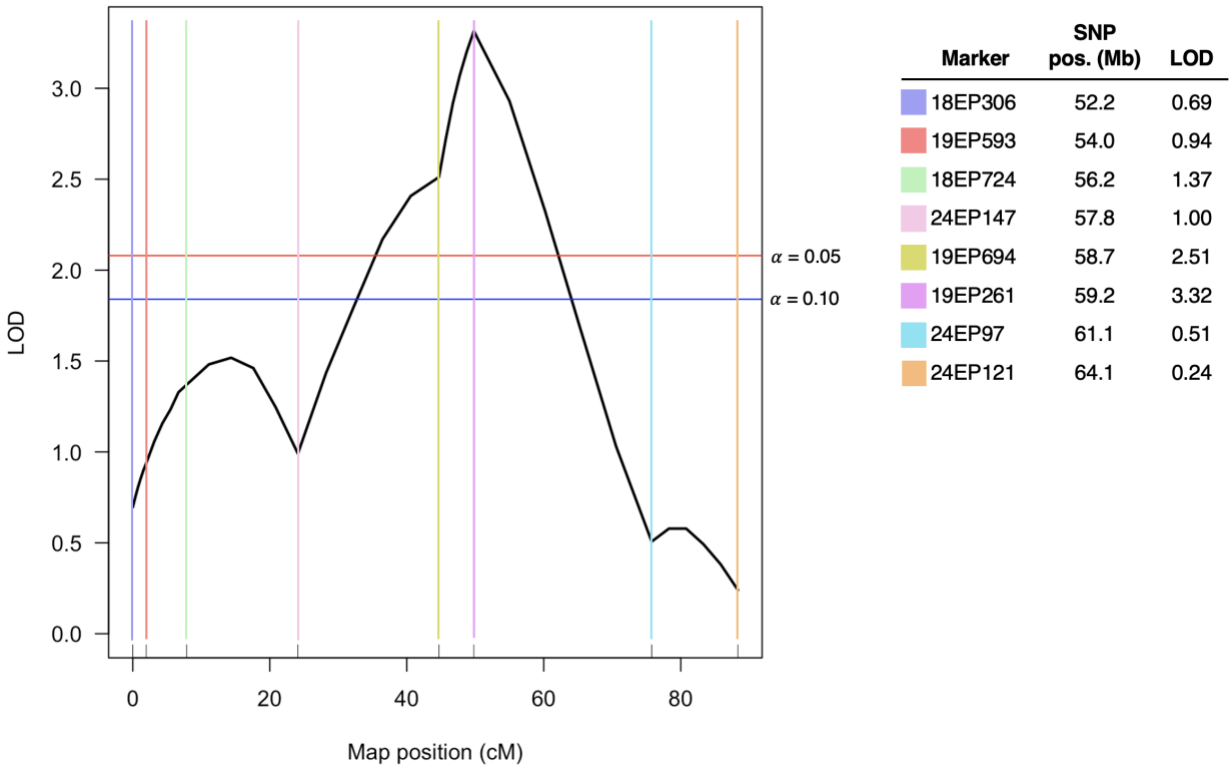


Fig. 6.11: Simple interval map showing the logarithm of odds (LOD) of a quantitative trait locus for the curved fruit shape index phenotype for each marker on chromosome 3.

Therefore, SL4.0ch03: 59187292 was confirmed as locus the most significantly correlated with the CFSI. The marker for this locus (19EP261) mapped approximately 25 kb upstream of the genomic region containing the fruit weight gene *fw3.2* (Chakrabarti et al., 2013). Therefore, it is likely that *fw3.2* is responsible for enhancing elongation of the fruits with *ovate* and *sun*. Previous research by van der Knaap et al. (2002) also identified a locus near *fw3.2* in Long John that was significantly correlated with fruit elongation. However, the primary distinction in the present study is both SA30 and Long John have *sun* and *ovate*, meaning the progeny were fixed for these mutant alleles. Therefore, the QTL seq of the most compact and most elongated bulked populations only identified differences in the genome which were not due to the impact of *sun* or *ovate*.

Ongoing research is investigating potential epistatic interactions between fruit weight and fruit shape genes, namely *fw2.2*, *fw3.2*, *ovate*, and *sun*. Because *ovate* and *sun* contribute to elongation in other genera, future research should also investigate whether the loci identified in the present research is responsible for elongation of other fruits. Furthermore, future research should determine consumer interest in elongated, pear-shaped fruit to inform breeding research and marketing to release new cultivars to the market.

Conclusion

Both the 23S76 and 24S54 F_2 populations were segregating with a wide, continuous distribution of the CFSI. Furthermore, there was a significant correlation between the CFSI and PER, which estimates fruit weight ($r = 0.63$, $p < 0.0001$), indicating that larger fruits were more likely to have a greater CFSI. The marker most significantly correlated with the CFSI (19EP261) was located at SL4.0ch03: 59187292, which is located near a known fruit weight gene. Future research should investigate potential interactions between fruit weight and fruit shape genes.

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Author Contributions

All authors listed have made substantial direct and intellectual contribution to the work and approved it for inclusion on this dissertation. Specifically, R.C.I. Maynard phenotyped and

genotyped the plants from each population, analyzed the data, and summarized the findings in this report. L. Zhang advised the research and contributed to DNA extraction and marker development. Y. Topcu advised the research and conducted the QTL Seq. E. van der Knaap advised the research and funded the experiment.

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CHAPTER 7

IDENTIFYING FACULTY OPINIONS ABOUT IMPLEMENTING AN ONLINE, NON-THESIS MASTER'S DEGREE

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Abstract

Although distance education is a growing field, there are benefits and challenges in creating and implementing new online degrees. Faculty play a critical role in forming new online programs and hold differing perceptions about online learning. The purpose of this research was to identify faculty opinions about implementing an online, non-thesis master's degree as an alternative to the existing thesis-based degree. In this study, 17 faculty members in the field of horticulture were surveyed at a major university in the southeastern United States. Q methodology was selected as the research tool to identify the primary opinions faculty held about an online, non-thesis master's degree. Once participants were surveyed, factor analysis was used to reduce the response into three perspectives. Three distinct perspectives were summarized as: (A) in-person instruction is more effective than online education, (B) online programs increase accessibility to graduate degrees, and (C) successful online programs require independent learners. Participants holding the first perspective believed an online program would not benefit student learning and would increase the workload of faculty. Participants with perspectives B and C agreed that an online program would be beneficial in reaching a broader audience of students. Although perspective C placed a high importance on independent learning, perspectives A and B indicated engaging with students was critical to student learning. All factors agreed an online degree would help our department reach non-research-oriented students. However, there was discrepancy in faculty willingness to support the program. Based on the identified faculty perspectives, it is believed that an online, non-thesis master's would be successful if faculty who are willing to participate in the online degree were trained to be effective online educators and if they encouraged students to hold a deeper level of engagement with the content.

Keywords: faculty opinions, mixed methods, non-thesis, online degree, Q methodology

1. Introduction

Distance education is an increasingly popular option with abundant access to online resources and emerging technologies which allow for collaborative learning in an asynchronous format (Lee, 2017). Graduate students report selecting distance education as the best option for continuing their education while employed full time and are drawn to online programs for their convenience and flexibility (Harris and Martin, 2012; Ilgaz and Gulbahar, 2017). In Fall 2021, 3.2 million students were enrolled in graduate degree programs in the United States, and 40% of those students were exclusively taking online courses (U.S. Department of Education, 2021).

Although online graduate degrees are common, there is a striking lack of availability for students interested in pursuing a graduate degree in horticulture. As of the 2021-2022 academic year, 23 institutions in the United States offered a master's in horticulture. Of these programs, only four institutions had a fully online, non-thesis option causing a discrepancy in career requirements and educational opportunity (Institute of Education Statistics, 2022). For example, many positions such as Extension Agents, directors of botanical gardens, and instructors at community and technical colleges require a master's degree without the need for research-based skills (O*Net OnLine, 2023; Seed Your Future, 2020). Therefore, these positions may easily be filled by candidates with online degrees.

An online, non-thesis master's degree option would broaden the educational opportunities for students pursuing specialized, non-research positions. Additionally, distance education would make a department's graduate program more accessible to employed individuals with limited schedule flexibility (Coleman and Berge, 2018). One important variable

impacting the availability of online graduate degrees is support from the department's faculty. One third of leaders in higher education institutions in the United States reported that faculty attitudes towards online education is a significant hinderance to the growth of online programs (Elaine et al., 2016). Therefore, this research aimed to investigate what factors influence faculty support or disagreement with online programs. This study identified the perceived impact of implementing a new online, non-thesis master's degree by surveying horticulture faculty at a major university in the southeastern United States.

2. Literature Review

2.1. Student Perspectives of Online Graduate Programs

Several studies have investigated the success of graduate students in online programs. In Malaysa, online learning is a new option, which gave researchers a unique opportunity to study graduate students' experiences with a newly developed online program. The study surveyed online Master of Education students enrolled part time or full time. Although the respondents found it more challenging to get help from the instructor in an online format, they reported the online format did not decrease their motivation to learn. Also, most respondents said online learning was supportive of their individual learning needs. However, many students were not comfortable with this new instructive method and preferred face-to-face programs (Tareen and Haand, 2020).

A study of online graduate business students at a university in Australia identified a correlation between student self-efficacy and success in online programs. Two factors contributing to self-efficacy were the attitude of the student and digital literacy. Students who had high self-efficacy demonstrated greater engagement with other students in the course and

interacted more with the learning management system. It was recommended that educators should take care to improve the capacity of all students to effectively use the digital technologies selected in their classroom to improve their chance of success in an online program (Prior et al., 2016).

