PLANT GROWTH REGULATORS AND TRANSPLANT MANAGEMENT EFFECTS ON GERMINATION, ROOT TRAITS, YIELD AND QUALITY

A Thesis

by

MARIA ANDREA MACIAS LEON

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Chair of Committee,	Daniel I. Leskovar
Co-Chair of Committee,	Kevin Crosby
Committee Members,	Scott Finlayson
	Vijay Joshi
Head of Department,	Dan Lineberger

December 2017

Major Subject: Horticulture

ABSTRACT

Onions (*Allium cepa L*.) are known for their slow germination and relative growth rate, due to their unique germination mechanism. In addition, onions lack root hairs and only develop a few lateral roots.

A first study was conducted to evaluate onion seed germination and root growth traits of two cultivars Don Victor (yellow) and Lambada (red). Seeds were soaked in various hormonal solutions at low, medium, high and combination of concentrations of IAA, *trans*-zeatin, ethephon and ACC for ten hours; then dried under a laminar hood with lights on and continuous air flow for two days. Seeds treated with tZ had significantly increased root length (RL) as compared to control, while seeds with ethephon had reduced RL. Conversely, root surface area (RSA) increased with ethephon RL and significantly increased by the combination of ACC + tZ and ACC + tZ + Eth. The benefits of enhancing early seed germination and early root growth components in onion may improve stand establishment, water and nutrient uptake efficiency and ultimately increase in yield.

A second study was conducted to evaluate the effect of seedling density (direct seeding, DS; one plant per cell, T1; and three plants per cells, T3), planting date (Nov 14, early; Dec 8, mid; and Jan 9, late) and transplants growing environments (Alamo, TX and Ruskin, FL) in Uvalde, TX during 2016 – 2017 on three onion cultivars (Caramelo, yellow; Don Victor, yellow; and Lambada) DS treatments and early plantings required more days to reach maturity than mid and late plantings. However plant height, leaf number and fresh weight were greater in late-transplanted onions. Early planted transplants and DS resulted in higher yields compared to mid and late-season transplants. No significant differences in final yield were observed across the three plant density treatments. However, DS and T1 produced a higher numbers of more valuable jumbo and colossal bulb sizes compared to T3. Onion quality was only affected by cultivar, with cv. Lambada expressing the highest levels of total soluble solids, pyruvic acid and anthocyanins.

The technique of establishing onions from transplants produced from 1 plant per cell (1 seed per cell) or multiple plants (2 or more seeds per cell) transplants provides a practical and economical alternative to produce earlier crops reducing the time of exposure to biotic and abiotic stresses during stand establishment in field conditions.

DEDICATION

I dedicate all my efforts and accomplishments during my Master’s degree to my parents Alonso Macias and Berenice Leon, to my sister and brother Berenice and Alonso Macias who have always been there to applaud my success.

For their love and support, I will always be grateful.

ACKNOWLEDGEMENTS

I would like to express my most sincere gratitude to my committee chair, Dr. Leskovar to provide me the opportunity to work under his guidance. I appreciate your counsel, support and valuable inputs. I will always be grateful to have had the opportunity to learn from you. To my committee members, Dr. Crosby, Dr. Finlayson and Dr. Joshi, for their guidance and support throughout this research.

Thanks also go to my friends and colleagues and the department faculty and staff for making my time at Texas A&M University a great experience. Thanks to Naining Chi, Karla Arredondo, Ramon Gonzalez and Kuan Qin for making my college experience more enjoyable and pleasant. To my friends and family in Mexico my most sincere appreciation.

I extend my gratitude to the people at the Texas A&M Agrilife Research Center in Uvalde, TX. To Christine Thompson, Liza Silva, Lisa Samaripa, Megan Logan and Isabel Hernandez for yours continued efforts to cheer me up during my time spent in Uvalde. To Carrie Hensarling and Madhumita Joshi for your support during my long hours at the lab. Also, I would like to thank Manuel Figueroa, Michael Tidwell, Sixto Silva, Ben Puerta and Ray King for their constant help in the field. To Desire Djdonou for wiliness to share experiences and provide guidance, I am sure you will reach all the goals you have set.

To my boyfriend Matt Eager, for his love and kindness during working hours. Finally, thanks to my mother and father for their encouragement and to my brother and sister for their patience and love.

CONTRIBUTORS AND FUNDING SOURCES

This work was supervised by a thesis committee consisting of Dr. Daniel Leskovar (advisor), Dr. Crosby (co-advisor) and Dr. Joshi of the Department of Horticultural Sciences and Dr. Finlayson of the Department of Soil and Crop Sciences.

Thanks to Junho Yang graduate student at the Statistic Department for helping me to conduct the statistical analysis of my experiments.

This work was partially funded by a Cropping System Seed Grant, Texas A&M University. I also thank the Lamar Fleming scholarship for graduate studies support.

I extend my gratitude to the Speedling Company in Alamo, TX and Ruskin, FL and their staff for their collaboration and support during this research.

NOMENCLATURE

ABW	Average bulb weight
ACC	1-aminocyclopropane-1-carboxilic acid
LN	Average leaf number
AOA	Antioxidant activity
BD	Bulb diameter
BDW	Bulb dry weight
BFW	Bulb fresh dry weight
BL	Bulb length
BN	Bulb number
cv	Cultivar
DPPH	2,2-diphenyl-1-picrlhydrazyl
F – C	Folin – Ciocalteu
IAA	Indole-3-acetic acid
LDW	Leaf dry weight
LFW	Leaf fresh weight
MDG	Mean days of germination
ND	Neck diameter
P	Plant density
T1	One plant per cell
T3	Three plants per cell

PD	Planting date
PyA	Pyruvic acid
PH	Plant height
RD	Root diameter
RL	Root length
RSA	Root surface area
SD	Direct seeding
OC	Onion cultivar
TGP	Total germination percentage
TSS	Total soluble content
tZ	*trans*-Zeatin

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION

Onion (*Allium cepa*) is an important horticultural crop with a worldwide production of 84, 758,191 tons, with China, India, United States, Iran and Russia as the top 5 producers (FAOSTAT, 2014). In 2015, U.S. onion production was 3,369,000 tons grown on 61,326 hectares (USDA, 2016). The onion industry represents the fifth most valuable vegetable in the U.S. with a fresh-market value of $ 969 million (USDA, 2016).

Despite the high domestic production, the U.S. imported over 436,952 tons of onion in 2013 (FAOSTAT, 2014). The leading exporting country into the U.S. is Mexico, following by Peru, Canada, the Netherlands and China. In 2015, the U.S imported 12.7% of the Mexican fresh onion production which represents 73.4% of the total onion imports (Trademap, 2016).

In Texas, onion is a leading vegetable crop bringing $70 to $100 million annually. Growers plant short-day varieties from early October and harvest in May-June with a typical plant population ranging from 250,000 to 500,000 plants per hectare (Brewster, 2008). In general, high densities can result in higher total yield and early maturity, but smaller bulbs. Conversely, low densities result in lesser total yield and late maturity, but larger bulbs (Rubatzky and Yamaguchi, 1997).

The overall goal of this project is two-fold: 1) Improve onion seedling quality (emphasis on root growth) through screening and selection of exogenous phyto-hormones, and 2) Improve growth, yield and quality of onion transplants based on two nursery growing environments (Alamo, TX and Ruskin, FL) We expect the results of this research will provide farmers new production alternatives to reduce risks and production costs and to increase yield and profitability.

CHAPTER II

LITERATURE REVIEW

Plant Growth Regulator Effects on Seed Germination and Root Growth Traits

Auxin

In *Arabidopsis*, auxin can control root growth by modulating gibberellins (GAs) status in the plant. GAs promote root growth by antagonizing the effects of DELLA proteins. DELLA is a family of nuclear proteins identified as growth repressors. GAs have the ability to destabilize, reduce concentrations and prevent the production of DELLA proteins (Silverstone et al., 2001), inhibiting the detrimental effects on root growth. In roots, auxin is involved in lateral and adventitious root formation, and root apical dominance (Hodge et al., 2009).

In *Arabidopsis*, auxin accumulation in root pericycle cells triggers lateral root initiation by transforming these cells into lateral root founders (Dubrovsky et al., 2008), hence normal levels of endogenous auxins are necessary for lateral root initiation.

Indole-3-acetic acid (IAA), 1-naphthalene acetic acid (NAA) and tri-iodobenzoic acid (TIBA) inhibit primary root growth by reducing the length of the growth zone rather than reducing cell production rate. Other auxins such as 2-4-dichlorophenoxy acetic acid (2-4-D) and naphthylphthalamic acid (NPA) inhibit root growth by decreasing cell production rates (Rahman et al., 2007). Furthermore, in *Arabidopsis thaliana*, IAA at 30 nM and NAA at 100 nM applications displayed a cell length reduction proportional to root elongation inhibition; this means as cell length was reduced so was root length. Although auxins are known to constrain root growth, there is a lack of knowledge on the contributions of auxins to cell division and root elongation. Moreover, we have limited knowledge of the hormonal pathways modulating root length and development in specific crops, such as onions.

Cytokinins

It is well documented that cytokinins (CTKs) are involved in root to shoot signal communication, regulating many plant growth and development mechanisms such as apical dominance, the formation of apical shoot meristems, leaf senescence, nutrient mobilization, seed formation and pathogen responses (Werner et al., 2003). In lettuce (*Lactuca sativa* L.), cytokinins overcame the inhibitory effects of ABA promoting seed germination (Black et al., 1974). Moreover, exogenous applications of cytokinins reduced lateral root formation. Transgenic *Arabidopsis* plants with a reduced cytokinin synthesis exhibited an increase of root branching and promoted primary root growth (Werner et al., 2003). Lateral root spacing appears to be controlled by cytokinin gene – overexpression; mutations of CYP735A genes required for *trans*-zeatin biosynthesis results in strong defects of lateral root positioning (Chang et al., 2015). Regarding primary root growth, etiolated pea seedlings displayed inhibition of primary root elongation and lateral root initiation when benzylaminopurine (BAP) and *trans*-zeatin at 0.01μM were applied (Bertell and Eliasson 1992). BAP treated plants appeared to cause a moderate increase in ethylene production, which could explain the effects of cytokinins effects on root inhibition.

Ethylene and ACC

Ethylene (C_2H_4) has the simplest biochemical structure among plant hormones. Despite this, it influences a wide range of plant activities (Arteca and Arteca, 2008). There are many reports of how ethylene affects seed germination; however it is not yet clearly understood. Some researchers presume ethylene is necessary for seed germination whereas others claim that ethylene is produced as a result of seed germination (Matilla, 2000; Petruzzelli et al., 2000). Asano et al. (1999) and Borghetti et al. (2002) suggested ethylene can remove seed dormancy by regulating the expression of cysteine-proteinase genes, triggering seed protein degradation during the first germination stages.

Furthermore, ethylene represents an important signal in root growth and development. In *Zea maize,* ACC (ethylene precursor) and ethylene production have been associated with root growth inhibition (Sarquis et al., 1991) by preventing root cell expansion (Swarup et al., 2007). Conversely, ethylene is involved in lateral root formation. In rice, ethylene is known to be the hormonal signal in adventitious roots emergence under flooding conditions (Mergemann and Sauter, 2000). Ethylene promotes epidermal cell death facilitating root emergence (Mergemann and Sauter, 2000). The formation of a secondary root system could determine the plant's ability to survive.

Ethylene is implicated as a positive regulator of root hair development. *Arabidopsis* seedlings treated with ACC at 5 μM displayed a significant root hair elongation when compared to untreated seedlings. However, it can be argued that root hair promotion is due to a plant stress response (Pitts et al., 1998).

Stand Establishment Methods

Generally, onion is established by direct seeding, which is the least costly method of stand establishment (Rubatzky and Yamaguchi, 1997). Onion germination has a relatively different mechanism compared with most vegetable seeds. In these species, the cotyledon drives the embryonic root - shoot axes out of the seed, except the tip of the cotyledon, which remains in the seed continuing to absorb nutrients from the endosperm. Eventually, the tip of the cotyledon comes up, free from the seed coat. Because of this unique developmental mechanism, soil crusting is usually a problem for emergence, especially for seeds lacking vigor (Rubatzky and Yamaguchi, 1997). The slow germination and low relative growth rate cause onions to be easily outcompeted by weeds resulting in less homogenous populations and reduced bulb size (Brewster, 2008). Also, seedling growth is delayed by deficit or excess water availability, the presence of soil-borne diseases such as pink root (*Phoma terrestris* E.M. Hans), or seedling diseases such as Fusarium basal plate rot (*Fusarium oxysporum* Schlechtend.:Fr.F.sp.cepae (H.N.Hans) W.C. Syder & H.N. Hans), *Pythium* spp. and *Rhizoctonia solani* Kühn (Mohan and Howard, 1995).

Another propagation technique to establish the crop uses onion sets, which consists of a small propagule (less than 25 mm diameter), with a greater reserve of stored assimilates compared with seeds. This high storage reserve leads to a rapid plant development as compared to direct seeding, needing a shorter growing cycle of about 90 to 110 days after planting, compared to 150 days with transplants. Sets are usually prepared in spring for planting late in the fall (Corgan and Kedar, 1990). However, some drawbacks for sets include the presence of double bulbs or multiple centers as set size increases; susceptibility to bolting by exposure to long periods of cold temperatures (7ºC to 12ºC); soil-borne diseases and insect proliferation such as white rot (*Sclerotium cepivorum*) and bulb eelworm (*Ditlenchus dipsaci*). In addition, sets require storage, increasing the total cost of production (Brewster, 2008).

Alternative methods of establishment include bare-root transplants and containerized transplants. These are commonly used to reduce abiotic and biotic stresses, shorten the growing cycle and produce earlier and higher yields (Leskovar and Vavrina, 1999). Bare-root transplants can be produced in the field, glasshouses or shelter plastic tunnels. For that system, onion seeds are sown at a rate of 4,500 seeds m^{-2} in rows 7 to 10 cm apart and at 3 to 12 mm depth (Brewster, 1990). On the other hand, containerized transplants are produced in polystyrene or plastic trays molded into small cells, able to contain from 4 to 7.1 cm^3 of medium (Leskovar et al., 2002). Transplants are usually suitable for use after 8 to 12 weeks of growth when stem diameter reaches 3-4 mm (Brewster, 2008; Rubatzky and Yamaguchi, 1997). Compared with direct seeding, transplanting usually has a lower irrigation demand, herbicide control and pesticide inputs (Leskovar et al., 2002). However, the use of transplants is not a practice followed in all onion production regions due to the required use of multiple-cell tray containers and considerably higher costs than direct seeding (Leskovar et al., 2002). In Georgia, the estimated transplant cost of production and setting is about $1,370 per hectare (Boyhan et al, 2009). Multi-seeding of individual cells has been used as an alternative to providing a greater number of seedlings per unit area, thus reducing the cost of production (Rubatzky and Yamaguchi, 1997; Leskovar et al. 2004).

Factors Affecting Yield and Bulb Size Distribution

Transplant Quality

Containerized grown onion transplants have the potential to reduce post-planting stress, by providing an immediate, complete stand. A high-quality transplant can produce greater and more uniform bulbs at an earlier maturity as compared to direct seeding (Leskovar and Vavrina, 1999). Onion transplants grew under low light conditions (PAR < 800 mmol m^{-2} s^{-1}) and low temperatures in the UK showed a negative correlation between transplant size and time to onset of bulbing and maturity and positive correlation to bulb weight. On the contrary, transplants grown under high light intensities (PAR > 1500 mmol m^{-2} s^{-1}) and high temperatures in Sri Lanka showed a positive correlation between transplant size and onset of bulbing and maturity (Mettananda and Fordham, 1999). Therefore manipulation of transplant size during the nursery stage could optimize the time of bulbing and final bulb size. With favorable light and temperature conditions, small transplants should bulb earlier; whereas, it has been reported that large onion transplants should mature later and increase bulb size. Plant size at transplanting time can affect onion development and quality throughout the growing season until harvest (Gray and Steckel, 1993). Weather, different nursery conditions and locations influence plant size and subsequent will affect bulb quality and size.

Brewster (1990) reported that the most favorable temperatures for growing onion transplants are 15 ºC to 18ºC and 10ºC day and night respectively. Herison et al. (1993) also recommended 18ºC as mean day temperature, but suggested a night temperature of 13ºC instead. Since environmental factors and management such as daylength, temperature, nutrition and spacing can control onion bulbing (Currah and Proctor, 1990). Expanded understanding of how these factors impact bulbing could help growers manipulate final yield and bulb size distribution.