A study of graduate students in online thesis and non-thesis degrees in Turkey found that students were more prepared for online learning if they were self-directed and had control over the learning process (Demir Kaymak and Horzum, 2013). Additionally, students were more satisfied with their online classes when they could self-regulate their learning and efficiently manage their time (Kara et al., 2019; Landrum, 2020). Demir Kaymak and Horzum (2013) also found a negative interaction between student readiness and course structure meaning the more prepared students were to enter an online program, the less course structure they needed. The implications of this study are that if students are not ready to enter an online program, they will need more support from the instructor in terms of a well-designed course structure to be successful.

Another indicator of student success in online programs is how interactive they are. The more interaction students have with the content, instructor, and other students, the more likely they are to meet their learning needs (Demir Kaymak and Horzum, 2013). For example, online graduates enrolled in a Master of Arts program in the United States reported their best experiences with online learning involved sharing their knowledge with classmates (Holzweiss et al., 2014). Although it can be challenging to have consistent interactions with other students in an online class, student retention in online programs is greatest when they hold quality interactions with the instructor (Harris and Martin, 2012; Kara et al., 2019).

2.2. Faculty Perspectives of Online Graduate Programs

For online programs to be successful, graduate departments need willing and engaged faculty to teach online courses. However, several barriers have been identified that prevent instructors from beginning or continuing online courses. The first barrier is the time needed to create online content (Kellen and Kumar, 2021). Some time-consuming factors for new online classes include creating an effective instructional design and learning new software and technology (Rockwell et al., 1999). However, faculty who teach online and face-to-face courses reported their total workload did not differ between the two delivery formats (Thompson, 2004). Once an online course is established, faculty report the majority of their time is spent grading and communicating with students (Mandernach et al., 2013; Thompson, 2004).

Another concern for faculty is the perceived value of online instruction (Kellen and Kumar, 2021). This is evidenced by employers who do not view online and in-person degrees as equal. A study by Lennon (2021) indicated that employers are twice as likely to respond to applicants who were awarded an in-person degree as opposed to an online degree. Prospective employers perceived online degrees as less rigorous, lacking in-person interactions, and the potential for academic dishonesty. However, they also recognized student self-direction and discipline as unique benefits of online education (Columbaro and Monaghan, 2008). Despite faculty and employer apprehensions about the quality of an online degree, a multi-year study of graduate students enrolled in several courses in Scotland and Sweden indicated there was no performance difference between the online and face-to-face students (McPhee and Söderström, 2012).

Faculty also lack confidence in delivering online lectures (Kellen and Kumar, 2021). Because online classes create distance between the student and the instructor, appropriate use of

technology paired with constructive pedagogy is essential for effective online instruction. Also, since online courses lack non-verbal cues, communication between faculty and their students can be challenging (Holzweiss et al., 2014). One way instructors can improve communication with the student is by providing feedback in a video or audio format (Davis et al., 2019). Successful online education not only relies on the instructor to remain current with changing technology and communicate well with students, but the students must also take an active role in the learning process (Joshi et al., 2022). It has been shown that self-regulation is critical to student success in online courses (Ye and Pennisi, 2022). Whereas face-to-face courses rely on students self-reporting their engagement with course content, online learning management systems allow instructors to monitor the frequency and duration of student engagement with the content to ensure the students are making appropriate advancements through the course (Davis et al., 2019). Overall, students must self-regulate their learning, manage their time effectively, and receive sufficient feedback from their instructor to be successful in an online program (Lee, 2017).

In addition to the barriers that prevent faculty from starting an online course, several factors influence faculty satisfaction with online teaching. Satisfaction is higher when faculty have autonomy in designing their courses and more interactions with students. However, they are dissatisfied with student evaluations, which tend to be lower in online courses and can negatively contribute to promotion and tenure (Marasi et al., 2022). Faculty satisfaction with and perception of online instruction may be influenced by the lockdown period of the Covid-19 pandemic when many institutions were forced to transition online. This is particularly true for faculty who had no previous experience teaching in an online format. However, their abrupt transition online is not comparable with faculty who have well-constructed online courses (Marasi et al., 2022).

3. Materials and Methods

3.1. Q Methodology

This research aimed to determine faculty opinions on the addition of an online, non-thesis master's degree to the existing thesis option. Q methodology was selected as the research tool because it allows analysis of both divergent and mutual opinions. Because individual opinions are subjective and vary from person to person, they are challenging to study (Brown S.R., 2019). However, Q methodology, introduced by William Stephenson in the 1930s (Stephenson, 1935), is a way of studying human subjectivity in a quantitative, systematic manner (Herrington and Coogan, 2011; McKeown and Thomas, 2013). The goal of Q methodology is not to identify a single truth but to investigate the diversity of opinions held by many individuals (Cross, 2004). A drawback of other survey tools is they tend to report consensus statements and minimize periphery viewpoints. Conversely, Q methodology attempts to explore all viewpoints relating to the given topic and can derive greater meaning than Likert-scale studies (Lundberg et al., 2020). For the purpose of this study, it was important to recognize all opinions held by faculty in the department, making Q methodology a useful research tool.

Q methodology has been used extensively in education research. Lundberg et al. (2020) analyzed the use of Q methodology in 74 studies conducted in the United States, the United Kingdom, Australia, and South Korea about compulsory education. These studies used Q methodology as a research tool to investigate teachers' and students' understanding of a subject, attitudes and values, critical reflection, evaluation of educational issues, preference in responding to issues, and method of decision making (Lundberg et al., 2020). Q methodology has also been used at the university level in many studies including investigating students' motivation for

learning (Zheng et al., 2020), faculty opinions about student evaluations (Wu and Wang, 2021), and faculty opinions on using technology in the classroom (Clausen et al., 2021).

One study used Q methodology to determine faculty opinions about shifting classes online during the Covid-19 pandemic (Ramlo, 2021). The study identified three distinct perspectives. The first perspective was comprised of faculty who enjoy teaching and are proficient with using technology. They had previous experience teaching online and transitioned well to fully online classrooms but valued hands-on experiences in face-to-face classrooms. Another group of faculty generally experienced feeling overwhelmed during the pandemic, but not necessarily because of shifting their courses online. The third group of faculty had a strong preference towards in-person instruction and were challenged by having to learn a new way of teaching (Ramlo, 2021). Although the subject of the Ramlo (2021) study is similar to the present research, one clear distinction is the motivation for moving to online instruction. Whereas during the lockdown period of the pandemic, instructors were forced to move into an exclusively online format, the present study investigates the willingness or reluctance of faculty to adopt an online program. The significance of this distinction is faculty would have time to prepare and be trained for online instruction.

3.2. Research Instrument

The initial step in Q methodology is the development of a Q concourse. The concourse is a collection of opinion-based statements representing all possible opinions on the subject, which makes it theoretically limitless (Brown S.R., 2019). The concourse is traditionally developed from focus groups and interviews, but it can also be formed from previous research articles (Brown M.M., 2004). For this study, statements for the Q concourse were collected from three

different sources. Statements were inspired from major themes in previous research including time required to develop online courses, lack of confidence in teaching online, the perceived reduction in value of online courses compared to face-to-face instruction, the need for external support, the convenience of online programs for students, the students' needs for quality interactions, and the ability of students to take an active role in the learning process (Demir Kaymak and Horzum, 2013; Elaine et al., 2016; Harris and Martin, 2012; Holzweiss et al., 2014; Ilgaz and Gulbahar, 2017; Joshi et al., 2022; Kara et al., 2019; Kellen and Kumar, 2021; Landrum, 2020; Rockwell et al., 1999; Ye and Pennisi, 2022).

Next, a focus group was formed during the strategic planning of an online, non-thesis option for a master's in horticulture to identify additional themes of interest. In this focus group, several potential benefits and challenges of this new degree were recognized including creating new interest in the field of horticulture by making the graduate degree more accessible, the ability of a new degree option to bring more funding to the department, and whether a new program should be the shared responsibility of all graduate faculty in the department. These ideas were recorded as a list of opinion-based statements and added to the Q concourse.

Finally, to ensure faculty opinions were expressed in the concourse, all graduate faculty in the department were surveyed. Before the survey, they were told the basic structure of a potential online master's which included converting the same courses required for the thesis-based degree into an online format, requiring twice as many course credit hours, and removing the thesis requirement. Since the online master's had not been developed yet, an anonymous survey was distributed to all graduate faculty asking: "What would the implementation of an online, non-thesis master's degree mean to you?" Many faculty responses to the survey were similar, but each statement was recorded individually.

From the Q concourse, five themes were identified including the growth of the department, quality of the degree, faculty responsibility, implications for teaching, and impact on the student. Based on these five categories, the concourse was then reduced in an attempt to summarize the primary viewpoints (Stephenson, 1978). Each of the 50 statements from the Q concourse was considered individually. Statements with similar meanings were combined, but any statement that represented a unique perspective was maintained in the list. The Q concourse was reduced to a list of 34 representative statements forming the Q set. The Q set was reviewed by the department head, two faculty members, and a graduate researcher to ensure its completeness and clarity (Table S.7.1).

3.3. Study Population

The graduate faculty invited to participate in this study included 31 members with varying appointments in instruction, extension, and research. Twenty-six of them taught courses for graduate students and six had fully online courses that were developed outside of the Covid-19 lockdown period. Seventeen faculty had formal academic responsibility with a teaching appointment ranging from 5% to 86%; however, only six had a high teaching responsibility. Because all graduate faculty, regardless of their instruction appointment, may interact with graduate students in an advisory capacity, all members of the graduate faculty were invited to participate in the study. The faculty included fifteen Professors, seven Associate Professors, eight Assistant Professors, and one Senior Public Service Associate with a full appointment in extension. The age of faculty also varied with five aged 30-40, seven aged 41-50, twelve aged 51-60, and seven aged 61-70. The teaching experience of the faculty was expected to differ based on the wide age range and difference in teaching responsibility within the department.

Although all graduate faculty were invited to participate in the study, only 17 responded, giving a response rate of 55%. Because some faculty chose not to participate, the response rate may be a limitation of the study. Without complete participation, it is challenging to infer what percentage of the faculty population falls under each of the perspectives analyzed. However, the number of participants was deemed sufficient based on Watts and Stenner's (2012) recommendation of having half as many participants as Q statements. In general, Q research studies do not require a large participant population because the aim is to identify distinct viewpoints and analyze them further. Watts and Stenner (2012) state that unique perspectives identified by factors may be identified with as few as one participant significantly loading onto the factor (Watts and Stenner, 2012).

3.4. Data Collection

In Q methodology, data is collected by participants sorting statements in the Q set. This sorting is often conducted in person, but to maintain the anonymity of research participants and minimize subjective bias, the Q sort was administered online. The EQ Web Sort platform (version 1.0.2), created by Shawn Banasick and made available through GitHub, was used to configure this Q study in an online format. All graduate faculty in the department were provided with a URL link to the Q set and asked to initially sort the randomized statements into three categories: statements they agreed with, had neutral feelings about, or disagreed with. After the initial sorting, participants were asked to re-sort the statements into a forced, quasi-normal distribution from "most agree" (+4) to "least agree" (-4) (Fig. 7.1), forming the individual Q sort (Brown S.R., 1993). It is important to note that statements ranked with a negative value do not necessarily mean the individual disagreed with the statement. Rather, their opinions were more

closely aligned with statements ranked with a higher value. An example of the completed Q sort is shown in Fig. 7.1.

Least Agree							Most Agree	
-4	-3	-2	-1	0	1	2	3	4
15	3	9	2	6	1	4	16	29
18	17	13	11	8	5	7	23	33
	21	24	20	12	26	10	28	
		30	25	19	27	14		
			32	22	34			
				31				

Fig. 7.1: A quasi-normal distribution ranging from most agree (+4) to least agree (-4) was used for the Q sort in this study. Each square on the distribution is a placeholder for a statement number. This figure shows an example of a completed Q sort from a participant in Factor B. The forced distribution ensures participants consider the importance of each statement with respect to every other statement.

Once participants completed the Q sort, they were asked to explain the statements they agreed the most and least with through an open-ended question in the online survey. Demographic information was collected through an anonymous survey to identify potential relationships between the factors and teaching experiences of the participant. Questions included their instruction appointment, whether they teach graduate classes, whether they have experience teaching online classes, how many graduate students they advise, how many graduate committees they serve on, and how many professional development activities related to teaching they had participated in the past 5 years.

3.5. Statistical Analysis

A distinguishing feature of Q methodology is its use of factor analysis to group individual responses into fewer viewpoints. A participant's Q sort represents the overall perceptions of the individual. If other participants Q sorts are highly correlated, they are grouped together to form a factor. Individuals within a factor will have a similar perspective, whereas each factor represents a different viewpoint (Watts and Stenner, 2005). Although factor analysis attempts to explain as much of the variance among Q sorts as possible, reducing the number of factors analyzed also reduces the number of viewpoints that can be described. Therefore, Q methodology is a mixed methods approach using quantitative statistical analysis combined with qualitative abductive reasoning to extract the appropriate number of factors.

Results from the Q sorts were analyzed using the KADE software (version 1.2.1) (Banasick, 2019). First, the Q set and all faculty responses were uploaded to the software, and a correlation matrix was formed to compare how similar or dissimilar individual sorts were to one another (Brown M.M., 2004). Next, principal component analysis with forced Q sorting was used to isolate factors. Eight factors were initially identified by the software, and the Kaiser-Guttman criterion recommends maintaining all factors with an eigenvalue greater than 1.00 (Shrestha, 2021). In this study, six factors had an eigenvalue greater than 1.00. However, three of the six factors only had one Q sort significantly loading onto the factor. Because factor analysis is intended to reduce the number of variables in a data set, it is not useful to form factors with fewer than two Q sorts (Watts and Stenner, 2005). Yeomans and Golder (1982) identified that the Kaiser-Guttman criterion can result in an inaccurate prediction of the appropriate number of factors. Therefore, only three factors were selected for analysis to account for as much of the variance of each Q sort as possible (Table 7.1).

Table 7.1: Factor analysis was used to group faculty opinions on the implementation of an online, non-thesis master's in horticulture. Of the 17 survey participants, 14 faculty significantly loaded onto three factors (A, B, and C). These factors explain 50% of the total study variance.

	Factors		
Q sort	**A**	**B**	**C**
Participants loading onto factor (no.)	6	4	4
Study variance explained (%)	28	12	10

Once three factors were selected for analysis, a varimax rotation was used to reduce the many opinions expressed in the Q sorts into a few core ideas (McKeown and Thomas, 2013). Varimax rotations present the most mathematically significant factor loadings by representing as much study variance as possible in the factors. Additionally, varimax rotations minimize researcher bias by preventing manual, judgmental rotations (Watts and Stenner, 2005). Although the software automatically loaded participants into the three factors, the factor loadings were only considered statistically significant at $p \leq 0.05$. Therefore, three factors with several significantly loading participants were created using this method of factor analysis. Next, composite Q sorts were created for each factor which are weighted averages of each of the factor loadings showing how each statement was ranked by the factor. Therefore, an individual factor is an idealized Q sort comprised of several individual Q sorts representing the shared perspective of all participants within a factor (Stephenson, 1978).

The perspective of each factor was interpreted using the crib sheet method described by Watts and Stenner (2012), which uses abductive reasoning to identify key characteristics of each factor. This method examines the highest and lowest ranking statements in each factor as well as each of the statements ranked at more extreme values than any other factor. The crib sheet method was used to isolate defining statements for each of the factors. Additionally, the post-sort survey responses were used to confirm placement of individuals into each factor. Each of the distinguishing statements identified with the crib sheet method and the survey responses were

used to form a theory about the entire factor. Therefore, both quantitative data from the factor analysis and qualitative data from the abductive reasoning were used to generate the factors.

4. Results

The three analyzed factors explained 50% of the study variance. Six participants significantly loaded onto factor A and four participants significantly loaded onto Factors B and C. Three Q sorts did not significantly align with any of the factors. Rather, they shared opinions with more than one factor and were excluded from individual factor analysis. Responses to post-sort survey questions were used to support the perspectives of each factor.

Of the 17 participants, only seven responded to the follow-up survey about their teaching experience. For this survey, one respondent loaded onto Factor A, two respondents loaded onto Factor B, three respondents loaded onto Factor C, and one did not load significantly onto any factor. Due to low participation in the post-sort survey, sufficient demographic information was not available to describe each of the factors, which was one limitation of the study. Therefore, the teaching experience survey was not included in the factor analysis. The perspectives expressed in the Q sort from each of the three factors are described below using (statement no.: Q sort value).

4.1. Perspective A: In-Person Instruction is More Effective Than Online Education

Factor A has an eigenvalue of 4.70 and explains 28% of the study variance with six participants significantly aligning with the factor. A primary concern of Factor A was the increased workload for current faculty (statement no. 14: Q sort value +4) (Table S.7.1). Therefore, Factor A considered that additional faculty and staff support would be required for the

new program (16: +2; 19: +2). Additionally, Factor A regarded that student learning depends on level of engagement (25: +1; 31: +2; 34: +4). Overall, participants did not consider an online degree valuable for the department (1: 0; 2: -1; 5: -2; 15: +3) or the students (7: -2; 8: -3; 11: 0). Thus, Factor A did not acknowledge a current need for the program (6: -3) and were not willing to contribute to the program (24: -4). When participants from Factor A were asked to explain their Q sort, the dominant response was they felt the program would require significant work without sufficient financial return to the department. Additionally, they reiterated the belief that in-person instruction is essential to a successful education. The distinguishing statements of extreme viewpoints for Factor A with p value < 0.05 are listed in Table 7.2.

Table 7.2: Statements which were ranked significantly differently ($p \leq 0.05$) by Factor A than by Factors B and C were considered distinguishing statements for Factor A. This table gives distinguishing statements ranked |3| or |4| by Factor A.

No.	Statement	Q sort value	p-value
Most agreed with:			
14	This degree would increase the workload of faculty members.	4	$p < 0.005$
34	Student learning depends on level of engagement in online courses.	4	$p < 0.01$
15	This degree would have minimal benefit to me.	3	$p < 0.0001$
Least agreed with:			
6	There is a current need for this degree in our department.	-3	$p < 0.0001$
24	I would be willing to develop an online course if I had technical assistance.	-4	$p < 0.05$

Fig. 7.2 shows statements that distinguish Factor A from either other factor and how those statements were ranked differently by Factors B and C. For example, Factor A, shown as the solid black line, strongly agreed that the addition of an online, non-thesis degree would increase faculty workload with minimal benefit to the faculty. Although Factors B and C somewhat agreed faculty workload would be increased, they found more personal benefit from

the program. Furthermore, Factor A strongly disagreed that there is a current need for the degree in our department; however, Factors B and C both recognized a need for the program. The results of a Q methodology study by Amaruzaman et al. (2017) were also represented in this way to visualize the differences in opinions between factors.