Seedling Density

Cultural practices such as plant density and arrangement influence plant shape, size and yield (Stoffella, 1996). Plant density is one of the most important factors for growth and development of onions (Weerasinghe and Fordham, 1993). A previous report by Herison et al. (1993) indicated that growing two plants per cell could optimize yields for large bulb size (> 76 cm). In a study conducted by Leskovar et al. (2004a) direct seeding showed a higher bulbing rate when compared to transplants.

In the same study, Leskovar et al. (2004) compared the effect of different establishment techniques in onion yield production including direct seeded, bare-root transplants and tray densities with one, two, and three seedlings in 228 cells per tray, separated and without separation at transplanting. They found that total yields were not statistically different among different densities in containerized transplants and bare-root transplants, but showed higher yields compared to direct seeding in the 60 – 70 mm bulb diameter category. For bulb size, two and three seeds per cell were the best in the 50 – 70 mm diameter category. At the nursery, planting multiple seeds in individual cells can therefore be an alternative and cost efficient system for onion production compared to direct seeding.

Planting Time

Onion bulbing is influenced by day-length and planting date. Planting date determines bulb size for short-day onions, which also influences plant survival, growth and yield. Previous research showed late plantings during fall usually result in a reduction of plant survival and yield (Allen et al., 1978; Fuqua, 1975) . Others observed that planting dates are critical for superior foliage development and bulb size (Gaskell, 1998). Bolting and seed germination are dependent on temperature (Corgan et al., 2000). An experiment conducted with short and intermediate-day onions in Queensland, Australia (lat. 27°35'S, long. 152°23'E) showed, April plantings producing the greatest yield as compared to February – March / May – June (Jackson et al., 1997)
Multiple centers arise from axillary buds development between leaves at the base of the plate, resulting in bulb splitting. The development of multiple centers is mainly controlled by genetics, however, it has been proven that environmental factors and any kind of stress could contribute to this phenomenon (Longbrake et al., 1987). Double bulb incidence can be reduced with later sowing or transplanting. In Georgia (lat. 32°11'N, long. 82°17'W) multiple centers were prevented by sowing the first week of October rather than the last week of September and planting the first week of December rather than November (Boyhan et al., 2009).

Planting date also modifies bulb shape. In Weslaco, TX (26.15°N, 97.9°W), elongated bulbs were produced when intermediate-day varieties were transplanted early

November and mid-December. Short-day types were unaffected by planting dates, but significant differences were observed in bulb sizes (Longbrake, 1972). It has been suggested that a combination of planting dates and seedling densities should be used to determine the mean shape in onion breeding lines (Dowker and Fennell, 1974).

Onion Quality

Onions differ in bulb shape, skin color (white, yellow and red) and pungency (from mild and sweet to very pungent), bulb storage life and dry matter content (Brewster, 1994). Onions have many health benefits and are a great source of various phytonutrients such as anthocyanins, phenolic acids, flavonoids, thiosulfates and cepaenes (Fossen and Andersen, 2003). Flavonoids continue to attract attention due to their potential implication in cardiovascular diseases, inflammation and cancer (Middleton et al., 2000). Flavonols are the major flavonoids of outer pigmented scales in onion. There have been described at least 25 flavonols from onion bulbs including, quercetin, isorhamnetin and kaempferol. It is well known that phytonutrient content varies among onion cultivars (Slimestad et al., 2007; Lee et al., 2015).

Pyruvic Acid

Onion flavor chemistry is complex, and difficult to measure. Onion pungency is produced by the release of the enzyme alliinase (EC 4.4.1.4) when cells are disrupted. Alliinase reacts with flavor precursors S-alk(en)yl-cysteine sulfoxide to produce pyruvic acid, ammonia and S-volatile compounds (Lancaster and Boland, 1990). Therefore, pyruvic acid concentration in onion juice (μM/mL) is used as an indicator of onion pungency (Schwimmer and Weston, 1961). Wall and Corgan (1992) obtained a 0.95

correlation between taste perception of pungency and pyruvic acid concentration. Onion pungency can be divided into three groups according to the pyruvic acid concentration: mild onions, 2 to 4 μM, intermediate, 8 to 10 μM, and strong those above 15 μM (Schwimmer and Weston, 1961).

Onion pungency intensity and flavor have been attributed to genetic and environmental factors. The concentration of flavor precursors also differs by cultivars and allium species. S—methyl-1cysteine sulfoxide (MeCSO) is a major precursor in chive and Chinese chive (0.68 – 1.85 mg g^{-1} fresh wt.); while for garlic and giant garlic the major precursor is S-allyl-L-cysteine sulfoxide (AICSO) (3.2 – 9.8 mg g^{-1} fresh wt.); for onion, leek and green onions S-propenyl-L-cysteine sulfoxide (PeCSO) has been reported as the major flavor precursor (0.3 – 2.2 mg g^{-1} fresh wt. Previous studies reported that pungency is greatly affected by cultivar, soil type and environmental factors (Yoo and Pike, 1998). In an early study, Yoo et al. (2006) reported that among 15 onion cultivars grown in three locations in the Rio Grande Valley areas including Weslaco (lat. 26°15’N, long. 97°98’W) Edinburg (lat. 26°35’N, long. 98°25’W) and Donna (lat. 26°20’N long. 98°25’) 80% of differences in pungency were attributed to genetic factors rather than environmental effects. Platenius and Knott (1941) compared 16 onion varieties grown in Winter Haven, Texas (lat. 28°62’N, long. 99°85’W) and Ithaca, New York (lat. 42°26’N, long. 76°29’W and reported that onions grown at 20 to 27 ºC were three times more pungent compared to onions grown at 15 ºC.

Anthocyanin

The flavonoid group includes compounds such as anthocyanins, flavones, isoflavones, flavanones and catechins contributing to fruit and vegetable antioxidant capacity (Cao et al., 1997). In plant tissue, anthocyanins produce red, blue and purple colors. In onions, the red color is attributed to anthocyanins consisting mainly of cyanidin and peonidin glucosides (Mazza and Miniati, 1993). The anthocyanin content contributes to approximately 10% of the total flavonoid content of the fresh weight in onions (Slimestad et al., 2007) and is also known to be a source of antioxidant activity (Geetha et al., 2016). Pérez-Gregorio et al. (2010) suggested red onion consumption due to high levels of flavonols and anthocyanins compared to white and yellow varieties.

Antioxidant Capacity

Reactive oxygen species (ROS) are produced in cells by cellular metabolism and other environmental factors. The overproduction of ROS could lead to several diseases due to damage in nucleic acids, proteins, lipids and enzymes molecules (Stajner and Varga, 2003). Onions are known for having a high antioxidant activity (AOA), therefore onions are associated with a reduction of numerous human diseases (Sanderson et al., 1999)

Methodologies have been developed to measure AOA in plant extracts. The Folin-Ciocalteu (F-C) method is extensively used due to practicality and convenience, although this method was developed to measured phenolic content in protein, this assay can also be used to estimated AOA (Prior et al., 2005). Another well-known assay is the 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH color declines over time while reducing certain compounds, therefore two hours incubation in darkness is recommended to obtain accurate results (Yoo et al., 2011).

CHAPTER III

EFFECTS OF PLANT GROWTH REGULATORS ON SEED GERMINATION AND ROOT GROWTH TRAITS OF RED AND YELLOW ONION*

Introduction

Onion field production is subject to a wide range of challenges. Onions are easily outcompeted by weeds due to slow germination and relative growth rate, which results in bulb size reduction and less homogenous populations (Brewster, 2008). In addition, the un-branched root system with few lateral roots and absence of root hairs (Kamula et al., 1994) leads to inefficient nutrient uptake, which may result in nutrient deficiencies (Sullivan et al., 2001). Improving seed germination and root architecture may help to overcome nutrient and water limitations, and mitigate abiotic stresses.

Endogenous plant growth regulators (PGRs) control germination, seedling growth and root development (Locke et al., 2000). Priming treatments with plant growth regulators have proved to enhance the performance of vegetable crops. A usual priming response is the increase of emergence rate over a range of environments and temperatures, resulting in better crop stand and higher yields (Halmer, 2004).

*Reprinted with permission from "Plant growth regulator effects on germination and root traits of 'Lambada' and 'Don Victor' onion cultivars" by Maria A. Macias-Leon and Daniel I. Leskovar, in press. HortScience, Copyright 2017 by the American Society for Horticultural Science.

Auxin is known to be involved in germination and root development processes. During seed imbibition, the free natural auxin, indole-3-acetic acid (IAA), accumulates before the initiation of root elongation (Kucera et al., 2005). In beans, IAA synthesis increases during germination (Bialek et al., 1992). In addition, auxins play an essential role in promoting lateral roots by stimulating pericycle cells within elongating primary

roots to enter de-novo organogenesis, leading to the establishment of new lateral root meristems (Ivanchenko et al., 2008).

Cytokinins (CTKs) are also present in developing seeds, and are recognized for stimulating cell division, and promoting lateral bud growth and seed germination in certain crops (Wareing, 2016). In species, such as *Orobanche* and *Striga*, CTKs appear to contribute to breaking dormancy by promoting ethylene release (Kucera et al., 2005). Substantial quantities of CTKs are sourced from root tips, which are then distributed to leaves and the rest of the plant (Wareing, 2016). Moreover, both, auxins and CTKs are responsible for root gravitropism (Aloni et al., 2006).

The ethylene precursor, 1-aminocyclopropane-1-carboxilic acid (ACC), and ethylene releasing substance, 2-chloroethylphosphonic acid (ethephon, Eth), are involved in breaking seed dormancy (Kolářová et al., 2010; Shinohara et al., 2016) and in increasing the number and length of root hairs in Brassica species (Hasegawa et al., 2003) and globe artichoke (Hasegawa et al., 2003; Shinohara et al., 2016).

Whether onion root development and seed germination can be modulated by PGRs is unknown. Thus, there is a need to understand better how exogenous hormones, individually or in combination, affect root initiation and architecture, as well as the development of primary, lateral, and adventitious roots, and root hairs. The objective of this study was to evaluate the effect of selected plant growth regulators, applied singly or in combination, on seed germination and root traits of yellow and red onion seedlings.

Materials and Methods

Study 1. Effects of PGRs on Seed Germination and Root Growth at Low, Medium and High Concentrations

Seeds of onion (*Allium cepa*) cultivars, cvs. Don Victor and Lambada (Nunhems USA Inc, Parma, Idaho) were used as experimental materials. Don Victor is a gold-yellow, globe shaped cultivar with a growing season of 170 -175 days, and Lambada is an early maturing red, round shaped cultivar, with a growing season between 160-165 days. Both cultivars are widely used for bulb production across the southwest United States and Mexico. Five grams of dry seeds were soaked in 30 mL each of the following hormone solutions 1, 3, and 10 μM IAA (a.i. 98.0% indole-3-acetic acid, Sigma-Aldrich®, Milwaukee, WI), 1, 3, and 10 μM trans-Zeatin (a.i. 97.0% trans-Zeatin Sigma-Aldrich®, Milwaukee, WI); 10, 30, and 100 μM of ACC (a.i. 98.0% 1-aminocyclopropane-1-carboxylic acid, Sigma-Aldrich®, Milwaukee, WI) and 10, 30, and 100 μM of ethephon (FLOREL®, a.i. 3.9% 2-chloroethylphosphonic acid; Lawn and Garden Products, INC., Fresno, CA); a combination of 10 μM IAA and 100 μM ethephon; a combination of 1μM *trans*-Zeatin and 100 μM ACC (Kakei et al., 2015); and water (hydro-priming), for 10 hours. Dried seeds were also included as a control. After treatment, seeds were washed three times with distilled water and re-dried under a laminar hood, with lights on and a continuous air flow for 48 hours until the original weight was re-established (Irfan et al., 2005)

For germination evaluation, four Petri dishes containing 25 seeds per treatment were placed on one layer of germination paper imbibed with 3 to 5 mL of distilled water. Seeds were incubated at 20ºC in the dark for nine days. Germination was recorded every 12 hours to calculate mean germination time (MGT), according to the formula $\sum Dn / \sum n$ (Ellis and Roberts, 1981), where Dn is the number of newly germinated seeds on day D and n is the number of seeds. Total germination percentage (TGP) was calculated nine days after incubation as described in Duclos et al. (2013).

Root length (RL), root surface area (RSA) and root diameter (RD) were measured and recorded using WINRHIZO LA-1600 (Regent instruments Inc., Quebec, Canada) with a resolution of 400 dpi.

Study 2. Synergistic Effects of Plant Growth Regulators on Seed Germination and Root Traits

Six priming treatments including, 3 μM IAA and 250 μM ACC (IAA + ACC); 3 μM IAA, 250 μM ACC, 0.5 μM tZ and 20μM Eth (IAA + ACC + tZ + Eth); 250 μM ACC and 0.5μM tZ (ACC + tZ); 250 μM ACC, 0.5μM tZ and 20 μM Eth (ACC + tZ + Eth); hydro-priming (hydro); and control were used. Treatments were selected based on the results of study 1 and incorporated modifications based on the literature. Since study 1 showed that tZ reduced root lengths at higher concentrations (1 μM to 10 μM), levels were reduced by 50% compared to the lowest treatment. Ethephon treatments displayed a high root length reduction for concentrations above 30 μM; therefore, for study 2 the concentration was reduced to 20 μM. ACC treatments did not show any significant response in the range 10 to 30 μM, therefore, the concentration tested was 250 μM. Seeds were primed and evaluated as previously described.

Statistical Analysis

Both incubation studies were conducted using a completely randomized block design (RCBD) with four Petri dishes per PGR treatment. Each study was replicated in the growth chambers three times (cycles) according to the methodology described by Langhans and Tibbitts (1997). All treatments were analyzed by ANOVA using JMP13. Differences among treatments were compared by LSD at $\alpha = 0.05$. If the Shaphiro-Wilk test for normality was significant, data were transformed for analysis.

Results

Study 1. Effects of PGRs on Seed Germination and Root Growth at Low, Medium and High Concentrations

Total Germination Percentage (TGP)

TGP showed significant differences among cultivars ($P = 0.002$). Averaged across all treatments, the red onion cv. Lambada had a TGP of 96% compared to 94% for the yellow cv. Don Victor. There was not a significant interaction for TGP between cultivar and treatment ($P = 0.488$). PGR treatments showed highly significant differences ($P = 0.001$). Control seeds had the lowest TGP (92%), and 3 μM tZ had the highest TGP (97%). Other treatments did not provide significant differences when compared to the control (data not shown).

Mean Germination Time (MGT)

Mean germination times are presented in figure 1. Overall, cv. Don Victor showed a slower germination time (2.70 days) compared to cv. Lambada (2.35 days). MGT was significantly improved for all treatments compared to the control (P = < 0.001). Ethephon concentrations of 30 and 100 μM and the combination of 10 μM IAA and 100 μM ethephon significantly reduced MGT compared to the rest of the treatments, 2.25, 2.23 and 2.03 days, respectively. The rest of the treatments did not result in significant differences (Figure 1). There was not a significant interaction effect for MGT between treatment and cultivars.

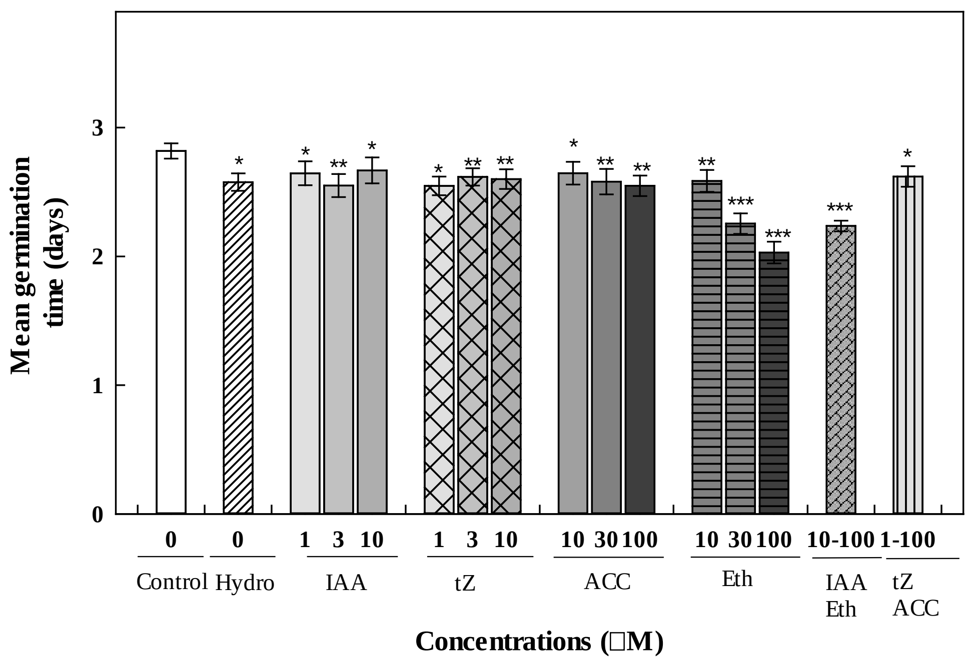

Figure 1. Mean germination time of onion seedlings in response to ethylene (Eth), trans-zeatin (tZ), ACC, IAA, tZ+ACC and Eth+IAA concentrations. Vertical bars indicate means ±SE (n = 25).
*,**,*** significantly different at *P* = 0.05, *P* = 0.01 and *P* =< 0.001 respectively compared to the control (LSD).
Since there were no significant interaction effects between cultivars (Don Victor, yellow; Lambada, red) and treatments; means displayed are averages of both cultivars.