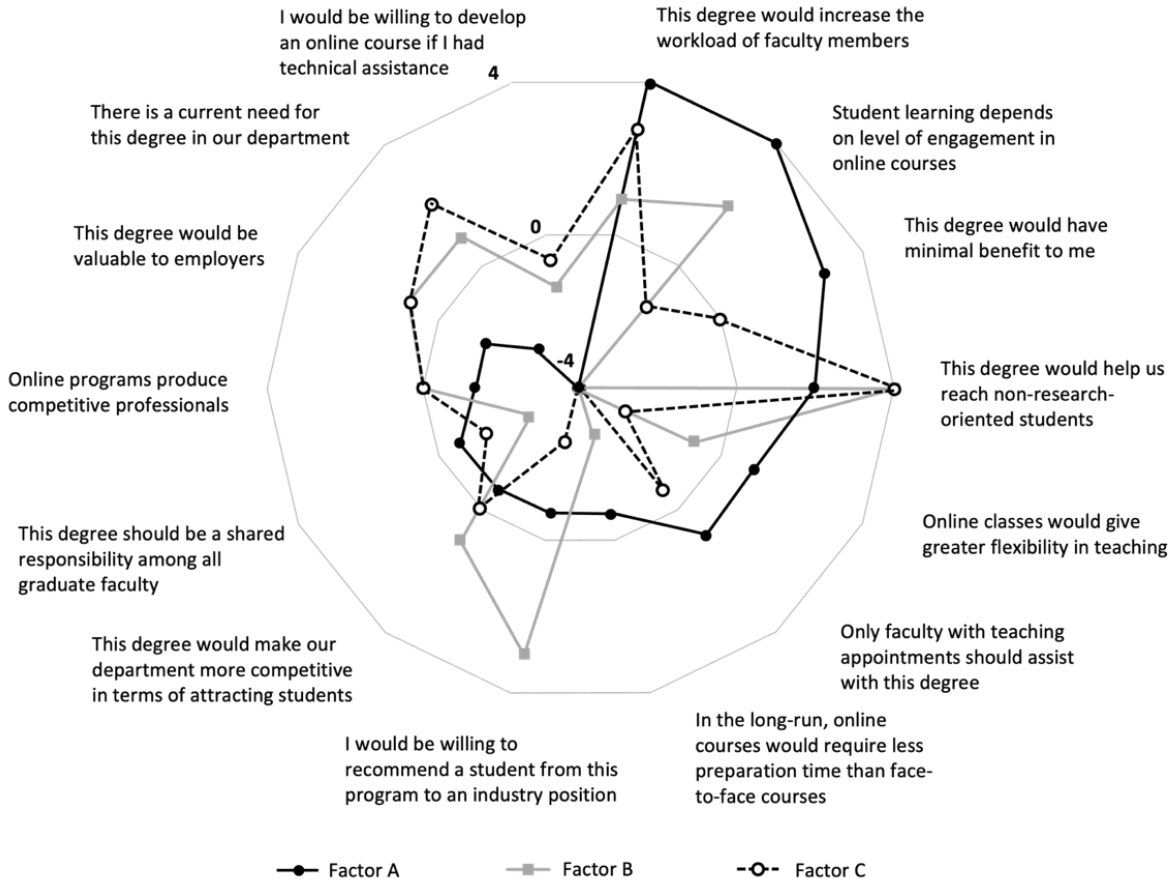

Fig. 7.2: The distinguishing statements for Factor A are shown with a solid black line on the diagram. The rings represent a gradient of Q sort values from +4 on the outer ring to -4 in the center of the diagram. Each marker corresponds to a statement on the perimeter of the diagram. The solid gray line and dashed black line indicate how Factors B and C ranked the distinguishing statements from Factor A.

4.2. Perspective B: Online Programs Increase Accessibility

Factor B has an eigenvalue of 2.01 and explains 12% of the overall study variance with four participants loading onto the factor. Respondents in Factor B primarily highlight the ability

of an online program to increase the accessibility of a graduate degree in horticulture, especially to non-research-oriented students (29: +4; 33: +4). Participants felt the program would be valuable for the department (2: +1) and the students (11: +2; 12: +3). Additionally, they expressed their willingness to personally support the program (12: +3; 15: -4; 20: +3). They responded that they were already comfortable teaching online classes (27: -3) but could benefit from additional training (23: -2). They did not believe all faculty should be involved in the program (17: -3), but they valued support from faculty without a teaching appointment (18: -4). Specifically, in the post-sort questions, participants identified that faculty with extension appointments would positively contribute to the program. Respondents in Factor B supported the program because they observed other successful online programs, and they reported the horticulture industry already expressed interest and support for this program. Table 7.3 summarizes the distinguishing statements of extreme views for Factor B with p value < 0.05.

Table 7.3: Statements which were ranked significantly differently ($p \leq 0.05$) by Factor B than by Factors A and C were considered distinguishing statements for Factor B. This table gives distinguishing statements ranked |3| or |4| by Factor B.

No.	Statement	Q Sort Value	p-Value
Most agreed with:			
29	This degree would allow more students to obtain a graduate degree	4	$p < 0.005$
20	I would be willing to serve as a graduate advisor for students in this degree	3	$p < 0.0001$
12	I would be willing to recommend a student from this program to an industry position	3	$p < 0.0001$
Least agreed with:			
27	I would need significant training to teach an asynchronous online course	-3	$p < 0.0001$
22	In the long-run, online courses would require less preparation time than face-to-face courses	-3	$p < 0.05$
15	This degree would have minimal benefit to me	-4	$p < 0.0001$
18	Only faculty with teaching appointments should assist with this degree	-4	$p < 0.0001$

Fig. 7.3 shows how distinguishing statements for Factor B were ranked by Factors A and C. Factor B, shown as the solid grapy line, strongly agreed the degree would allow more students to obtain a graduate degree and would be willing to serve as a graduate advisor for students in the program. Although Factors A and C somewhat agreed the degree would make our graduate program more accessible to students, neither were willing to personally serve in an advisory capacity. On the other hand, Factor B strongly disagreed that they would need training to teach an asynchronous, online class; however, Factors A and C were not confident teaching online classes without significant training.

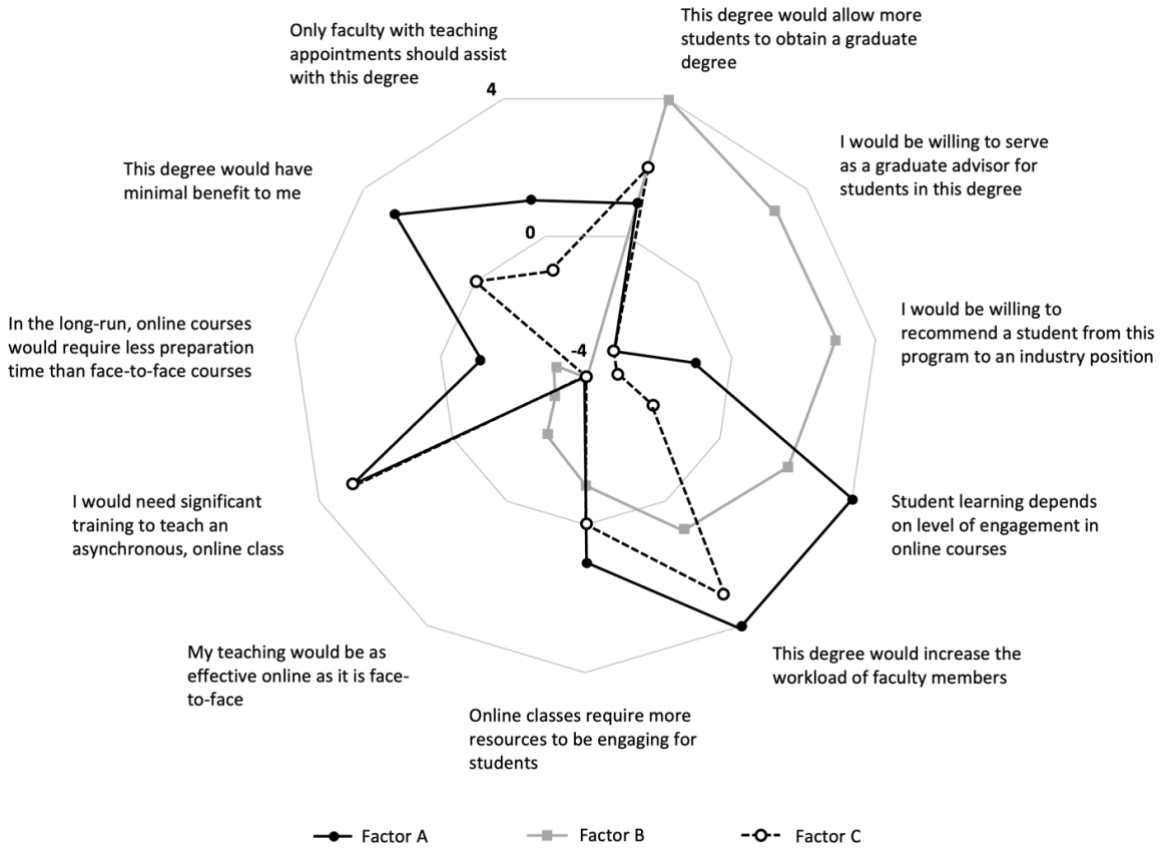

Fig. 7.3: The distinguishing statements for Factor B are shown with a solid gray line on the diagram. The rings represent a gradient of Q sort values from +4 on the outer ring to -4 in the center of the diagram. Each marker corresponds to a statement on the perimeter of the diagram. The solid black line and dashed black line indicate how Factors A and C ranked the distinguishing statements from Factor B.

4.3. Perspective C: Successful Online Programs Require Independent Learners

Factor C is a composite of four respondents, explaining 10% of the survey variance with an eigenvalue of 1.65. Respondents in Factor C strongly believe the program would benefit non-research-oriented students (33: +4). Specifically, individuals expressed the value the program would hold for professionals in the horticulture industry who are not research-minded but want to further their education. Factor C also highlighted the importance of students being independent learners in an online program (30: +4) and placed a lesser importance on direct engagement with the students (34: -2). Respondents in Factor C also believed the program would add value to the department (3: +3; 5: +2; 6: +2). A primary concern of Factor C was that although online classes would give flexibility to the student, it would increase the workload of faculty (14: +3; 21: -3; 22: -4). Finally, respondents noted the importance of appropriate training for faculty to be effective instructors online (23: -4; 27: +3). The distinguishing statements for extreme views of Factor C are summarized in Table 7.4.

Table 7.4: Statements which were ranked significantly differently ($p \leq 0.05$) by Factor C than by Factors A and B were considered distinguishing statements for Factor C. This table gives distinguishing statements ranked |3| or |4| by Factor C.