Root Length (RL)

Root lengths are provided in Table 14. There were significant interactions for RL between treatment and cultivar *(P* = 0.006). The Lambada control RL was 2.61 cm and was significantly increased with hydro-priming, 10 μM IAA, all tZ (1 -10 μM) and ACC (10 -100 μM) concentrations. The highest RL increase was achieved with ACC at 100 μM (3.89 cm), a 49% increase as compared to the control. For cv. Don Victor, RL was also significantly increased with hydro-priming, and all tZ and ACC concentrations compared to the control (2.44 cm), with the highest RL at 1 μM tZ (3.52 cm). Both onion cultivars showed a significant root length reduction with Eth at 30 and 100 μM as compared to control.

Root Surface Area (RSA)

Averaged across all treatments, the cv. Lambada showed a slightly greater RSA (0.586 cm^2) compared to cv. Don Victor (0.557 cm^2). There was not interaction effect for RSA between cultivars and treatment. RSA with the different treatments are given in Figure 2. RSA was significantly increased by Eth concentrations (10 to 100 μM). The IAA + Eth treatment showed the greatest RSA increase (0.677 cm^2) compared to the control (0.542 cm^2). RSA was also increased by 1 and 3 μM IAA. Significant differences in RSA were not observed with tZ or ACC treatments.

Root Diameter (RD)

In general, cv. Lambada showed a higher RD than cv. Don Victor (Table 1). Lambada displayed an increase in RD when exposed to hydro-priming, IAA at 1 μM and 10 μM, and all tZ and ACC concentrations. The highest increase in cv. Lambada RD was observed with tZ at 3 μM (0.75 mm). RD for cv. Don Victor was also significantly increased by hydro-priming, but only by tZ at 3 and 10 μM, ACC at 30 and 100 μM, and tZ + ACC. The highest average root diameter was observed with tZ at 1 μM in cv. Don Victor (0.63 mm). IAA treatments in cv. Don Victor showed no significant responses compared to the control. RD was significantly reduced by Eth at 30 and 100 μM and IAA + Eth treatment in both cultivars.

Table 1. Root length and average root diameter of two onion cultivars (Don Victor, yellow; Lambada, red) obtained after nine days of incubation at different concentrations of IAA, tZ, ACC, Eth, IAA + Eth and tZ + ACC priming treatments.

Treatment	Concentration (μM)	Root length (cm)				Root diameter (mm)			
		'Don Victor'		'Lambada'		'Don Victor'		'Lambada'	
Control	0	2.44	Ad	2.61	Ade	0.45	Acd	0.48	Aef
Hydro-priming	0	3.23	Aab	3.58	Aabc	0.59	Bab	0.69	Aabc
IAA	1	2.56	Bcd	3.43	Acd	0.43	Bd	0.62	Acd
	3	2.38	Bd	3.07	Acd	0.39	Bd	0.56	Ade
	10	2.36	Bd	3.31	Abc	0.43	Bd	0.62	Acd
tZ	1	3.52	Aa	3.74	Aab	0.63	Aa	0.69	Aabc
	3	3.07	Bb	3.85	Aa	0.55	Bb	0.75	Aa
	10	2.93	Bbc	3.48	Aabc	0.53	Bb	0.68	Aabc
ACC	10	2.88	Bbc	3.39	Aabc	0.52	Bbc	0.65	Abc
	30	2.93	Bbc	3.84	Aa	0.53	Bb	0.74	Aab
	100	3.01	Bb	3.89	Aa	0.53	Bb	0.73	Aab
Eth	10	2.24	Ade	2.54	Ae	0.39	Bd	0.46	Af
	30	1.95	Aef	1.91	Af	0.31	Ae	0.30	Ag
	100	1.39	Ag	1.46	Af	0.21	Af	0.22	Ag
IAA + Eth	10 - 100	1.58	Afg	1.64	Af	0.23	Af	0.25	Ag
tZ + ACC	1 - 100	3.04	Bb	3.83	Aa	0.53	Bb	0.71	Aabc

Shown are the mean root length and the root diameter of three replicates, 4 subsamples with 25 seeds each. Results that are not significantly different at the $P = 0.05$ level of significance (determined by LSD) followed by the same letter. Upper caser letters represent multiple comparisons of the means to the effect between cultivars. Lower case letters represent multiple comparisons for the effect due to PGR treatments.

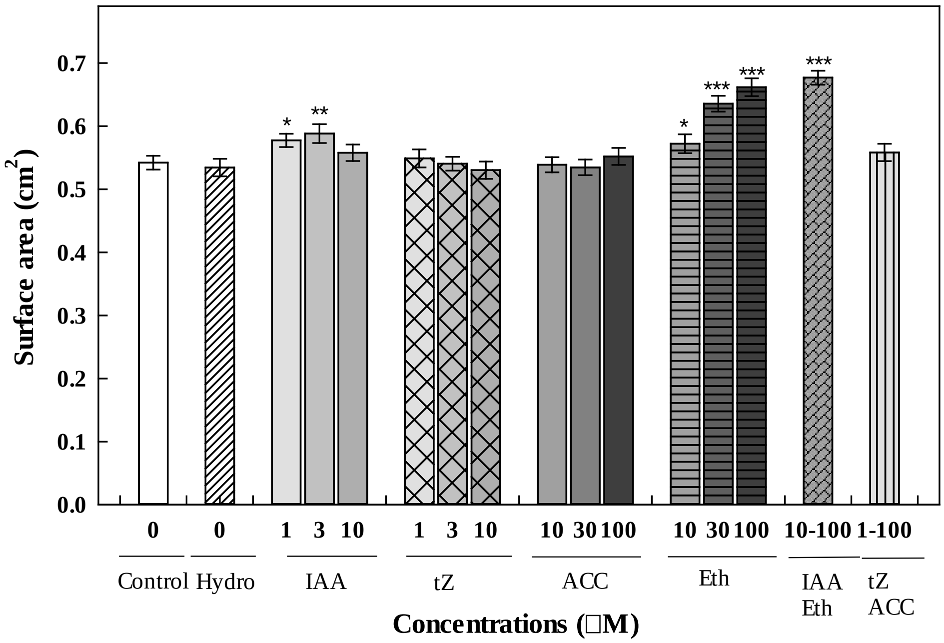

Figure 2. Surface area of onion seedlings in response to ethylene (Eth), trans-zeatin (tZ), ACC, IAA, tZ + ACC and Eth + IAA concentrations. Vertical bars indicate mean ±SE (n = 25).
*,**,*** significantly different at $P = 0.05$, $P = 0.01$ and $P =< 0.001$ respectively compared to the control (LSD).
Since there were no significant interaction effects between cultivars (Don Victor, yellow; Lambada, red) and treatments, means displayed are averages of both cultivars.

Study 2. Synergistic Effect of Plant Growth Regulators on Seed Germination and Root Traits

Total Germination Percentage (TGP)

The cv. Don Victor displayed a higher TGP (94.91%) compared to cv. Lambada (93.08%). All treatments showed a significant effect in increasing TGP (85.5 to 97%) compared to the control ($P = < 0.001$), but not when compared to hydro-priming.

Overall, the highest TGP was achieved with ACC + tZ + Eth (97.75%). There was no interaction effect between cultivar and treatment (*P* = 0.312).

Mean Germination Time (MGT)

The cv. Lambada displayed earlier mean germination time (2.97 days) compared to cv. Don Victor (3.16 days). MGT did not show an interaction effect between cultivar and treatments (*P* = 0.746). A significant decrease in the MGT was observed in all treatments (2.94 days) when compared to the control (3.38 days) with the fastest germination for the IAA + ACC + tZ + Eth treatment (data not shown).

Root Length (RL)

Root length (Figure 3) displayed an interaction effect between cultivar and treatment (*P* = 0.005). RL for cv. Don Victor was significantly increased by ACC + tZ and ACC + tZ + Eth, with 3.20 cm and 3.31 cm, respectively when compared to the control 2.41 cm RL. While the hydro-priming did not significantly increase root length in cv. Don Victor, there was a numerical increase when compared to the control. The cv. Lambada RL was significantly increased by all treatments, with IAA + ACC showing the highest increase at 3.44 cm compared to 2.02 cm for control.

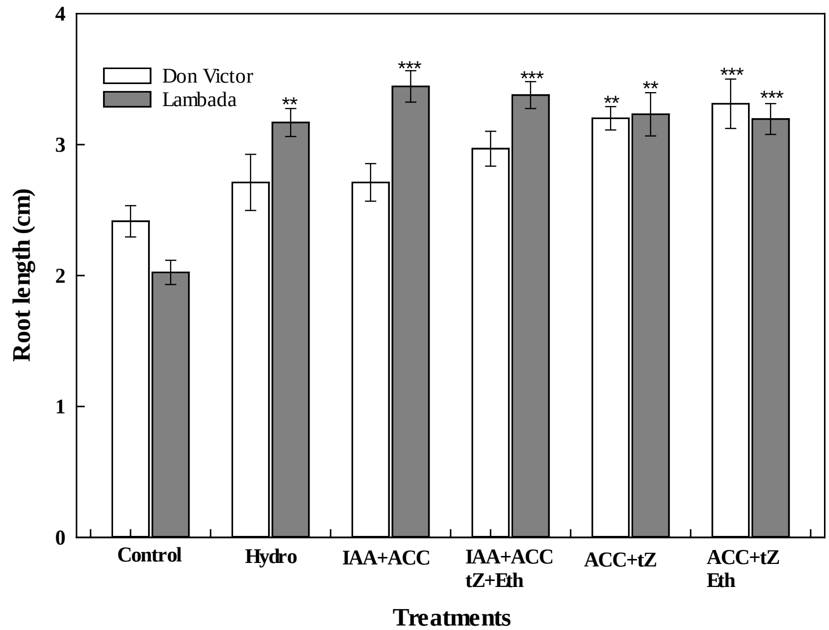

Figure 3. Root length of two onion cultivars (Don Victor, yellow; Lambada, red) in response to PGR applications. Vertical bars indicate means ±SE (n = 25). Hormonal concentrations: IAA 3μM; ACC 250 μM; tZ 0.5μM, and Eth 20μM.
*,**,*** significantly different at $P = 0.05$, $P = 0.01$ and $P =< 0.001$ respectively compared to the control (LSD).

Root Surface Area (RSA)

No significant differences in the root surface area were observed among the PGR treatments. Regarding cultivars, there was a slight significant difference with Don Victor having a higher RSA (0.533 cm^2) compared to cv. Lambada (0.483 cm^2).

Root Diameter (RD)

Root diameters are presented in Table 2. An interaction effect between cultivars and PGR treatments was observed in average root diameter ($P = 0.004$). The RD of Don Victor increased with ACC + tZ and ACC + tZ + Eth, while the other treatments showed no significant effect. Root diameter for the cv. Lambada increased with all treatments.

The highest increase was observed with IAA + ACC, with a RD of 0.703 mm, compared to 0.434 mm for control.

Table 2. Average root diameter of two onion cultivars (Don Victor, yellow; Lambada, red) obtained after nine days of incubation at different priming treatments: Control, Hydro-priming, IAA+ACC, IAA+ ACC + tZ + Eth, ACC + tZ and ACC + tZ + Eth.

Root parameter	Treatment	Onion cultivar			
		Don Victor		Lambada	
Diameter (mm)	Control	0.489	Ab	0.434	Ab
	Hydro-priming	0.509	Ab	0.665	Aa
	IAA + ACC	0.488	Bb	0.703	Aa
	IAA + ACC + tZ + Eth	0.560	Aab	0.682	Aa
	ACC + tZ	0.612	Aa	0.667	Aa
	ACC + tZ + Eth	0.621	Aa	0.676	Aa

Values are means of root diameter of two replicates, 4 subsamples with 25 seeds each. Results that are not significantly different at the $P = 0.05$ level of significance (determined by LSD) are followed by the same letter. Upper caser letters represent multiple comparisons of the means for cultivar effects. Lower case letters represent multiple comparisons for treatment means.
Hormonal concentrations: IAA 3μM; ACC 250 μM; tZ 0.05μM and Eth 20μM.

Discussion

Ethylene is known for inducing seed germination by promoting the rupturing of the testa and endosperm, while antagonistically interacting with inhibitory effects of ABA (Finch-Savage and Leubner-Metzger, 2006; Finkelstein et al., 2008). In *Arabidopsis* and *Lepidum sativum*, ethylene promotes cap and endosperm rupture by neutralizing ABA effects (Linkies et al., 2009). It is also well known that CTKs promote ethylene production, therefore, some of their effects might be mediated by ethylene (Stenlid, 1982). Nonetheless, explaining the crosstalk between ethylene and CTKs is complicated by the fact that ethylene is known to reduce endogenous auxin levels, while exogenous applications of CTKs increased IAA levels (Saleh, 1981). In pea plants, the ethylene precursor ACC has been shown to increase ethylene production in the radicle, and to promote radicle emergence (Petruzzelli et al., 2000; Petruzzelli et al., 2003)

The current results were partially consistent with the hypothesis that PGR priming treatments might improve germination percentage and timing. IAA concentrations did not affect total germination percentage, but increased the speed of germination compared to the control. On the other hand, tZ increased TGP but did not improve MGT. Ethephon at 30 and 100 μM increased MGT the greatest, but 100 μM reduced TGP. Finally, ACC priming treatments showed no significant effect in enhancing seed germination time or germination percentage. KeÇpczyński and KeÇpczyńska (1997) reported ACC effects on seed germination being less evident than ethylene, which could be attributed to due to the seeds inability to convert ACC to ethylene, an effect observed by Satoh and Esashi (1983) in dormant cocklebur seeds. Study 2 also suggested that PGR treatments increase the total germination percentage and decrease the mean germination time when compared to the control but not with respect to hydro-priming. In an early study by Brocklehurst and Dearman (1983), carrot, celery and onion displayed a MGT reduction when seeds were previously exposed to hydro-priming. Therefore, we cannot reject the assumption that the results obtained here could also be attributed to the benefits of hydro-priming itself and not to hormonal treatments.

According to Díez et al. (1970) root growth is characterized by: (i) lengthwise growth at different times and (ii) cell production and cell elongation as part of the growth components. Root growth is a consequence of cell division, taking place in the meristematic zone, and cell enlargement which takes place in the elongation zone.

Ethylene inhibited root elongation by decreasing root cell length at 2 to 4 mm from the root tip in maize (Whalen and Feldman, 1988). In *Arabidopsis thaliana*, Ivanchenko et al. (2008) also observed an inhibition of lateral root initiation and primary root growth and a decrease in elongation of the distal root zone when ethylene dose increased. Sunflower seedlings treated with ACC rapidly increased ethylene synthesis, inhibiting root elongation (Finlayson et al., 1996). Shinohara et al. (2016) observed no beneficial effects of ethephon, ACC or methionine on primary root length in globe artichoke. In study 1 ethephon promoted primary root inhibition (Table 1). However, there was no statistical evidence of root elongation inhibition when ACC, the ethylene precursor, was applied, which is contrary to what Finlayson et al. (1996) reported in sunflower. Conversely, study 2 displayed an increase in root elongation with IAA + ACC in cv. Lambada. This response could be attributed to the IAA counteracting the detrimental effects of ACC. In Arabidopsis, Negi et al. (2008) observed a similar response of IAA at 1 μM in a combination treatment with ACC at 1 μM, whereas auxin overcame the negative root growth inhibition due to ACC.