No.	Statement	Q sort value	p-value
Most agreed with:			
30	Students in an online program should have self-regulating study habits	4	$p < 0.0001$
3	This degree would bring additional funding to our department	3	$p < 0.0001$
14	This degree would increase the workload of faculty members	3	$p < 0.05$
Least agreed with:			
12	I would be willing to recommend a student from this program to an industry position	-3	$p < 0.005$
22	In the long-run, online courses would require less preparation time than face-to-face courses	-4	$p < 0.01$

Fig. 7.4 shows how distinguishing statements for Factor C were ranked by Factors A and B. It was the perspective of Factor C, shown as the dashed black line, that students in online program need self-regulating study habits. However, Factors A and B did not agree as strongly with this statement. Factor C also strongly disagreed that student learning depends on level of engagement in online classes. In contrast, Factors A and B strongly agreed that the learning process requires students to engage in online classes.

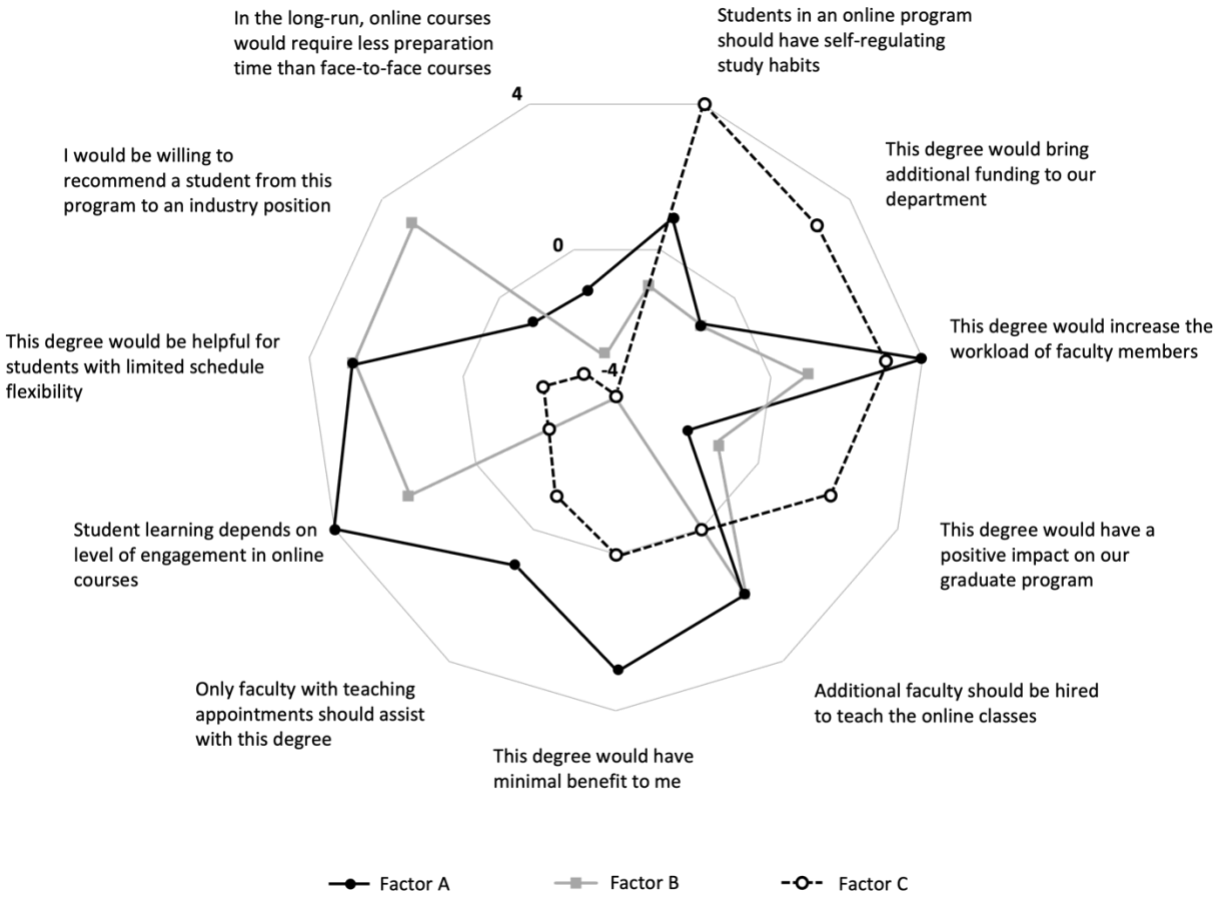

Fig. 7.4: The distinguishing statements for Factor C are shown with a dashed black line on the diagram. The rings represent a gradient of Q sort values from +4 on the outer ring to -4 in the center of the diagram. Each marker corresponds to a statement on the perimeter of the diagram. The solid black line and the solid gray line indicate how Factors A and B ranked the distinguishing statements from Factor C.

4.4. Consensus Statements Among Factors

This study also identified eight statements that were not significantly divergent among the three factors (Fig. 7.5). The responses to most of these statements were neutral, indicating they were not primary concerns for any factor. The most notable consensus was a strong agreement among all factors that the online degree would allow us to reach non-research-oriented students (33: 2/4/4), which was the primary objective of the program. Additionally, Factors A, B, and C agreed or responded neutrally that an online master's degree would make the department more visible (1: 0/1/1) and generate new interest in horticulture (4: 0/2/2). However, all three factors were neutral that the online degree would be valuable to students (10: 0/0/0) and did not agree that it would maintain the value of the current degree program (13: -2/-2/-1). Faculty also shared an unwillingness to learn more about online education (26: 0/-2/-2). Finally, each factor agreed that students should have networking opportunities (31: 2/0/1) but not necessarily involving faculty (32: 0/0/-1).

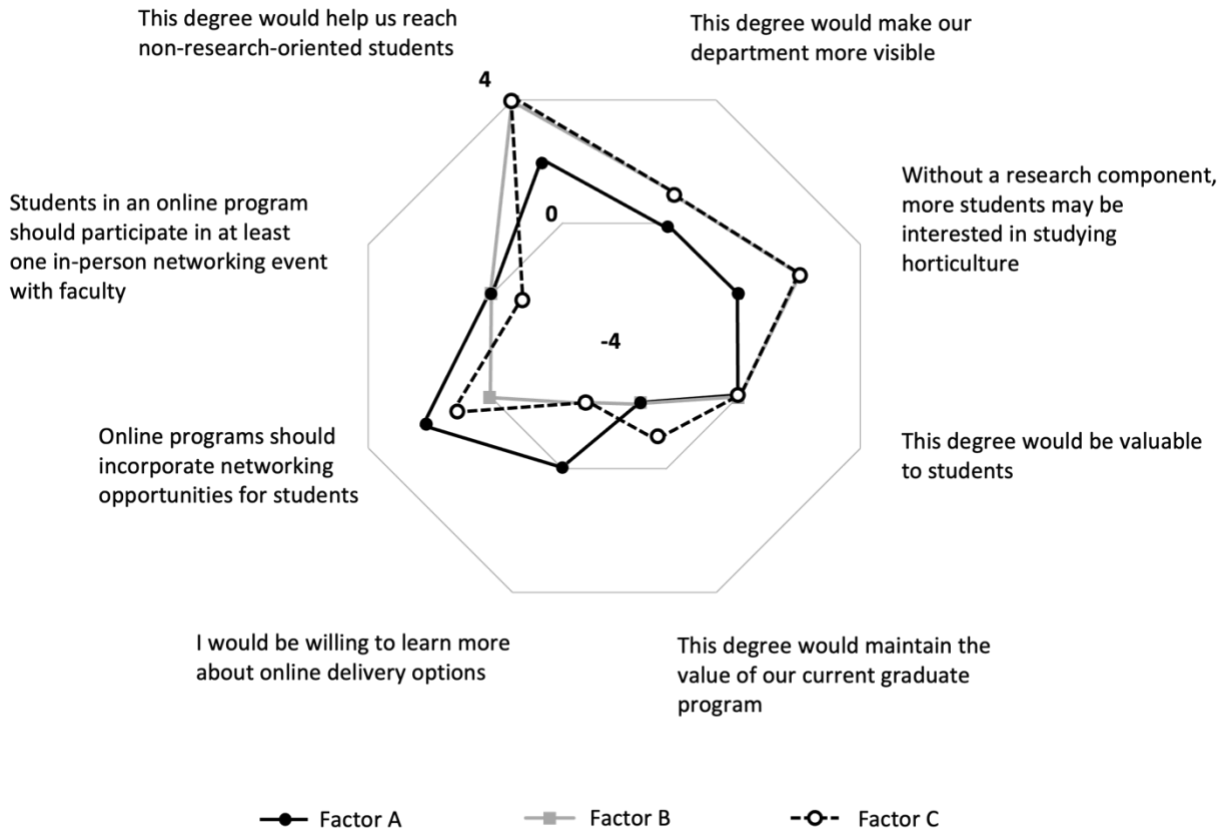

Fig. 7.5: This diagram shows the consensus statements among Factors A, B, and C using a solid black line, solid gray line, and dashed black line, respectively. The rings represent a gradient of Q sort values from +4 on the outer ring to -4 in the center of the diagram. Each marker corresponds to a statement on the perimeter of the diagram.

5. Discussion

Although the opinions expressed in this survey fell into three distinct factors, some perspectives were shared between two or more factors including faculty workload, effectiveness of teaching, and importance of student engagement. The primary objective of this research was to identify why there was a discrepancy between the educational need of graduate students entering the workforce and available educational opportunities. Since faculty play a critical role in the growth of online programs, our research attempted to identify the reason for faculty support of or disagreement with an online master's degree. One consensus statement among all factors indicated faculty strongly believed an online degree would help our department reach

non-research-oriented students. Therefore, we could improve educational opportunities for graduate students by offering an online degree if we can increase the willingness of faculty to support the program and equip them to be successful online educators.

A primary concern of Factors A and C was the potential for increased faculty workload. As seen in the consensus statements, all factors also shared an unwillingness to learn more about online programs, which was presumably related to their concern for additional work. Increased workload is a common deterrent for faculty considering online course offerings, particularly for those who are unfamiliar with online education (Mandernach et al., 2013; Thompson, 2004). Some useful strategies that faculty have found to efficiently manage time in online classes include automated grading for close-ended questions, use of general assignment comments for repetitive feedback, and using direct notifications to regularly communicate with the class to minimize student confusion (Cooper et al., 2019). However, the time invested in creating a new online course is a significant barrier for faculty to teach online (Kellen and Kumar, 2021). Because preparation for online teaching is time consuming, training and technical support are important for faculty who are new to online teaching (Elshami et al., 2021). Additionally, faculty satisfaction with online teaching is higher when they receive incentives (Marasi et al., 2022). Therefore, if faculty receive adequate technical support and incentives for establishing a new course, they may be less apprehensive about teaching online.

All three factors indicated faculty were concerned their teaching would be less effective in an online format and held concern about the value of an online program compared to the existing face-to face master's. Both are common concerns for university educators (Kellen and Kumar, 2021). Martin et al. (2019) studied faculty perception of their online teaching ability and found that faculty have high confidence in their ability to create assignments for online courses

but have less confidence in creating instructional videos. For course communication, faculty have confidence in their ability to respond to student emails but less confidence in their ability to make materials accessible for different student needs (Martin et al., 2019). Confidence in effective online instruction is likely tied to the perceived value of online programs. There has been a consistent trend of faculty being unwilling to accept the legitimacy of online programs, but there is a significant correlation between acceptance of online programs and student enrollment in online courses (Elaine et al., 2016). Although the present study did not investigate the reasons faculty lacked confidence in the effectiveness of their teaching and did not value online programs, future research could investigate specific ways to support faculty to improve their readiness for online instruction.

Each factor was based on faculty experiences and instructional preferences making the needs of each factor different. Faculty in Factor A, who value in-person instruction over online education, are not willing to teach online and do not currently value online education. Therefore, Factor A would benefit from seeing other successful online programs to give them more confidence in supporting a new online master's degree. Faculty in Factor B, who believe online programs increase accessibility, have previous experience teaching online and are willing to support the online program. Therefore, it would be essential to involve faculty who fall in Factor B in the development and implementation of a new online master's. Finally, faculty in Factor C, who believe successful online programs require independent learners, see the value of an online degree for the learners but need support in creating an online classroom that promotes independent learning.

6. Conclusion

The results of this research study highlight three distinct perspectives about the addition of an online, non-thesis master's program. These were: (A) in-person instruction is more effective than online education, (B) online programs increase accessibility, and (C) successful online programs require independent learners. The primary concerns for this program came from Factor A who are supporters of in-person instruction. Factor A was reluctant to support the online program because they strongly believed the degree would increase the workload of faculty and not provide significant benefit to prospective students or the department. However, both Factors B and C indicated support for the program. Individuals loading on Factor B believed the primary benefit of the online program would be increasing accessibility of higher education to non-research-oriented students. Factor C also recognized the benefits of this program for students but placed a higher emphasis on independent learning than Factor B. By recognizing the differences in perspectives and needs of faculty in each factor, the department is better equipped to overcome potential challenges in the implementation of a new online master's degree. Overall, a successful program would require the participation of willing faculty who are provided the necessary resources and pedagogical training to be effective online educators.

Although previous studies investigated student and faculty perspectives of online instruction, our research provides new insight on factors that influence faculty support of online master's programs. Q methodology, which was selected for this research, is a well-established mixed methods tool to study subjective opinions. It has commonly been used in education research but has not been extensively used to study the opinion of faculty in higher education. To our knowledge, this is the first application of Q methodology to gauge faculty opinions for informing departmental decisions. Although faculty participating in this study were from the

field of horticulture, the context of the study was not discipline-specific. Therefore, the methodology and presented findings are applicable to many other disciplines. Specifically, Q methodology can be used as a survey tool to better prepare departments to implement new programs by identifying what resources are needed to equip their faculty to be successful educators.

Acknowledgements

We would like to thank Dr. Lloyd Rieber from the Department of Workforce Education and Instructional Technology at the University of Georgia for his intellectual contributions to the article as well as his guidance on Q methodology.

Author Contributions

All authors listed have made substantial direct and intellectual contribution to the work and approved it for inclusion on this dissertation. Specifically, R.C.I. Maynard contributed to the experimental design, collected survey responses from faculty, analyzed the data, and summarized the findings in this report. S.V. Pennisi advised the research and completed the IRB approval process. L. Lombardini advised the research and funded the experiment.

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APPENDICES

APPENDIX A

SUPPLEMENTAL INFORMATION FOR CHAPTER 2

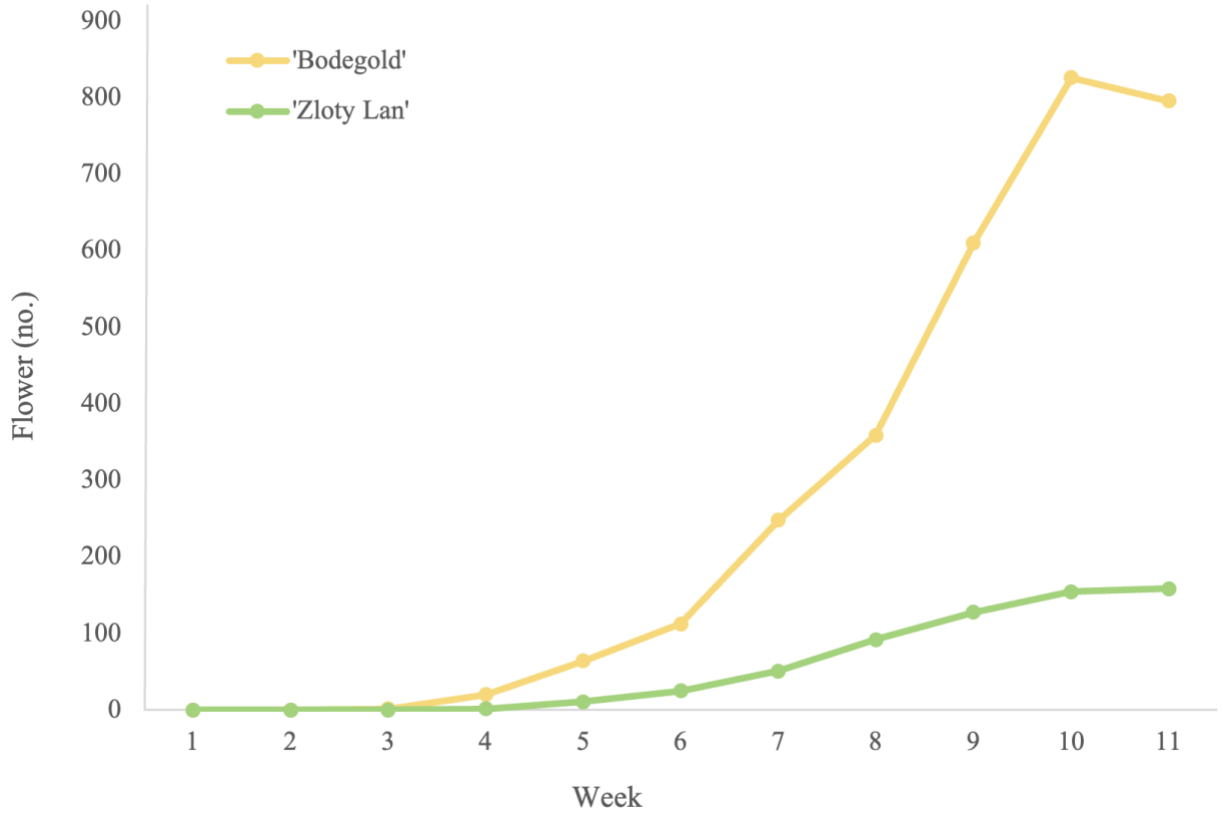


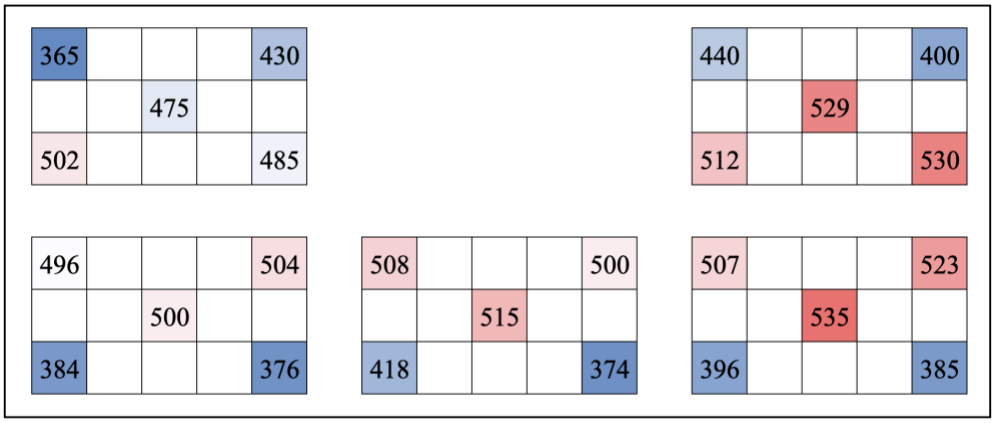
Fig. S.2.1: Number of chamomile (*Matricaria recutita)* flowers harvest each week after transplant to the vertical farm.

Table S.2.1: Dried biomass of shoot and root tissue in chamomile (*Matricaria recutita)* and parsley (*Petroselinum crispum*) cultivars with calculated percent of root biomass compared to the total biomass.