Auxin has been described as a positive regulator of lateral root initiation and root development at or above 0.1 μM (Poupart et al., 2005). IAA is known to regulate the development of primary and lateral roots (Casimiro et al., 2003; Taiz et al., 2010). In study 1, IAA applied at 10 μM to cv. Lambada (red type) increased RL compared to the control; however, cv. Don Victor (yellow type) showed neither an increase nor a reduction of primary root growth. In onions, the auxin 1-Naphthaleneacetic acid (NAA) applied at 0.01 μM promoted lateral root development but inhibited primary root elongation (Lloret and Pulgarín, 1992). In a study by Zhang et al. (2012) indole-3-butyric acid (IBA) and NAA promoted adventitious root development. IBA induced adventitious root formation and proliferation at optimum concentrations (1 to 2 mg/L, 4.9 to 9.8 μM), in *Periploca sepium*. Selected root explants were cultured on a medium supplemented with auxins (IBA, NAA). Zhang et al. (2012) observed a production of about 10 to 15 adventitious roots on every root explant when IBA was added, whereas, NAA addition only generated callus formation on the root explants. In our study, it may be possible that the lack of onion root responses to IAA concentrations might be due to their low sensitivity to this type of auxin.

Other work (Ivanchenko et al., 2008) suggests that ethylene and auxin interact to suppress lateral root initiation. Crosstalk between ethylene and auxin has been demonstrated but their interactions on root branching have yet not been described. In Arabidopsis, application of low levels of ACC, the ethylene precursor, promotes the initiation of lateral root primordia (Ivanchenko et al., 2008). Higher ACC concentrations inhibit the ability of pericycle cells to initiate new lateral root primordia, but promotes the emergence of existing lateral root primordia (Ivanchenko et al., 2008). Tanimoto (2005) observed an increase in root hair cells when *Arabidopsis* seedlings were sprayed with ACC. Even though, root hair development was not quantified in these studies, onion roots treated with Ethephon at 30 and 100 μM displayed a higher root surface area, which could be attributed to the increased root hair development (Figure 4).

However, it was not possible to validate if ethylene or ACC promoted lateral root development, since none of our treatments in study 1 or 2 affected lateral root initiation.

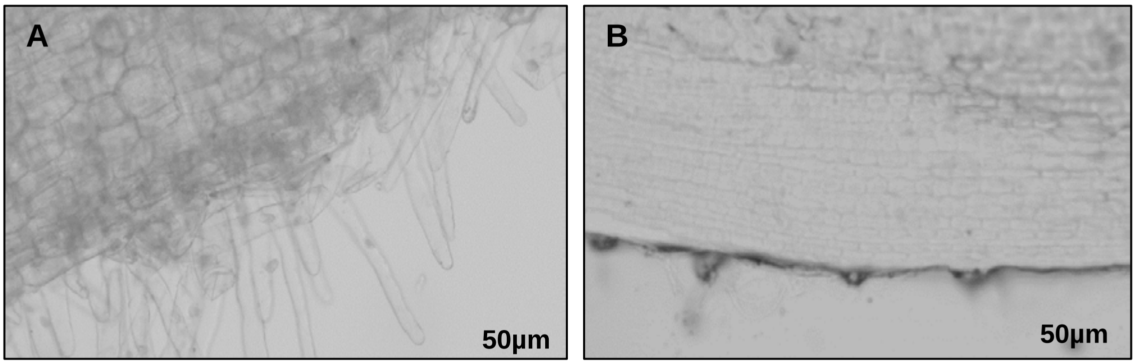

Figure 4. Onion root morphology in response to 100 μM ethephon (A) and control (B), 9 days after incubation.

Cytokinins are negative regulators of root growth and development (Werner et al., 2001). Bertell and Eliasson (1992) showed a significant root reduction in pea roots treated at 1 μM *trans*-zeatin, and as previously reported in maize roots (Bourquin and Pilet, 1990). However, our study in onion suggests an increase in primary root length at all tZ concentrations (1 to 10 μM) when compared to the control (Table 1). The highest RL increase was observed with *trans*-zeatin at 1 μM. There is evidence that lateral root formation is affected by the interaction between CTKs and auxins (Aloni et al., 2006). In our onion study, there was no indication of lateral or adventitious roots formation in any of the treatments; however, all tZ concentrations, and combinations with other hormones showed an increase in root diameter in both studies (Table 1, 2). It has been reported that exogenous applications of CTK increased root diameter by swelling the root elongation zone, which is due to the expansion of cortex cells and the formation of large intercellular spaces (Kappler and Kristen, 1986).

PGRs can positively regulate seed germination, root growth and development. From the findings of this research with PGRs in onion the following conclusions could be drawn: 1) ethephon proved beneficial to increasing onion root surface area at a moderate concentration but reduced root length, 2) addition of IAA could potentially control the reductions in root length and diameter in response to a single application of ethephon, 3) combining tZ and ACC increased root length, and 4) hydro-priming proved to be a simple and viable method to improve onion germination and root traits. There is a need for a better understanding of the hormonal pathways and molecular interactions underlying seed germination and root growth traits (Miransari and Smith, 2014). This is particularly important in onions since bulb development, uniformity and final size are greatly affected by the time spread of seedling emergence during early establishment (Benjamin, 1990).

CHAPTER IV

EVALUATION OF GROWTH, YIELD AND QUALITY OF ONION BASED ON SEEDLING DENSITY, PLANTING DATE AND NURSERY GROWING ENVIRONMENTS

Introduction

Onions are classified into short, intermediate and long day types. This refers to the minimum daylength required to bulb formation. Short day onions can initiate bulb development when daylength exceeds 11 to 12 hours, while intermediate onions need 13 to 14 hours and long day types more than 16 hours (Brewster, 1994). Overall, longer days enhance leaf growth and earlier bulb maturity. In the U.S. short day onions are planted in the field as seeds between early October to early November, and grown to maturity until March and May (Pike, 1986). Most onion varieties are limited by climatic adaptation. Temperatures between 21ºC and 27ºC contribute to bulb development when daylength conditions are favorable. Bulb formation will not be promoted at temperatures below 10º C, regardless of daylength (Brewster, 1977; Longbrake et al., 1987).

Onions are usually established by direct seeding. Nonetheless, onions established by seeds have a higher susceptibility to soil-borne diseases and stresses under extreme conditions, often reducing plant survival, yield and quality (Leskovar et al., 2004). In some regions, onions are produced from transplants grown in greenhouses or sets produced the previous year (Brewster, 1994).

Bulb size is an important trait in onion as it determines the size grade distribution and final market destination of the bulb. Several studies have been conducted to evaluate bulb size distribution in response to direct seeding and transplant establishment. In Sweden, onion transplants increased yield by 96% and improved bulb quality compared to direct seeding (Ascard and Fogelberg, 2008). Comparisons between direct seeded and transplants grown from one or three seeds per cell in 228 cell flats showed a greater percentage of large onion size (< 76 mm diameter) when onions are grown one seed per cell, compared to direct seeded, and three seeds per cell (Leskovar et al., 2004).

Brewster (1990) recommends early plantings to prevent bulb size reduction and “thick-necked” plants. In New Mexico, early September plantings usually result in high levels of bolting and low yields; mid to late September reduces bolting and increases yields; while mid-October produces no bolting, but reduces yields (Corgan and Kedar, 1990). Thus, determining optimal sowing and transplanting dates are critical to maximizing yield and bulb quality.

In Georgia, onions can be transplanted starting the first week of November until the last week of December and still obtained reasonable yield and quality. There, onions transplanted in December 2004 had a lower yield compared to onions transplanted in November 2004, but showed no differences from the onions transplanted in January 2005 (Boyhan et al., 2009). Similar results were observed in Egypt by El-Rehim et al. (1997), in where higher yields were obtained from transplanting in November rather than February.

Onion value is attributed to its unique pungent flavor and the ability to enhance other food flavors. The pungency is produced by the release of the enzyme allinase (EC 4..4.14) reacting with the flavor precursors S-alk(en)yl-cysteine sulfoxide when cells are disrupted (Lancaster and Boland, 1990). In addition, onions have many health benefits and are a great source of various phytonutrients such as anthocyanins, phenolic acids, flavonoids, thiosulfates and cepaenes which have a high level of antioxidant activity (Fossen and Andersen, 2003). Onions also have significant medicinal properties such as anticarcigenic, antiasthmatic, antibiotic effects, antithrombotic activity and antiplatelet effects (Griffiths et al., 2002). Antioxidants can inhibit oxidative reactions and help in functional performance of enzyme systems for self-defense mechanisms in cells (Lee et al., 2004).

Onion seedling density (number of plants per container cell), planting dates and cultivars can influence growth, yield, bulb size distribution and onion quality. However, to our knowledge there are not studies th**at** evaluated influence of the combination of these three factors on such parameters. This study aims to evaluate the effects of planting date and seedling density of three onion cultivars growing in two nursery environments on growth, yield and quality.

Materials and Methods

Plant Material

This experiment was conducted using three short-day cultivars Don Victor, Lambada and Caramelo (Nunhems, Germany). Don Victor is a gold-yellow globe shaped cultivar with a growing season of 170 - 175 days; Lambada is an early maturing red round shaped cultivar, with a growing season between 160 - 165 days; and Caramelo is a yellow granex cultivar with a growing season of 170 – 175 days. Onion cultivars were sown in Speedling trays containing 392 cells ($4.84cm^2$ x 6.35cm and $14.75cm^3$) with one plant per cell (T1) and three plants per cell (T3) on a Speedling tobacco peat lite mix media (Speedling, Sun City, FL) and covered with vermiculite grade 2-3-4 (W.R. Grace, Cambridge, MA) at Ruskin, Florida and Alamo, Texas nurseries. The sowing scheme is shown in Table 3. Seeding started 8 September 2016 at both locations, followed with second and third sowings 8 October and 8 November 2016, respectively. Onion seedlings were transplanted at three dates early (November 14), mid (December 8), and late (January 9) season.

Table 3. Seeding in Texas and Florida nurseries and planting schemes.

Cultivar	# Trays	# seeds/cell	Nursery sowing date	Field planting date
Caramelo	3	1	8-Sep	14-Nov
Caramelo	3	1	8-Oct	8-Dec
Caramelo	3	1	8-Nov	9-Jan
Caramelo	2	3	8-Sep	14-Nov
Caramelo	2	3	8-Oct	8-Dec
Caramelo	2	3	8-Nov	9-Jan
Lambada	3	1	8-Sep	14-Nov
Lambada	3	1	8-Oct	8-Dec
Lambada	3	1	8-Nov	9-Jan
Lambada	2	3	8-Sep	14-Nov
Lambada	2	3	8-Oct	8-Dec
Lambada	2	3	8-Nov	9-Jan
Don Victor	3	1	8-Sep	14-Nov
Don Victor	3	1	8-Oct	8-Dec
Don Victor	3	1	8-Nov	9-Jan

Don Victor	2	3	8-Sep	14-Nov
Don Victor	2	3	8-Oct	8-Dec
Don Victor	2	3	8-Nov	9-Jan

Experimental Location

Seedlings were manually transplanted into the field at the Texas AgriLife Research and Extension Center at Uvalde, Texas (29°12’57.6’’N, 99°45’21.6’’W) to assess the effect of seedling density, onion cultivar and planting date on transplants grown in two nurseries. The location has semi-arid to sub-humid conditions. During the study period, the total seasonal precipitation was 770 mm. Seasonal average temperature, relative humidity and daily light integral were 18.8 °C, 68.8%, 58.2 mol/m^2/day, respectively (Figure 5). Soil chemical properties are presented in Table 4.

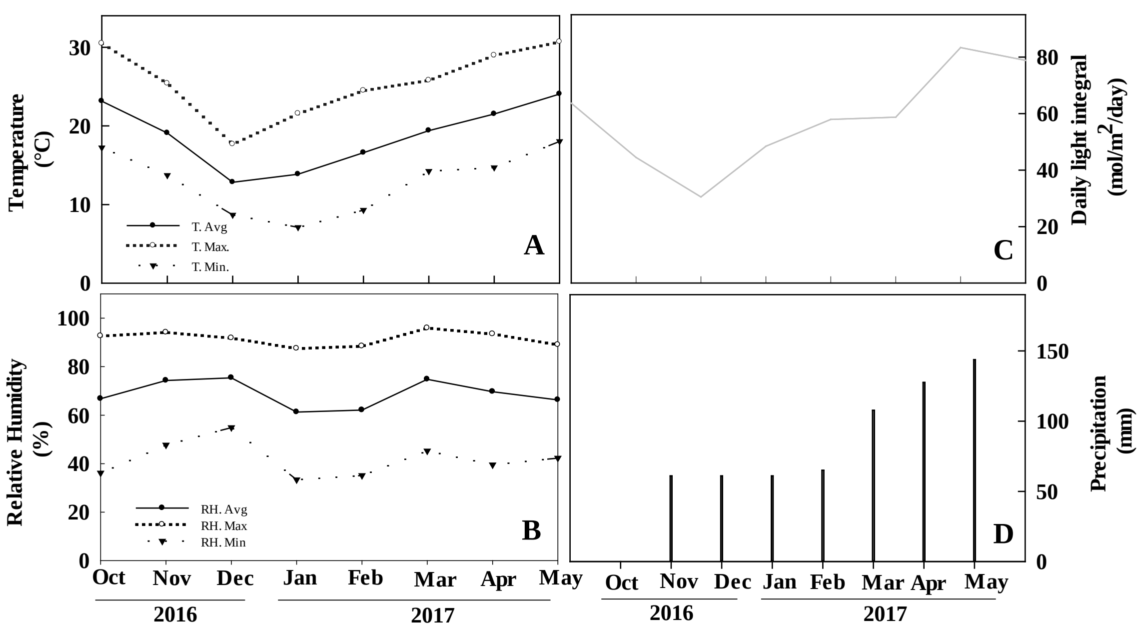

Figure 5. Seasonal growing conditions at Texas A&M Agrilife Research and Extension Center, Uvalde, TX. A) Maximum (T.max), minimum (T.min) and average (T.avg)

temperature; B) Maximum (RH.max), minimum (RH.min) and average (RH.avg) relative humidity; C) Daily light integral; and D) Cumulative precipitation.

Table 4. Soil physical and chemical properties at the Texas AgriLife Research and Extension Center, Uvalde, Texas.

Soil properties	Base analysis	Units
Clay	47.00	%
Sand	28.00	%
Silt	25.00	%
pH	8.10	-
Conductivity	532.00	umho/cm
Nitrate-N	19.50	mg/kg
Phosphorus	51.00	mg/kg
Potassium	770.50	mg/kg
Calcium	11710.75	mg/kg
Magnesium	290.25	mg/kg
Sulfur	11.00	mg/kg
Sodium	6.75	mg/kg
Iron	6.19	mg/kg
Zinc	0.30	mg/kg
Manganese	6.82	mg/kg
Cooper	0.55	mg/kg
Boron	0.75	mg/kg
Organic matter	3.56	%

Seedlings were planted on single raised beds 6 m long, with two beds per plot. Each bed was separated by 0.96 m centers and two rows per bed. Seedlings were placed 12 cm between row spacing and 10 cm within plants, with 120 plants per experimental unit (6m x 1m x 2 rows) and four replications. Additionally, direct seeded (DS) plots were planted on October 13, with the same bed layout but four instead of two rows per bed were used. Four lines per bed are considered a standard practice in Texas for direct seeding. Fertilization was provided before to transplanting at 56 kg/ha N and 84 kg/ha P_2O_5 by incorporating into beds. Total seasonal fertilization was 134 kg/ha N and 114 kg/ha P and 84 kg/ha K_2O.

Growth Parameters

Five plants were randomly selected per plot and plant height (PH), neck diameter (ND) and a number of visible green leaves (LN) were measured at 15, 90, 60 and 120 days after transplanting (DAT). Bulb length (BL), bulb diameter (BD), bulb fresh weight (BFW), leaf fresh weight (LFW), bulb dry weight (BDW) and leaf dry weight (LDW) were also measured at 60 and 120 DAT. Plants were harvested from 4.5 m plots when an average of 80% of the pseudostems bent over. At harvest, bulbs were sorted by diameter into classes: small (35 – 50 mm) medium (50 – 76 mm), large (76 – 102 mm) or jumbo (>102 mm).

After sorting, six onion bulbs per replicate were collected and individually measured for bulb diameter and height, and stored for chemical analysis for 1 to 2 weeks.

Shape Index (SI)

SI was calculated by the ratio of diameter to height to determine whether onions were flat (index > 1), globe (index = 1) or torpedo (index < 1) (Leskovar et al., 2004)

Pyruvic Acid (PyA) and Total Soluble Solids (TSS) Measurements

For the chemical analysis, first the outer onion scales, basal plate and the neck were removed. Then the central part of the onion was blended in a home mixer without adding water, and juice collected after filtering the puree through a filter paper no. 41 (GE Healthcare Life Sciences, WhatmanTM, UK). Then, the onion juice was frozen and kept at -20ºC until analysis (Yoo et al., 1995).