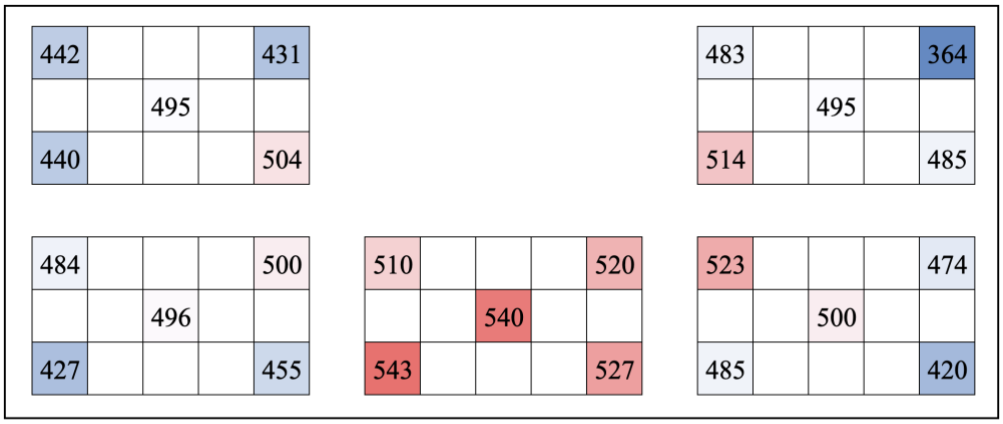
Cultivar	**Shoot biomass (g)**	**Root biomass (g)**	**Root %**
M. recutita 'Bodegold'	202.4	14.44	6.70%
M. recutita 'Zloty Lan'	100.44	9.22	8.40%
P. crispum 'Darki'	29.43	7.42	20.10%
P. crispum 'Giant of Italy'	84.26	20.53	19.60%
P. crispum 'Wega'	46.94	12.74	21.40%

APPENDIX B

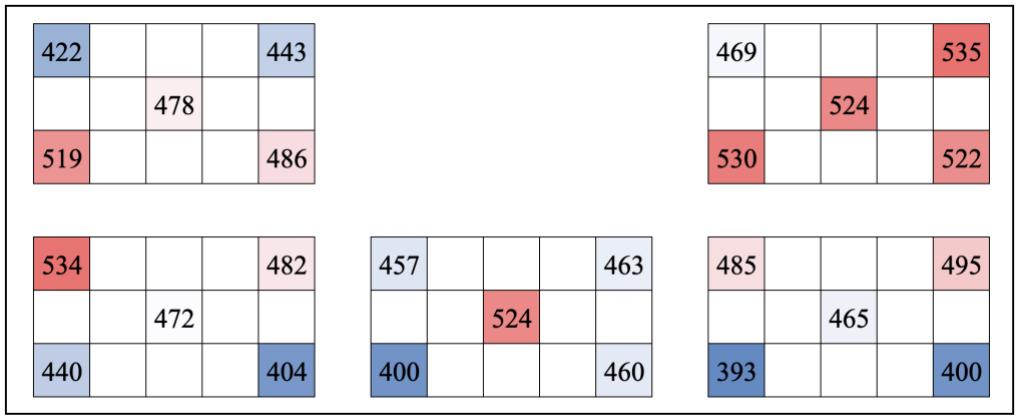
SUPPLEMENTAL INFORMATION FOR CHAPTER 3



Growth Chamber 1



Growth Chamber 2



Growth Chamber 3

Fig. S.3.1: Distribution and intensity in mW cm^{-2} of UV-B light measured at 310 nm across the three growth chambers.

APPENDIX C

SUPPLEMENTAL INFORMATION FOR CHAPTER 4

9	19	12	11	6
15	12	12	11	19
18	6	10	12	10
15	10	6	20	13

	Germination	
µmol L^{-1}	Mean	Percent
0	19.0	95%
50	12.3	64%
100	9.5	50%
150	10.0	53%
200	10.8	57%

Fig. S.4.1: Germination of parsley (*Petroselinum crispum*) seeds treated with 0, 50, 100, 150, or 200 µmol L^{-1} of oryzalin for 24 hours in a completely randomized design.

Rep 1	18	14	12	12	3	7	15
Rep 2	19	15	13	15	8	14	10
Rep 3	20	15	9	12	12	12	8
Rep 4	18	9	10	5	7	5	9

	Germination	
µmol L^{-1}	Mean	Percent
0	18.8	94%
25	13.3	66%
50	11.0	55%
100	11.0	55%
200	7.5	38%
400	9.5	48%
800	10.5	53%

Fig. S.4.2: Germination of parsley (*Petroselinum crispum*) seeds treated with 0, 25, 50, 100, 200, 400, or 800 µmol L^{-1} of oryzalin for 24 hours.

Rep 1

18	11	11
12	14	20
12	19	14
10	9	11

Rep 2

13	15	20
10	12	9
12	11	19
18	10	9

Rep 3

19	20	11
15	14	12
18	13	18
12	11	11

Rep 4

15	13	6
15	5	19
10	11	12
5	10	3

Exposure		Germination	
(µmol L^{-1})	(h)	Mean	Percent
0	24	16.5	83%
0	48	19.3	96%
0	72	17.8	89%
50	24	13.5	68%
50	48	12.3	61%
50	72	9.5	48%
100	24	12.5	63%
100	48	9.8	49%
100	72	9.3	46%
150	24	12.5	63%
150	48	11.0	55%
150	72	10.5	53%

Fig. S.4.3: Germination of parsley (*Petroselinum crispum*) seeds treated with 0, 50, 100, or 150 µmol L^{-1} of oryzalin for 24, 48, or 72 hours in a randomized complete block design.

APPENDIX D

SUPPLEMENTAL INFORMATION FOR CHAPTER 6

Table S.6.1: KASP markers designed with allele-specific forward primers and a common reverse primer to genotype the 23S76 segregating F_2 population.

Primer	SNP Position (Mb)	Sequence (5' → 3')
18EP83	SL4.0 ch02:41673863	**FAM**ACCAAAATATCAGCAACAGAAACCT**T**
18EP84		**HEX**ACCAAAATATCAGCAACAGAAACCT**C**
18EP85		CTTGGAGTTCTATTTGCATAATTGAATG
24EP67	SL4.0 ch02:43001395	**FAM**TCATCGGACGGGTTAGTAAC**G**
24EP68		**HEX**TCATCGGACGGGTTAGTAAC**A**
24EP69		TGTTGGCGTGCCAAGTCAAACTG
24EP52	SL4.0 ch02:45000039	**FAM**TTTATCAGTGGCCATATTTGCTGT**G**
24EP53		**HEX**TTTATCAGTGGCCATATTTGCTGT**C**
24EP54		AAAGAACACGCTGAACTCGAATTC
24EP70	SL4.0 ch02:46996095	**FAM**TCCAGAGGTCGATTCTCGATAC**A**
24EP71		**HEX**TCCAGAGGTCGATTCTCGATAC**T**
24EP72		ACTACTGTGGCCTCCCGGA
21EP240	SL4.0 ch02:49422957	**FAM**ATGCTGAAGATACAGCTATTCAACC**A**
21EP241		**HEX**TGCTGAAGATACAGCTATTCAACC**C**
21EP242		CTCTGCTGCCTTTTTAGCGATT
24EP76	SL4.0 ch03:50253031	**FAM**CGTGGCATCGTGAGTGTG**C**
24EP77		**HEX**CGTGGCATCGTGAGTGTG**T**
24EP78		TTTTGTGCCTGTATGGGTGGTATTATGTAC
18EP306	SL4.0 ch03:52233481	**FAM**TTGGTATTAATCAACAAAAGGTCACA**G**
18EP307		**HEX**TTGGTATTAATCAACAAAAGGTCACA**T**
18EP308		TGTATAACGACCGTAATCCTTTTAAGAAA
19EP593	SL4.0 ch03:54030362	**FAM**CACGACTAAGGAACCAATCTCATC**T**
19EP594		**HEX**ACGACTAAGGAACCAATCTCATC**C**
19EP595		CTCTGATGAATTTTCGGCTAACAC
18EP724	SL4.0 ch03:56245711	**FAM**AAGTTCATTCATTGCTGTAAGTAGG**C**
18EP725		**HEX**CAAGTTCATTCATTGCTGTAAGTAGG**T**
18EP726		CATTGTGTCTCTGAGCAGTGATGT
23EP366	SL4.0 ch03:56602634	**FAM**GTGCTGCCAAAGCTATGTATGAT**C**
23EP367		**HEX**GTGCTGCCAAAGCTATGTATGAT**T**
23EP368		TATTCCAAGTATCGAGCTGCTCC
19EP261	SL4.0 ch03:59187292	**FAM**AAGAAAAGCTTTCCAACTCACC**A**
19EP262		**HEX**AAGAAAAGCTTTCCAACTCACC**G**
19EP263		GCAGATCTGAAATTGGAGATGAGTT

24EP97	SL4.0 ch03:61109212	**FAM**ACTTGATGGATCTGAACGATGAA**G**
24EP98		**HEX**ACTTGATGGATCTGAACGATGAA**A**
24EP99		ACAAATGCCACAAGAGGGATCT
24EP121	SL4.0 ch03:64141817	**FAM**CCATAATCCGCACTTGACTC**G**
24EP122		**HEX**CCATAATCCGCACTTGACTC**A**
24EP123		GTGTGTGGCCCATTCAGGTAG

FAM sequence: GAAGGTGACCAAGTTCATGCT
HEX sequence: GAAGGTCGGAGTCAACGGAT

Table S.6.2: Additional primers used to screen the 24S54 F_2 population.

Primer	**SNP Position (Mb)**	**Sequence (5' → 3')**
24EP147	SL4.0 ch03:57844138	**FAM**GGATTGAGTCAGGACGATGAATTCCA**C**
24EP148		**HEX**GGATTGAGTCAGGACGATGAATTCCA**T**
24EP149		CGAACGAAAATGCAAGCACTGCTG
20EP694	SL4.0 ch03:58651039	**FAM**GTGACCTCCAAACATCTCCCA**C**
20EP695		**HEX**GTGACCTCCAAACATCTCCCA**T**
20EP696		CCCCCTAAACTCATTTTTGTGTAATTGT

FAM sequence: GAAGGTGACCAAGTTCATGCT
HEX sequence: GAAGGTCGGAGTCAACGGATT

Table S.6.3: Genotypic analysis of the 24S54 F_2 hybrids recombinant between 18EP724 (56.2 Mb) and 24EP97 (61.1 Mb).