The pyruvic acid content of onion juice was measured by the automated method described by Yoo et al. (2011). Samples and reagents volumes were adjusted for the reaction to fit in a one mL vials. Ten microliters of undiluted onion juice were combined in cuvettes with 300 μL DNPH (125 mg/L in 2N HCl) solution using an auto dispenser EasyChem Plus (Chinchilla Scientific, Oak Brook, IL, U.S.A.). The mixture was incubated for 10 min, then 600 μL NaOH (1.0 N) was added and incubated for 51 seconds. Absorbance was read at 405 nm. Total pyruvic acid concentration was calculated using the standard curve for pyruvic acid in a range between 2 μmol/mL and 10 μmol/mL. Results were expressed as μmol of pyruvic acid equivalents (PAE) per mL (Lee et al., 2015).

A drop of the aliquot of the onion juice was used for sugar content analysis using a digital refractometer (Palette-PR32α, ATAGO, USA).

Anthocyanin Content

An aliquot of the onion juice prepared for the pyruvic acid measurement was used. Two hundred micro-liters of the juice was mixed with 2 mL of 1% HCl diluted in 100% methanol. The absorbance of the mixture was measured with a spectrophotometer at 530 nm. Total anthocyanin concentration was calculated by using the molar coefficient factor of cyanidin-3glucoside (anthocyanin extracted from *chrysanthemum*) of 29,600. Results were expressed as μg of cyanidin-glucoside equivalents per mL (Lee et al., 2015).

Antioxidant Capacity

Folin-Ciocalteu, (F-C) Assay

The F-C assay was modified in a 10:1 scale reduction. A 20μL of aliquot onion juice was mixed with 1.9 mL of F-C reagent for 5 min. Then, 400 μL of sodium carbonate (NA_2CO_3) (20g/100mL) was added, and the solution was mixed and incubated for two hours at room temperature (24º C). The absorbance of the mixture was measured with a spectrophotometer at 760 nm. Total phenolic content was calculated using the standard curve for gallic acid in a range between 100 μg/mL and 600 μg/mL. Results were expressed as μg of gallic acid equivalents (GAE) per mL (Lee et al., 2015).

DPPH Assay

The antioxidant activity (AOA) for the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was determined by the spectrophotometry method described by Lee et al. (2015) with some modifications. The reaction solution was prepared by dissolving 20 mg of DPPH in 1 L of methanol (100%). Then, 2 mL of the reaction solution was added to 20μL of onion juice and incubated for two hours in the dark. The absorbance of the mixture was measured with a spectrophotometer at 515 nm. The AOA was calculated using the standard curve in gallic acid in a range between 10 μg/mL and 60 μg/mL. Methanol 100% was used as a blank. Results were expressed as μg of gallic acid equivalents (GAE) per mL (Lee et al., 2015).

Statistical Analysis

A completely randomized block design with three factors (3 onion cultivars, OC; 3 plant densities, D; 3 planting dates, PD) and 4 replications was conducted using transplant from each growing location. All treatments and growth parameters were analyzed by ANOVA using JMP13 (SAS Institute, Cary, NC). Mean differences among treatments were compared by LSD at $\alpha = 0.05$.

Results and Discussion

Alamo, Texas

Growth Parameters

In Southern western region of the U.S. short-day onion cultivars have more potential to increase yield compared to intermediate and long day cultivars due to the extensive top growth before bulbing begins. Larger and more extensive tops are important in determining bulb sizes primarily in cultivars intended for medium, large and jumbo bulb production (Corgan, 1988). For this experiment plant foliage was measured during the growing season at 15, 60, 90 and 120 days after transplanting (DAT), time when bulb maturity was reached.

Plant size base on of leaf number, area and plant weight strongly affects the response to a bulbing stimulus. Early plant establishment will have a continued impact on plant growth and development throughout the season. Larger plants have an advantage over smaller plants of the same age in terms of yield, bulb size distribution and quality (Mettananda and Fordham, 1999).

Plant Height (PH)

Figure 6 depicts plant height growth in response to onion cultivar and plant density at three planting dates (early, mid and late). In early planting, all three cultivars overall had a greater PH with T1 and DS as compared to T3 during the growing season. In contrast, at mid and late transplanting, only DS showed a greater PH in all three cultivars. Overall, the highest plant height was observed in early transplants at 120DAT. The same tendency was observed in Egypt 2010 and 2011 by Kandil et al. (2013) in Early Grano and Giza cultivars, with early transplanted onions (November) displaying the greatest PH at 90 and 120 DAT. Moreover, in New Mexico, onions seeded early (September 9) were larger and had more leaves than plants seeded late (September 30). As the season progressed, late seeded plants became comparable in plant height to those planted early (Cramer, 2003). In this study, mid and late transplanted onions (T1 and T3) showed a PH reduction when reaching 90 and 60 DAT, respectively. Comparing cultivars, Caramelo and Don Victor displayed a greater PH than Lambada across plant densities and planting dates.

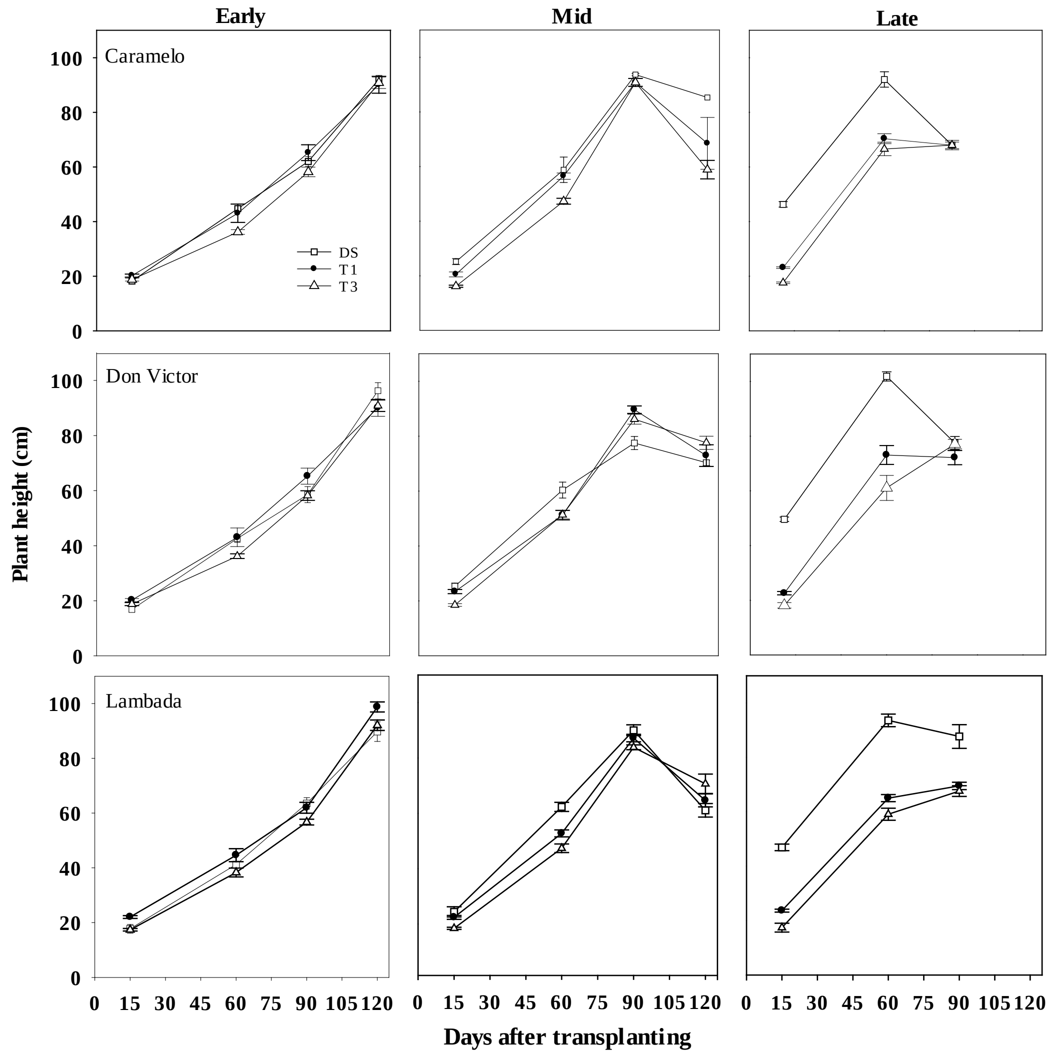

Figure 6. Plant height in response to onion cultivar (Caramelo, Don Victor and Lambada) and plant density (direct seeding, DS; one seed per cell, T1; and three seeds per cell, T3) at three planting dates (Early, Nov 14; mid, Dec; and late, Jan 9).

Neck Diameter (ND)

During early and mid-development (15 to 90 DAT), ND showed differences among density treatments in early planting; with thicker ND for DS and T1 as compared to T3 treatments for all the three cultivars. ND has been associated to bulb maturity; as bulbs get closer to harvest ND starts closing (Brewster, et al., 1987). In mid and plantings, ND reduction started when reaching 90 DAT. Our results suggested, mid and late plantings promoted ND closeness in a shorter period of time than DS and early transplants, reducing days to reach bulb maturity. Overall, the cv. Don Victor had the greatest ND among cultivars.

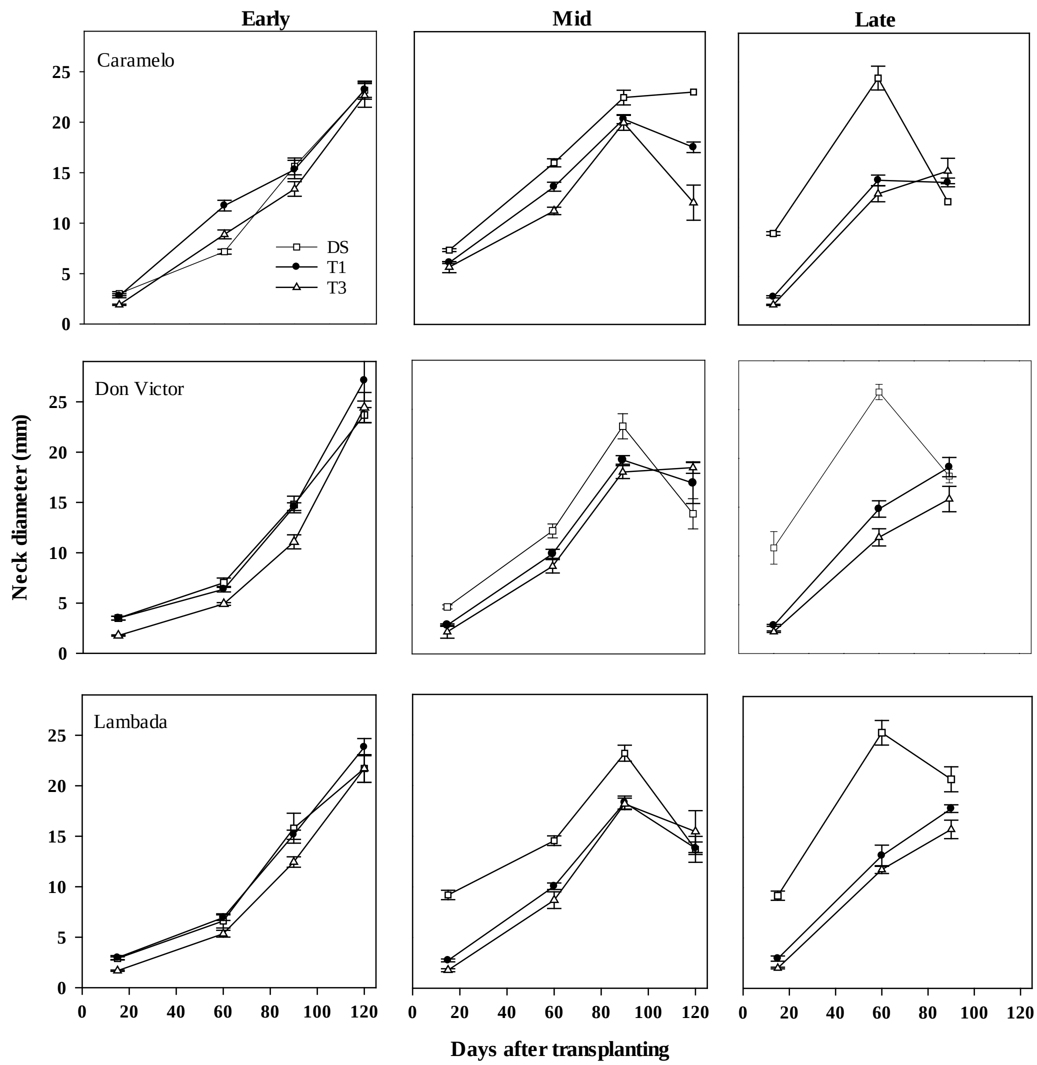

Figure 7. Neck diameter in response to onion cultivar (Caramelo, Don Victor and Lambada) and plant density (direct seeding, DS; one seed per cell, T1; and three seeds per cell, T3) at three planting dates (Early, Nov 14; mid, Dec; and late, Jan 9).

Average Leaf Number (LN)

In terms of LN during early growth (15 DAT) the cv. Caramelo had the greatest LN compared to cvs. Don Victor and Lambada ($P < 0.001$) with 2.89, 2.76 and 2.68 leaves respectively. During mid development (60DAT), DS and late transplanted onions displayed the highest LN. These results could be attributed to the temperature effect on seedling relative growth rate. Onion relative growth rate increases linearly over the range of 10 to 19ºC in the field and controlled environment conditions (Brewster, 1990). In our study, DS and late transplanted onions were exposed to higher temperatures during the early growth compared to early and mid-transplanted onions (Figure 5).

During mid growing season (60 to 90 DAT), DS and T1 had a greater LN in early plantings. An early study showed that early sown onion resulted in larger plants with more leaves compared to onions late seeded (Cramer, 2003). At reaching maturity the cv. Caramelo slightly reduced LN in T3 treatment, while the cv. Don Victor increased LN. Overall, the cv. Lambada displayed a reduction LN compared to cvs. Caramelo and Don Victor (Figure 8).

In an early onion study comparing growth of direct seeding vs. transplants, Leskovar et al. (2004) observed that LN was higher in direct seeded seedlings at early development compared to LN measured from transplants grown from one plant per cell or three plants per cell. It is well known that, leaf size, number and age influence bulb size and yield. Leaves are implicated in the photosynthetic process, light absorption and accumulation of sucrose in bulbs (Brewster, 1994). The lower number in LN in direct seeded plants might be the consequence of early bulb development, which in turn has a direct impact on competition with the renewal and maintenance of canopy growth. Plants of different sizes (LN, PH and plant weight) differed very little in their total number of leaves (Brewster, 1990). However, early seedling growth will determine growth rate and time to scale initiation, therefore is important an immediate and efficient stand establishment (Mettananda and Fordham, 1999).

Onions transplanted in January reached maturity at 113 DAT.

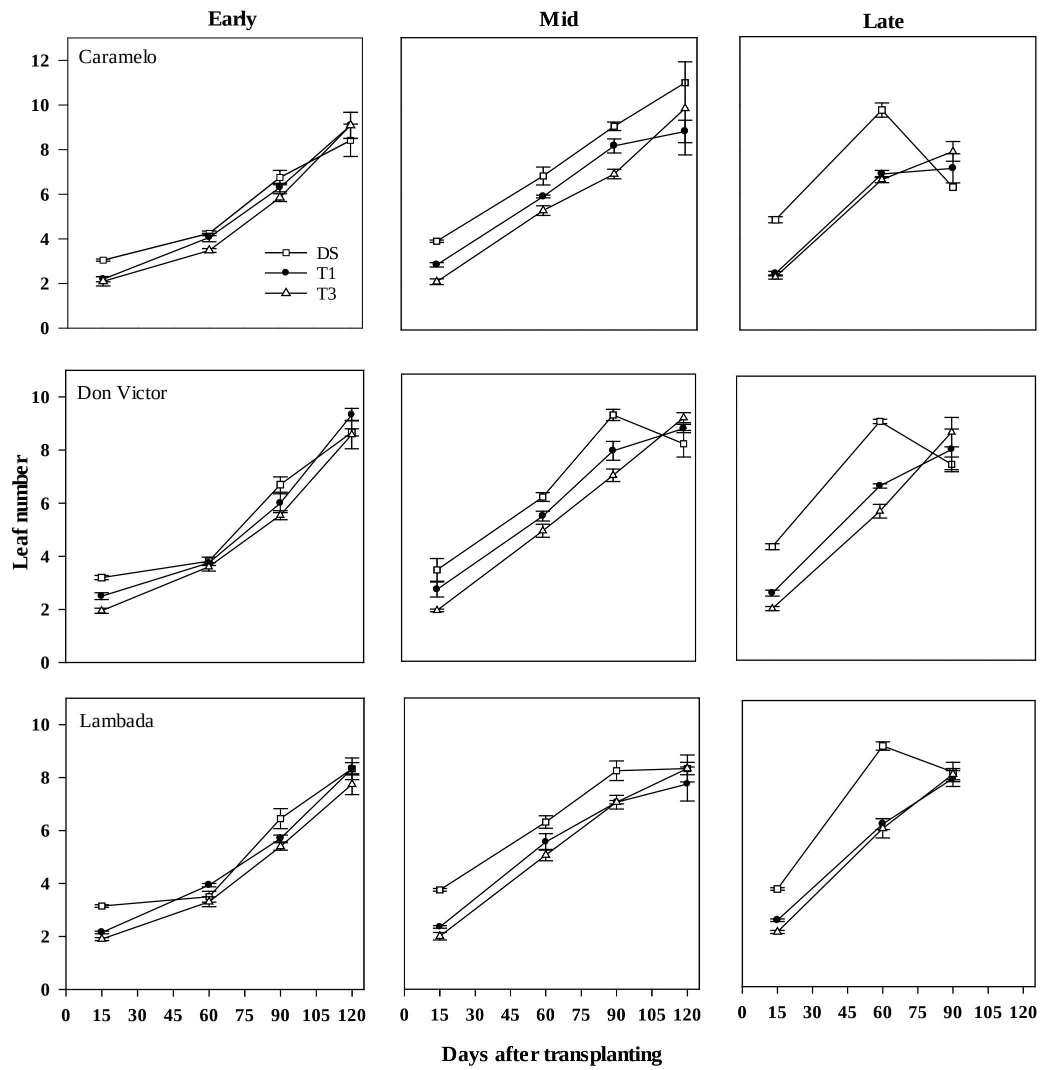

Figure 8. Leaf number in response to onion cultivar (Caramelo, Don Victor and Lambada) and plant density (direct seeding, DS; one seed per cell, T1; and three seeds per cell, T3) at three planting dates (Early, Nov 14; mid, Dec 8; and late, Jan 9).

Bulb Length (BL) and Diameter BD).

At 60 DAT, BL showed no significant differences across treatments. In contrast, BD showed a three-way cultivar × plant density × planting date interaction. The significant interaction showed no effects regardless of onion cultivars at early and mid-plantings. DS showed no significant differences compared to T1 in early planting (November). However, T3 had a significant BD reduction during the three planting dates across cultivars. In early plantings, T1 and T3 reduced BD by about 8 to 12% compared to DS; in mid planting the reduction was about 12 to 25%; and late plantings the reduction increased to25 to 60% in BD. As expected, the cv. Caramelo, granex type, had the greatest BD compared to cvs. Don Victor and Lambada (Figure 9).

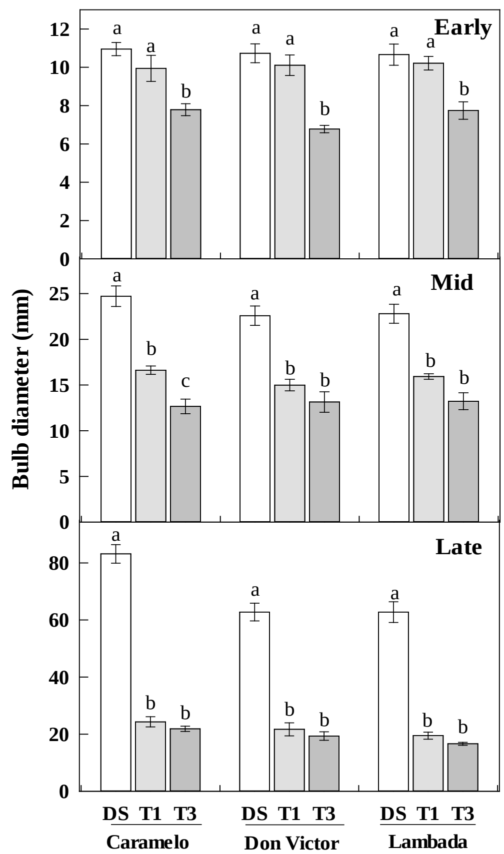

Figure 9. Bulb diameter in response to three plant densities, three onion cultivars, and three planting dates. Results that are significantly different at the P = 0.05 level of significance (determined by LSD) are identified with different letters. For treatments description refer to Figure 6.

Later during the growing season (120DAT) bulb length (BL) was significantly affected by planting date × onion cultivar interaction. The cv. Caramelo showed a significant BL reduction compared to the cv. Don Victor when early transplanted, response that was attributed to the granex phenotype of the cv. Caramelo. The highest BL in the two planting dates was observed in the cv. Don Victor (Table 5).

Table 5. Bulb length after 120 DAT. Shown are the means of three plant densities.

Planting date	Cultivar	Bulb length (mm)	
Early	Caramelo	52.1	Bb
	Don Victor	57.6	Ba
	Lambada	53.6	Bab
Mid	Caramelo	75.8	Aa
	Don Victor	82.5	Aa
	Lambada	67.7	Ab

Results that are significantly different at the $P = 0.05$ level of significance were determined by LSD. Upper caser letters represent multiple comparisons of the means for the effect between planting dates. Lower case letters represent multiple comparisons for the effect due to onion cultivar.

BD was significantly affected by cultivar and two-way interactions (PD*OC, PD*D). Overall, the cv. Caramelo had the greatest BD among cultivars in both planting dates (early and mid). In early planting, T1 displayed the greatest BD, while in December DS had biggest BD (Table 6).

Onions transplanted in January reached bulb maturity at 113 DAT.

Table 6. Bulb diameter after 120 DAT. Shown are the means of three plant densities (A) and three onion cultivars (B).

A)

Planting date	Cultivar	Bulb diameter (mm)	
Early	Caramelo	62.2	Ba
	Don Victor	47.1	Bb
	Lambada	48.2	Bb
Mid	Caramelo	85.1	Aa
	Don Victor	83	Aa
	Lambada	75.8	Ab

B)

Planting date	Plant density	Bulb diameter (mm)	
Early	Direct seeding	54.2	Ba
	1 plant/cell	56.6	Ba
	3 plants/cell	46.7	Bb
Mid	Direct seeding	89	Aa
	1 plant/cell	77.5	Ab
	3 plants/cell	77.4	Ab

Results that are significantly different at the P = 0.05 level of significance determined by LSD. Upper caser letters represent multiple comparisons of the mean to the effect between planting dates. Lower case letters represent multiple comparisons for the effect due plant density.

Leaf (LFW) and Bulb Fresh (BFW) and Leaf (LDW) and Bulb Dry Weight (BDW)

There was a significant onion cultivar × plant density × planting date interaction at 60 DAT for LFW and BFW (Figure 10). DS had the greatest LFW across planting dates and cultivars. Overall, the cv. Don Victor displayed the greatest LFW among planting dates. BFW displayed the same tendency as LFW for plant densities across planting dates. DS had the greatest BFW. The cv. Caramelo late transplanted had the greatest BFW, followed by Don Victor and Lambada. The lowest BFW was observed on onions transplanted November 14 (Figure 10). There is a proven correlation between LFW and BFW, in an experiment conducted in Sri Lanka (lat. 7ºN, long. 80ºE) 91% of the BFW was attributed to LN and LFW (Mettananda and Fordham, 1999). Our results suggested that the increase in BFW in DS treatments could also be associated with the high LFW observed at 60 DAT. The significant effect of plant density × planting date interaction for LDW suggested that late- transplanted onions rapidly accumulate higher dry matter than early and mid-transplanted onions response. The high radiation together with low high perception would explain higher dry matter accumulation in late transplants (Tei, et al., 1996). The accumulation of leaf fresh and dry weight in late transplants could be associated with LN superiority observed at 60 DAT (Figure 8). Moreover, the dry matter of a crop is the product of the radiation intercept, translated as the mean efficiency to convert intercepted radiation to dry matter (Brewster, 1990). Presuming, a non-linear photosynthetic response, then the distribution of light perception over a greater proportion of leaf area would be more efficient, therefore a higher dry matter accumulation (Aikman, 189). The onion cultivar × plant density × planting date interaction showed BDW was greater in DS onions compared to T1 and T3 transplanted late (Figure 11). The higher dry matter accumulation during early development in mid and late plantings was attributed to the exposure to higher temperature and longer days during early development compared to DS and early transplants. Moreover, in our study, the increase in LN at 60 DAT in late transplants is a reflection of the high solar irradiance intercepted by the leaf canopy that results in higher LDW and BDW. However, leaf production tends to be more energy demanding, in terms of higher protein content competing for the available resources against root and bulb growth (Penning De Vries et al, 1989), which later on it will be translated as bulb size reduction.

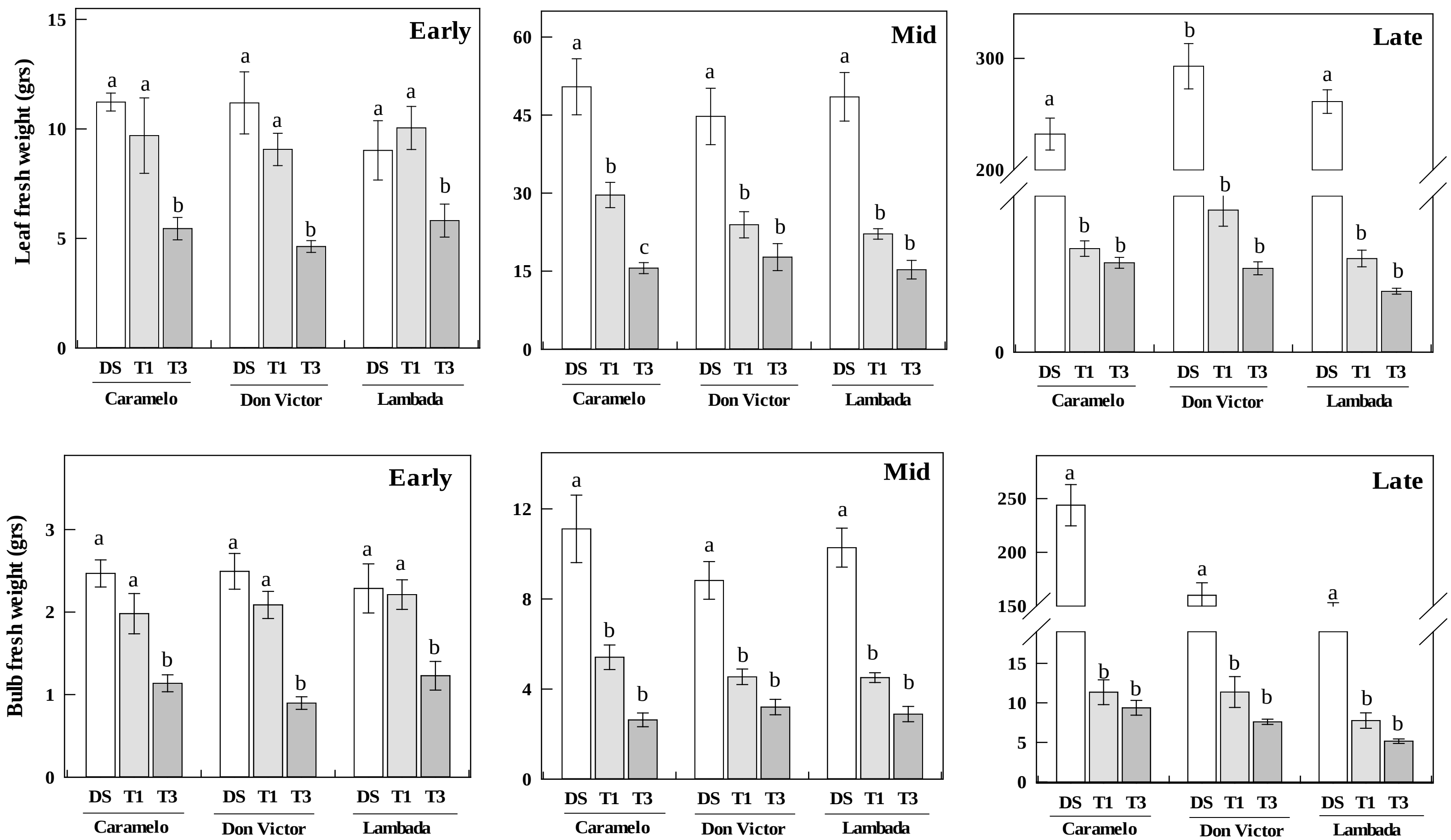

Figure 10. Leaf and bulb fresh weight in response to onion cultivar, plant density and planting date at 60 days after transplanting. Results that are significantly different at the $P = 0.05$ level of significance (determined by LSD) are identified with different letters. For treatments descriptions refer to Figure 6.

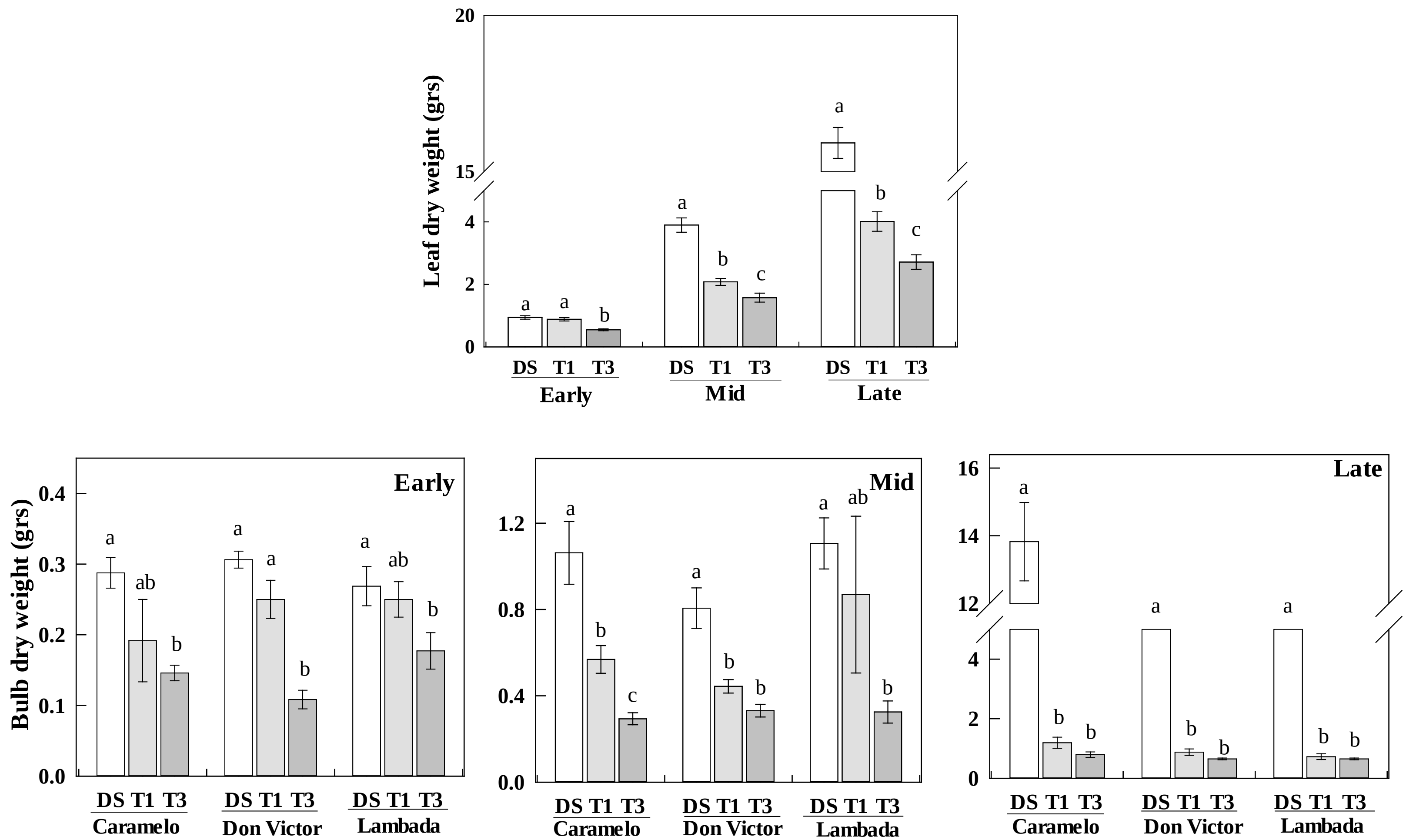

Figure 11. Leaf and bulb dry weight in response to onion cultivar, plant density and planting date at 60 days after transplanting. Results that are significantly different at the $P = 0.05$ level of significance (determined by LSD) are identified with different letters. For treatments descriptions refer to Figure 6.