		18EP306	19EP593	18EP724	24EP147	20EP694	19EP261	24EP97	24EP121
Accession	**CFSI**	**52233481**	**54030362**	**56245711**	**57844138**	**58651039**	**59187292**	**61109212**	**64141817**
24S54_022	6.52	H	H	H	LJ	LJ	LJ	H	H
24S54_073	2.44	H	H	H	SA	SA	SA	H	H
24S54_160	3.34	H	H	H	H	SA	SA	H	H
24S54_180	6.86	H	H	H	SA	SA	SA	H	H
24S54_006	NA	LJ	LJ	LJ	LJ	LJ	LJ	H	H
24S54_009	5.69	LJ	LJ	LJ	LJ	H	H	H	H
24S54_030	6.74	LJ	LJ	LJ	LJ	LJ	LJ	H	SA
24S54_031	7.30	LJ	LJ	LJ	H	H	H	H	H
24S54_051	5.65	H	H	LJ	H	H	H	H	H
24S54_055	6.46	LJ	LJ	LJ	LJ	H	H	H	SA
24S54_078	4.89	LJ	LJ	LJ	LJ	H	H	H	H
24S54_116	10.51	LJ	LJ	LJ	H	H	H	H	SA
24S54_121	4.88	LJ	LJ	LJ	H	H	H	H	H
24S54_147	5.59	H	H	LJ	LJ	LJ	LJ	H	H
24S54_168	5.13	LJ	LJ	LJ	H	H	H	H	LJ
24S54_190	4.05	LJ	LJ	LJ	LJ	LJ	LJ	H	SA
24S54_208	4.47	LJ	LJ	LJ	LJ	H	H	H	H
24S54_183	5.20	LJ	LJ	NA	LJ	LJ	H	H	H
24S54_186	2.71	LJ	LJ	NA	LJ	H	H	H	H
24S54_005	4.30	SA	SA	SA	SA	SA	H	H	H
24S54_011	4.90	SA	SA	SA	SA	SA	SA	H	H
24S54_015	7.37	H	H	SA	SA	H	H	H	H
24S54_025	6.20	SA	SA	SA	H	H	H	H	H

24S54_033	6.23	SA	SA	SA	H	H	H	H	H
24S54_061	5.51	SA	SA	SA	SA	SA	SA	H	H
24S54_079	4.05	SA	SA	SA	SA	H	H	H	H
24S54_080	2.91	SA	SA	SA	SA	SA	SA	H	H
24S54_089	4.41	SA	SA	SA	NA	SA	H	H	LJ
24S54_090	2.48	SA	SA	SA	NA	H	H	H	H
24S54_098	4.17	H	H	SA	SA	H	H	H	H
24S54_100	3.08	SA	SA	SA	SA	SA	SA	H	H
24S54_106	2.61	SA	SA	SA	SA	SA	SA	H	H
24S54_109	3.61	SA	SA	SA	H	H	H	H	H
24S54_112	4.48	SA	SA	SA	SA	SA	SA	H	H
24S54_114	5.29	SA	SA	SA	H	H	H	H	SA
24S54_118	2.97	H	SA	SA	SA	SA	SA	H	SA
24S54_127	3.92	SA	SA	SA	SA	H	H	H	H
24S54_129	6.63	SA	SA	SA	H	H	H	H	H
24S54_140	4.18	SA	NA	SA	SA	SA	SA	H	LJ
24S54_184	4.78	SA	SA	SA	SA	SA	H	H	H
24S54_204	5.03	SA	SA	SA	SA	H	H	H	LJ
24S54_209	4.41	SA	SA	SA	H	H	H	H	H
24S54_014	3.95	H	H	H	H	H	H	LJ	LJ
24S54_044	9.03	SA	SA	H	H	H	LJ	LJ	LJ
24S54_082	6.16	H	H	H	H	LJ	LJ	LJ	NA
24S54_095	6.34	NA	NA	H	H	LJ	LJ	LJ	LJ
24S54_113	6.04	H	H	H	LJ	LJ	LJ	LJ	LJ
24S54_132	4.03	H	H	H	H	LJ	LJ	LJ	H
24S54_158	5.43	H	H	H	H	H	H	LJ	H
24S54_166	5.65	H	H	H	H	H	LJ	LJ	H

24S54_169	5.71	H	H	H	H	H	H	LJ	LJ
24S54_175	5.70	H	H	H	H	LJ	LJ	LJ	H
24S54_178	4.11	SA	H	H	H	H	H	LJ	LJ
24S54_193	6.65	H	H	H	H	H	H	LJ	LJ
24S54_201	4.39	H	H	H	H	H	H	LJ	LJ
24S54_202	5.09	SA	SA	H	H	LJ	LJ	LJ	LJ
24S54_211	5.96	SA	SA	H	H	LJ	LJ	LJ	LJ
24S54_086	4.45	NA	NA	SA	SA	H	H	LJ	LJ
24S54_126	3.54	SA	SA	SA	SA	SA	SA	LJ	LJ
24S54_134	2.99	SA	NA	SA	SA	H	H	LJ	H
24S54_150	4.40	SA	SA	SA	H	H	H	LJ	LJ
24S54_164	5.88	SA	SA	SA	SA	H	H	LJ	LJ
24S54_173	3.98	SA	SA	SA	H	LJ	LJ	LJ	NA
24S54_004	3.08	H	H	H	H	SA	SA	SA	SA
24S54_010	4.46	H	H	H	SA	SA	SA	SA	SA
24S54_021	5.29	LJ	LJ	H	SA	SA	SA	SA	SA
24S54_062	2.66	H	H	H	SA	NA	SA	SA	SA
24S54_074	3.24	NA	H	H	H	SA	SA	SA	SA
24S54_075	3.55	H	H	H	H	H	SA	SA	SA
24S54_123	NA	H	H	H	H	H	H	SA	SA
24S54_131	3.69	NA	H	H	NA	H	SA	SA	SA
24S54_145	6.21	SA	SA	H	SA	SA	SA	SA	H
24S54_151	3.68	LJ	H	H	H	SA	SA	SA	SA
24S54_161	3.17	H	H	H	H	SA	SA	SA	SA
24S54_176	5.40	H	H	H	H	H	H	SA	NA
24S54_210	3.04	H	H	H	H	SA	SA	SA	H
24S54_212	7.86	H	H	H	SA	SA	SA	SA	H

24S54_213	NA	H	H	H	SA	SA	SA	SA	SA
24S54_115	5.71	LJ	LJ	LJ	NA	LJ	LJ	SA	SA
24S54_143	5.78	LJ	LJ	LJ	LJ	H	H	SA	SA
24S54_203	4.81	LJ	LJ	LJ	LJ	H	H	SA	SA

APPENDIX E

SUPPLEMENTAL INFORMATION FOR CHAPTER 7

Table S.7.1: A set of 34 neutral, subjective statements were compiled to address different opinions faculty may hold about the implementation of an online, non-thesis master's degree. These statements were the Q set used to survey faculty opinions in the Department of Horticulture at the University of Georgia. Faculty opinions on the implementation of an online, non-thesis master's in horticulture were grouped into three factors. Each factor corresponded to a composite Q sort, which is a weighted average of the opinions expressed in individual Q sorts. Statements from the Q set are listed below with their assigned value from the Q sort yielding the composite Q sort for Factors A, B, and C.

		Factors		
	Growth of the department	**A**	**B**	**C**
1	This degree would make our department more visible.	0	1	1
2	This degree would make our department more competitive in terms of attracting students.	-1	1	0
3	This degree would bring additional funding to our department.	-1	-1	3
4	Without a research component, more students may be interested in studying horticulture.	0	2	2
5	This degree would have a minimal impact on our graduate program.	-2	-1	2
6	There is a current need for this degree in our department.	-3	1	2
7	Online programs produce competitive professionals.	-2	0	0
8	Non-thesis programs produce competitive professionals.	-3	0	-1
	Quality of the degree			
9	This degree would be valuable to employers.	-2	1	1
10	This degree would be valuable for students.	0	0	0
11	This degree would prepare students to obtain a non-research industry position.	0	2	1
12	I would be willing to recommend a student from this program to an industry position.	-1	3	-3
13	This degree would maintain the value of our current graduate program.	-2	-2	-1
	Faculty responsibility			
14	This degree would increase the workload of faculty members.	4	1	3
15	This degree would have minimal benefit to me.	3	-4	0
16	Additional faculty should be hired to teach the online classes.	2	2	0
17	This degree should be a shared responsibility among all graduate faculty.	-1	-3	-2
18	Only faculty with teaching appointments should assist with this degree.	1	-4	-1
19	Additional staff should be hired to recruit students.	2	0	1

20	Additional staff should be hired to advise students.	-3	3	-3
	Implications for teaching			
21	Online classes would give greater flexibility in teaching.	1	-1	-3
22	In the long-run, online courses would require less preparation time.	-1	-3	-4
23	My teaching would be as effective online as it is face-to-face.	-4	-2	-4
24	I would be willing to develop an online course if I had technical assistance.	-4	-2	-1
25	Online classes require more resources to be engaging for students.	1	-1	0
26	I would be willing to learn more about online delivery options.	0	-2	-2
27	I would need significant training to teach an asynchronous online course.	3	-3	3
	Impact on the student			
28	This degree would be helpful for students with limited schedule flexibility.	3	3	-2
29	This degree would allow more students to obtain a graduate degree.	1	4	2
30	Students in an online program should have self-regulating study habits.	1	-1	4
31	Online programs should incorporate networking opportunities for students.	2	0	1
32	Students in an online program should participate in at least one in-person networking event with faculty.	0	0	-1
33	This degree would help us reach non-research-oriented students.	2	4	4
34	Student learning depends on level of engagement in online courses.	4	2	-2