At 120 DAT LFW was significantly affected by planting date × plant density interaction and onion cultivar. During early and mid-plantings, T1 had the highest LFW accumulation compared to DS and T3. Overall, during the three planting dates the cv. Caramelo had the highest LFW compared to cvs. Don Victor and Lambada. BFW was affected by planting date. Early transplanted onions displayed a higher BFW that those transplanted mid-season, again this can be attributed to the strong positive correlation between LFW and BDW ($R^2 = 0.91$) described by Mettananda and Fordham (1999). LDW showed no significant differences among treatments. BDW was significantly affected by two-way interactions (PD*P, PD*OC). Onions transplanted early showed a significant increase in dry matter accumulation with DS. While, T1 showed the greatest BDW in mid-planting. The cv. Caramelo displayed the greatest BDW when compared to the rest of the cultivars; the same response was observed in onions transplanted in December (Table 7).

Onions transplanted in January reached maturity at 113 DAT.

Table 7. Leaf fresh weight and bulb fresh and dry weight at 120 after transplanting.

Planting date	Plant density	Leaf fresh weight (grs)		Bulb dry weight (grs)		Cultivar	Bulb dry weight (grs)	
Early	Direct seeding	203.0	Aa	6.4	Ba	Caramelo	16.9	Aa
	1 plant/cell	211.5	Aa	5.9	Bab	Don Victor	15.3	Aab
	3 plants/cell	173.6	Ab	4.08	Bb	Lambada	13.2	Ab
Mid	Direct seeding	68.9	Ba	11.9	Ac	Caramelo	7	Ba
	1 plant/cell	77.8	Ba	18.9	Aa	Don Victor	4.2	Bab
	3 plants/cell	55.1	Ba	14.6	Ab	Lambada	5.2	Bb

Cultivar	Leaf fresh weight (grs)		Planting date	Bulb fresh weight (grs)	
Caramelo	148.8	a	Early	233.2	a
Don Victor	129.1	b	Mid	87.1	b
Lambada	117.0	b			

Results that are not significantly different were determined at the $P = 0.05$ level of significance by LSD. Upper caser letters represent multiple comparisons of the mean to the effect between planting dates. Lower case letters represent multiple comparisons for the effect due to plant density or onion cultivar.

Maturity

Onions from all the three planting dates (early, mid and late) were harvested when they reached physiological maturity. Direct seeded treatments were harvested 193 days after sowing (April 24); onions transplanted in November (Early) were harvested 176 DAT (April 24); those transplanted in December (Mid) were harvested 162 DAT (May 9); and those transplanted in January (Late) were harvested 130 DAT (May 19) even though maturity was reached at 113DAT, due to field conditions (Fig 12).

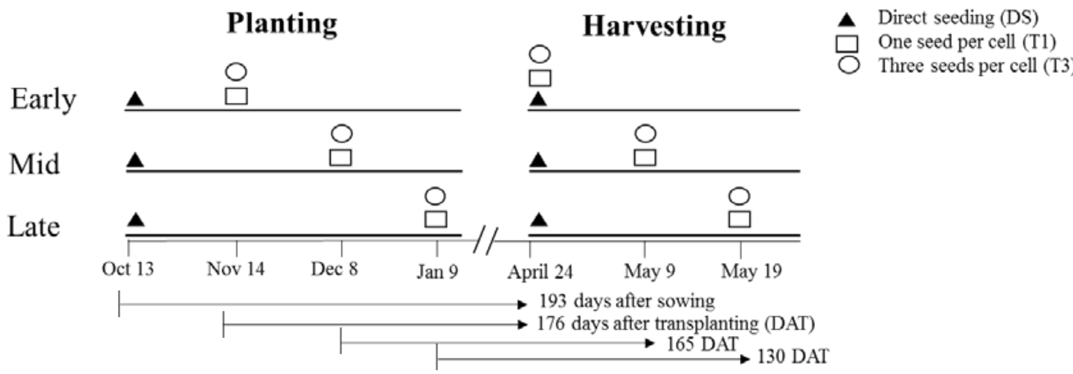

Figure 12. Scheme for planting and harvesting time for three onion cultivars in response to planting time and plant density.

A difference of approximately one month between direct seeding (October 13) and early planting (November 14) resulted in a 17-day difference to reach maturity, 31-day difference between mid-planting (December 8), and 63-day difference between to late planting (January 9).

Accordingly, early planting fits more closely the mature profile of cvs. Don Victor and Caramelo (170 to 175 days). On the other hand, the cv. Lambada reached its maturity profile when mid-transplanted.

Bulb initiation is mediated by photoperiod and daily temperatures (Brewster, 1990). The three cultivars used in this study showed an increase in days to maturity when transplanted early in the season. In Rio Grande City, TX Arce (1995) observed similar results, for three short-day onion cultivars. Onions planted on September 22 required more days to reach physiological maturity than those planted on October 12 and 29. Arce (1995) attributed early bulb setting to a rise in temperatures and daylength increase with the approaching of the spring season. Thus, intermediate and late plantings reached maturity sooner.

Yield

Total yield was affected by onion cultivar × plant density and planting date × plant density interaction. Average bulb weight (ABW) was affected by plant density × planting date. A total number of bulbs (BN) was significantly affected by on cultivar × planting date interaction.

Overall, the cv. Don Victor had the highest yield among all cultivars and planting dates with 58.9 tons/ha, followed by cvs. Caramelo with 53.8 tons/ha and Lambada with 37.5 tons/ha. Yield from onions transplanted early and mid – season showed no significant differences among plant densities; however, in late planting T1 and T3 had a significant yield reduction compared with DS. Onions transplanted on January 8 suffered freezing temperatures (Table 10). The onion capacity to survive the winter varies by cultivar and plant age. In New Mexico plants survived at -10ºC but died at -14ºC. The temperature for 50% killing was reported to be -10.5ºC. Onion freezing tolerance increases with timing; the longer the time they have been in the field, the higher the freezing tolerance (Peffley et al., 1981). In our study, transplants were exposed to two nights of freezing temperatures (-7°C) for an extended period of five hours immediately after transplanting decreasing survival by 30%.

Overall, the highest yield was obtained in early plantings. Similar results were observed in India by Patil et al. (2012) onions transplanted on November 15 displayed the maximum yield while the lowest yield was recorded in onion transplanted on January 15.

The cv. Don Victor showed the greatest ABW (401 grams) among the three planting dates and direct seeding, followed by cvs. Caramelo (299 grams) and Lambada (246 grams). T1 and T3 early-transplanted onions showed a greater ABW compared to DS. Contrary to the results obtained during early planting date, DS onions had a greater ABW than T1 and T3 in mid and late plantings (Table 8).

No differences were observed on BN for onions transplanted in early planting. For mid-season transplants cvs. Caramelo and Lambada had a greater BN compared to cv. Don Victor. Similar results were observed in onions transplanted late. Early and mid-plantings displayed a higher BN than late planting. Final yield results were attributed to both BN and ABW with significant correlations, R = 0.78 and R = 0.56, respectively as shown in Table 9.

Table 8. Alamo, TX transplants. Bulb number, bulb weight and total marketable yield in response to cultivar, plant density and planting date.

		Early (Nov 14)			**Mid (Dec 8)**			**Late (Jan 9)**		
Onion cultivar	**Plant density**	**Bulb number (No/Ha)**	**Bulb weight (grams)**	**Yield (tons/ha)**	**Bulb number (No/Ha)**	**Bulb weight (grams)**	**Yield (tons/ha)**	**Bulb number (No/Ha)**	**Bulb weight (grams)**	**Yield (tons/ha)**
	1 plant/cell	169,720	296	50.09	173,491	340	58.51	167,565	248	40.91
	3plants/cell	172,953	295	50.65	166,487	280	46.69	177,261	234	41.13
	1 plant/cell	155,172	379	59.02	161,245	410	65.37	178,340	283	50.51
	3plants/cell	178,340	350	62.33	156,789	366	57.45	126,616	281	35.04
	1 plant/cell	170,259	277	46.76	175,108	226	39.43	149,246	243	33.14
	3plants/cell	165,409	221	36.25	164,871	212	35.01	138,470	167	23.12

P values

	Bulb number	Bulb weight	Yield
Onion cultivar x plant density	0.001	0.608	0.002
Onion cultivar x planting date	0.585	0.070	0.399
Plant density x planting date	0.282	0.005	0.004
Onion cultivar x plant density x planting date	0.288	0.161	0.183

Table 9. Alamo, TX transplants. Correlations between bulb number (No/Ha), average bulb weight (grams) and yield (tons/Ha).

	Bulb number	Bulb weight	Yield
Bulb number	1.000	-0.05***	0.78**
Bulb weight		1.000	0.56***
Yield			1.000

*,** ,*** significantly different at *P* = 0.05, *P* = 0.01 and *P* =< 0.001 respectively compared to the control (LSD).

Bulb Size

According to the cultivar description by Nunhems Seed Company under optimal growing conditions, the cv. Don Victor is expected to produce jumbo and colossal sized bulbs; and cvs. Caramelo and Lambada produce more jumbo and medium sized bulbs. As previously mentioned, the importance of bulb size relies on the potential onion market demand and uses.

Figure 13 presents bulb size distribution for the different categories (small, medium, jumbo and colossal) according to bulb diameter.

For early- transplanted onions, the cv. Caramelo DS gave the highest production of small bulbs (14.7%) and the cv. Don Victor T1 displayed in the lowest (2 %). The cv. Lambada T1 displayed the greatest percentage of medium sized bulbs (49.5%) and the lowest the cv. Don Victor T1 (23.8%). The cv. Don Victor T1 had the highest percentage of jumbo bulbs (58.3%). The greatest percentage of colossal size was obtained from the cv. Don Victor at DS (5.7%). During this planting date cvs. Don Victor and Lambada produced bulb sizes within the categories described by Nunhems. Unexpectedly, the cv. Caramelo displayed the greatest percentage of small bulbs.

For mid-season transplants, the cv. Caramelo T3 gave in the highest production of small sized bulbs (20.6%) and the cv. Don Victor T1 the lowest (1.02 %). The cv. Caramelo DS displayed the greatest percentage of medium sized bulbs (43.2%) and the cv. Don Victor DS (20.6%) the lowest. The 59.8% of the cv. Don Victor T1 total yield was attributed to the jumbo bulbs. The greatest percentage of colossal bulbs was obtained by the cv. Don Victor DS (19.93%).

For late- transplanted onions, the cv. Caramelo T3 resulted in the highest production of small sized bulbs (28.6%) and the cv. Don Victor DS (2.1 %) in the lowest. In contrast, the cv. Don Victor T1 displayed the greatest percentage of medium sized bulb (52.7%) and the cv. Lambada T3 the lowest (9.2%). 53.9% of the cv. Don Victor DS total yield was attributed to the jumbo bulbs. The greatest percentage of colossal bulbs was observed in the cv. Caramelo DS (34.4%).

For late planting, even though cv. Caramelo had the greatest percentage in the small bulb category among all cultivars, it also displayed the greatest percentage of the most valuable colossal category. Values obtained from the late-planting showed a high variability among bulb categories, probably due to the effect of freezing temperatures affecting young transplants. This event agrees with Richwine (1990), who observed that onions transplanted late in the season were more susceptible to overwinter losses,

Overall, the percentage of the colossal bulbs was low for all three cultivars, except in cvs. Don Victor DS in mid and late plantings and Caramelo in late planting.

An early study determined that plant density can be an efficient strategy to control bulb size and yield (Hatridge-Esh and Bennett, 1980), with an increase in plant density increasing the number of bulbs in the medium size category. Nonetheless, in our study bulb size distribution was mostly affected by the interaction of planting date × plant density rather that plant density by itself. Furthermore, Brewster (1990) suggested that the variability of bulb size distribution is mainly due to the variability in the weights of individual scales within the bulb rather than a number of scales. Unfortunately, our experiment did not evaluate the effect of bulb scale weight in bulb size distribution.

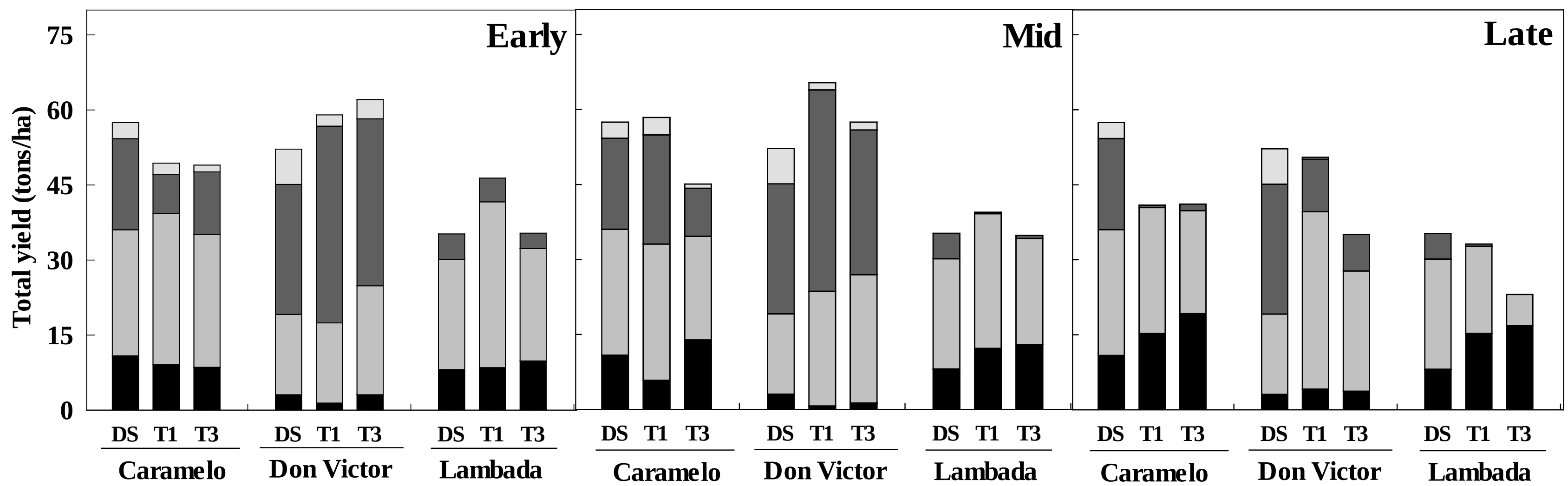

Figure 13. Alamo, TX transplants**.** Effect of planting date (Early, Nov 14; mid, Dec 8 late, Jan 9) and plant density (Direct seeding, DS; one plant per cell, T1; three plants per cell, T3) of marketable grade bulbs of three onion short day cultivars (Caramelo, Don Victor and Lambada**).**

Quality

Shape Index (SI)

SI was significantly affected by onion cultivar and plant density. The cv. Caramelo had a SI of 1.61 and was classified as a flat onion. Don Victor had a SI of 1.03 and was classified as a round onion. Lambada with a SI of 1.15 was slightly flat. T3 showed a higher tendency to develop a flatter onion than DS and T1 (Table 10). Leskovar et al., (2004) reported more elongated shapes for small classes in DS and transplants. It is unknown is this morphological response is related to root competition or the interaction between plants during early development (Leskovar et al., 2004).

Table 10. Alamo, TX transplants. Onion shape index.

	Shape index		
Onion cultivar	Caramelo	1.61	a
	Don Victor	1.03	c
	Lambada	1.15	b
Plant density	Direct seeding	1.22	b
	1 plant/cell	1.24	b
	3 plants/cell	1.30	a

Results are means of three planting times. Results that are significantly different at P = 0.05 are identified with different letters (determined by LSD).

Total Soluble Solids (TSS) and Pyruvic Acid (PyA)

TSS was significant affected by onion cultivar ($P < 0.001$) and planting date ($P = 0.009$). Among all cultivars, the cv. Lambada displayed the highest TSS content (7.93°Brix), followed by Caramelo, (6.43°Brix) and Don Victor (6.28°Brix). TSS did not show a clear tendency of increasing or decreasing with planting dates, onions transplanted in January showed the highest TSS content 7.08 °Brix followed by onions transplanted in November and December, 6.93 and 6.63 °Brix respectively (data not shown). In Egypt, Kandil et al. (2013) observed in Texas Early Grano and Giza cultivars the highest TSS levels on December 15, and attributed these results to the suitable weather conditions during this planting time. Similar results were obtained in India by Patil et al. (2012) in five short-day white onions cultivars, with the lowest TSS observed in early transplanting, (November 15) while the highest levels were obtained in late transplants (December 1). TSS content appears to have a negative correlation with growing temperature (Coolong and Randle, 2003). However, in Southern Italy (40°38 N, 14°58’) Caruso et al., (2014) observed a higher TSS content in onions transplanted on March 18 rather than February 1.

PyA content showed similar results to TSS content. PyA concentrations were affected by cultivar and planting date. The cv. Lambada displayed the highest PyA concentration, 4.38 µmol/mL, followed by cvs. Don Victor, 4.18 µmol/mL and Caramelo 3.52 µmol/mL. Onion pungency can greatly depend on cultivar, soil type and other environmental factors (Hamilton, et al., 1997). Lee et al., (2009) concluded that pungency levels varied between breeding lines and cultivars, providing a clear evidence of genetic variation influencing on pungency levels. Pyruvic acid concentrations of seven breeding lines and two commercial cultivars grown in Weslaco, TX (26°15’N, 97°98’W) on April 2005 varied among 1.9 and 8.3 µmol/mL (Lee et al., 2009).

As with TSS, PyA levels did not show a tendency of increasing or decreasing with planting timing, onions transplanted on November 14 showed the highest PyA content, 4.42 µmol/mL followed by onions transplanted on January 8 and December 9, 3.86 and 3.88 µmol/mL respectively (Figure 14 A, B). In a study by Arce (1995) early plantings during September 1993, displayed higher PyA levels (7.03 µmol/mL) compared to December plantings (6.48 µmol/mL), however no differences were found in onions planted during November. Pungency is associated with growing temperatures. Onions grown at lower temperatures had a greater pungency level (Platenius and Knott, 1941). However, it is rather difficult to predict precise relationships between environmental factors and onion pungency (Lancaster and Boland, 1990).

The cv. Lambada (red onion) had a higher TSS and PyA levels than cvs. Don Victor and Caramelo (yellow). These results are similar to those from a study by Lee et al. (2015), where they found TSS content had a broad range in white and yellow cultivars, suggesting that TSS is more related to genotypes rather than bulb scale color (Lee et al., 2015).

Correlation between TSS and PyA was low with a R^2 =0.09.

Anthocyanin Content

Anthocyanin content showed a significant cultivar × planting date interaction. As expected, cv. Lambada displayed the greatest anthocyanin content (19.97 µg/mL) as compared to cvs. DonVictor (3.82 µg/mL) and Caramelo (3.51 µg/mL) (Figure 14 C, D). Among all planting dates cv. Lambada had the highest levels of anthocyanin when transplanted early (27.54 µg/mL) and the lowest in onions transplanted late (15.85 µg/mL). In contrast, the cv. Caramelo increased anthocyanin when transplanted late from 2.85 to 4.98 µg/mL.

Anthocyanin content has not been associated with a distribution pattern across bulb scales and along the longitudinal axis (Pérez-Gregorio et al., 2010). It was suggested that small onions appear to have higher levels of anthocyanin and flavonoid content compared to larger onions (Pérez-Gregorio et al., 2010); however, our results did not agree since the cv. Lambada displayed the lowest levels of anthocyanin in late plantings when bulb sizes were smaller (Figure 13).

Folin-Ciocalteu, (F-C) Assay and DPPH Assay

The AOA levels determined by the F-C assay showed onion cultivar × date, and plant density × date interaction effects. Overall, among the three planting dates cv. Lambada had the highest AOA levels from the three cultivars with a range between 368.11 to 313.94 µg GAE /mL followed by cv. Don Victor and lastly cv. Caramelo. Plant density only displayed an effect on onions transplanted in December; with the highest levels obtained in DS onions. The AOA levels obtained in our study were lower than previously described. In a study by Lee et al., (2015), white onions showed 440 µg GAE /mL, yellow onions 500 to 750 µg GAE /mL and red onions 700 to 780 µg GAE/mL. Genetic variations have been associated with differences between yellow and red onion cultivars (34.44 and 47.3 mg.100g^{-1} fresh weight) rather than skin color (Benkeblia, 2005). Our results were attributed to genetic differences rather than skin color.

In the DPPH assay, the general levels of AOA were mainly affected by onion cultivar and planting date. The cv. Caramelo showed the lowest in AOA levels, 15.88 GAE µg/mL; the cv. Don Victor showed medium AOA levels, 19.83 GAE µg/mL; and cv. Lambada displayed the highest levels, 35.12 GAE µg/mL (Figure 8). When Li et al., 2011 evaluated AOA in white, yellow and red cultivars; they observed the same tendency with red onions displaying the highest AOA values (1.64 mg GAE/g FW). In general, the highest levels of AOA were obtained from onions transplanted in December, 25.48 GAE µg/mL followed by November 24.59 GAE µg/m; with low levels during January, 19.06 GAE µg/mL.

AOA levels obtained with the DPPH assay were lower than values obtained in the F-C assay. Correlation between F-C and DPPH was strong and positive R^2 =0.42 (Figure 14E).

A distinctive gradient of total flavonoid content has been observed between outer, central and inner edible scales and along the longitudinal axis of the onion. Flavonoid concentration decreased from outer to inner scales (Pérez-Gregorio et al., 2010). Therefore, low AOA values may be related to the sampling protocol since central scales have lower levels of flavonoids than outer scales.

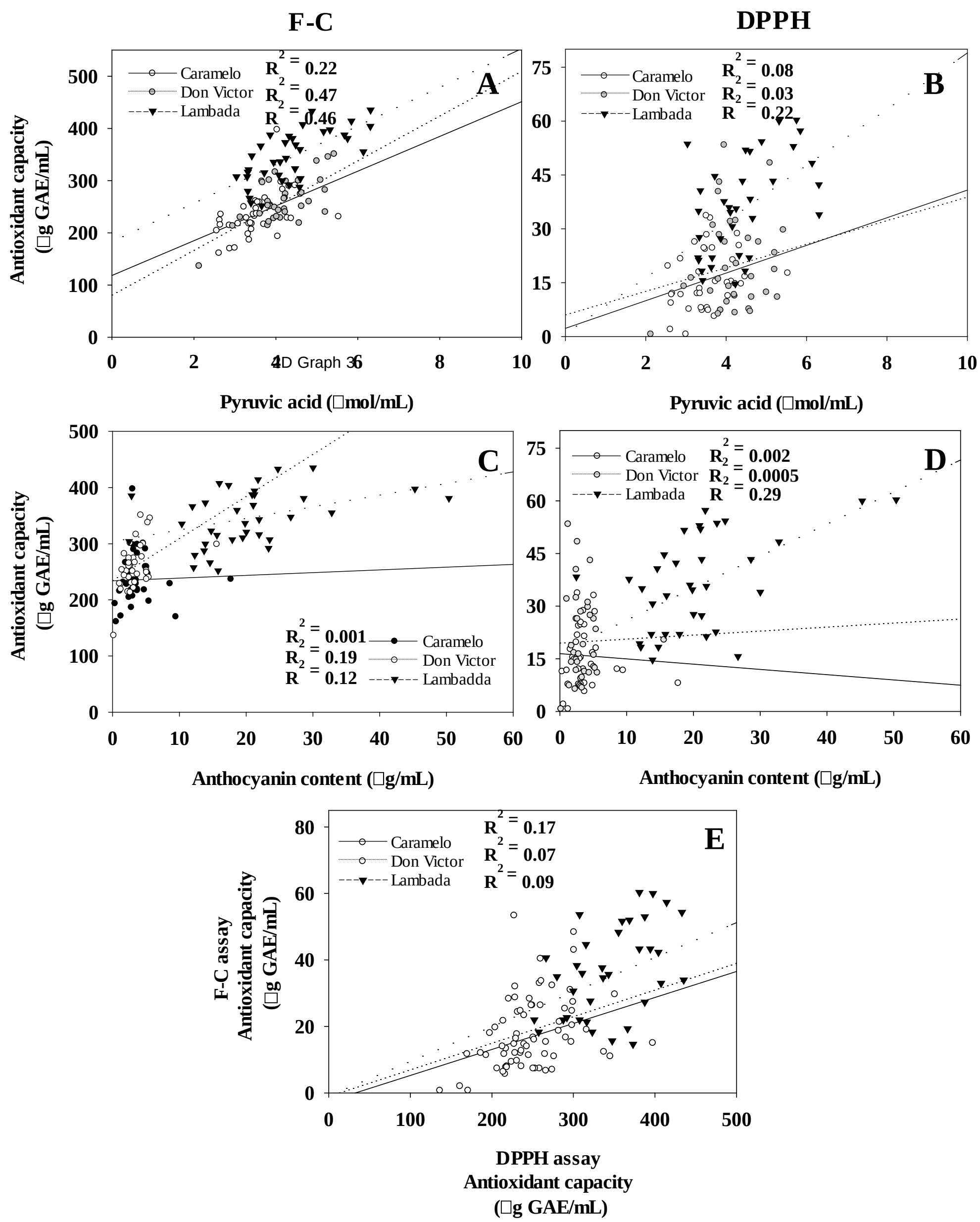

Figure 14. Correlation between the pyruvic acid content and antioxidant capacity by the F-C (A) and DPPH (B) method; the anthocyanin content and antioxidant activity by the F-C (C) and DPPH (D): and the correlation between the F-C and DPPH assays in two yellow and one red onion cultivars The regressions were y =75.5+50.8x (R^2=0.42) for (A) y =-9.14+8.12x (R^2=0.20) for (B) y =75.5+50.8 (R^2=0.46) for (C) y =14.9+.95x (R^2=0.38) for (D) y =219.4+2.58x (R^2=0.42) for (E).

Ruskin, Florida

Growth Parameters

Plant height, neck diameter, leaf number, bulb length and diameter, and leaf and bulb fresh and dry weight from transplants grown in the nursery in Ruskin, FL were similar to those observed in Alamo, TX transplants. Therefore, those results will not be presented here, except for differences in maturity, yield and quality.

Maturity

The maturity date in onion plants is defined as the time when 80% of the pseudostems bent over (Brewster 1990). All onions from the three planting dates were harvested independently when they reached physiological maturity. Direct seeded treatments were harvested 193 days after sowing; onions transplanted in November were harvested 176 DAT; those transplanted in December were harvested 162 DAT; and those transplanted in January were harvested 130 DAT. Time to maturity decreased as planting date was delayed (Figure 12). The same effect was observed in Weslaco, TX by Lopes (1987) in three short day cultivars (Special 38, Asgrow 429 and Texas Grano 1015Y). Days to maturity decreased from 194 days on the October 12 planting compared to 164 days on December 14.

Early transplanted T1, T3 and DS were harvested on April 24. Mid- transplanted T1 and T3 were harvested on May 9; and late-transplanted T1 and T3 were harvested on May 19. A difference of approximately one month between direct seeding (October 13) and early planting (November 14) resulted in a 17-day difference to maturity, 31-day difference between DS and mid planting (December 8), and 63-day difference between DS and late planting (January 9).

The three cultivars showed an increase in days to maturity when transplanted early in the season. As previously mentioned Arce (1995) observed similar results in three short-day onion types. Onion cultivars reduced days to reach maturity as sowing date was delayed. This response has been associated with higher temperatures and daylength increases promoting bulb initiation.

It appears that vegetative growth is influenced by a set of environmental conditions that characterize each agricultural location at a specific time of the year.

Yield

For any cultivar to achieve the highest yield potential, it must be grown in an area with the optimal environmental conditions such as temperature and daylength. In addition, fertilization, pest control, irrigation and weeding programs need to be maintained throughout the season. Onion yield varies with cultivar, season and management. In field production in the tropics yield can range from 15 to 55 tons/ha, while under experimental conditions 80 to 100 tons/ha have been recorded (Brewster, 1990b). Table 11 displayed total yield, ABW and BN affected by onion cultivar × plant density × planting date interaction.

The cv. Don Victor displayed the highest yield among treatments transplanted early and mid-season 44.57 tons/ha, 54.71 tons/ha respectively. In early transplants the cv. Don Victor T1 had the highest yield (62.3 tons/ha), while, mid and late season T1

(54.3 tons/ha) and T3 (49.7 tons/ha) showed a significant yield reduction compared to DS (60 tons/ha). Overall, for late planting, DS resulted in the highest yield.

The cv. Don Victor showed the greatest ABW among the three planting dates. The cv. Don Victor T1, early transplanted resulted in the greatest ABW (370 grams). In contrast, DS resulted in a greater ABW in mid and late plantings in all the three cultivars.

No differences were observed in bulb number (BN) for DS, T1 and T3 in early planting. T1 and T3 cvs. Caramelo and Lambada had a greater BN compared with the cv. Don Victor in mid-season transplants. BN was greatly affected by planting date. Onions transplanted late suffered freezing stress immediately after transplanting for two nights during five hours reducing plant survival, and therefore, final yield. Planting date determines plant size during the winter which could determine plant survival and consequently yield. Delaying sowing and transplanting dates usually increase plant losses (Lopes, 1987). As previously mentioned older transplants would stand freezing temperatures for an extended period of time compared to younger transplants (Peffley et al., 1981).

Moreover, yield response could also be attributed to the light perception by the leaf canopy. Increasing plant density either when established by direct seeding or containerized transplants reduces individual bulb size (McGeary, 1985; Stoffella, 1996). An explanation for the yield reduction observed in T3 transplants across cultivars and planting dates could be due to the plant competition during the early stages of development.

Onion yield and bulb average weight and size decreased significantly from early to late transplanting times. In Sele Plain, Italy (lat. 40°38’N, long. 14°52’E), it was observed that by delaying transplant date from February 1 to March 18, plant productivity was reduced from 58.3 to 49.2 tons/ha. Yield reduction was associated with the time frame reduction to reach bulb maturity and air temperature increase (Caruso et al., 2014).

In general, the final yield results were attributed to both ABW and BN, which resulted in a positive and strong correlation of R = 0.61 and R= 0.83, respectively (Table 12).

Table 11. Bulb number per hectare, average bulb weight and yield in response to onion cultivar and plant density across three planting dates from Ruskin, FL transplants.

		Early (Nov 14)			Mid (Dec 8)			Late (Jan 9)		
Onion cultivar	**Plant density**	**Bulb number (No/Ha)**	**Bulb weight (grams)**	**Yield (tons/ha)**	**Bulb number (No/Ha)**	**Bulb weight (grams)**	**Yield (tons/ha)**	**Bulb number (No/Ha)**	**Bulb weight (grams)**	**Yield (tons/ha)**
Caramelo	Direct seeding	209,321	294	59.92	209,321	294	59.92	209,321	294	59.92
	1 plant/cell	185,883	315	58.38	139,009	262	36.23	123,563	308	35.98
	3plants/cell	161,638	320	51.91	171,875	226	39.21	63,578	218	14.41
Don Victor	Direct seeding	158,136	335	52.84	158,136	335	52.84	158,136	335	52.84
	1 plant/cell	168,648	371	62.32	147,629	367	54.32	86,207	341	28.84
	3plants/cell	176,724	330	58.20	144,935	340	49.77	64,655	257	15.05
Lambada	Direct seeding	155,711	235	36.45	155,711	230	36.45	155,711	230	36.45
	1 plant/cell	167,026	267	44.46	161,099	243	38.69	133,621	226	30.26
	3plants/cell	176,185	223	39.33	145,474	230	31.40	84,590	214	16.54
					P values					
Onion cultivar x plant density					< 0.001	0.813	< 0.001			
Onion cultivar x planting date					0.496	0.062	0.002			
Plant density x planting date					< 0.001	0.023	< 0.001			
Onion cultivar x plant density x planting date					0.238	0.212	0.131			

Table 12. Correlation between bulb number (No/Ha), average bulb weight (grams) and yield (tons/Ha) from Ruskin, FL transplants.

	Bulb number	Bulb weight	Yield
Bulb number	1.000	0.10^{NS}	0.83^{***}
Bulb weight		1.0000	0.61^{***}
Yield			1.000

^{NS}, *, **, *** no significant, significantly different at P = 0.05, P = 0.01 and P =< 0.001 respectively compared to the control (LSD).

Bulb Size

The cv. Don Victor is expected to produce jumbo and colossal sized bulbs. While, cvs. Caramelo and Lambada produce more jumbo and medium sized bulbs. These sizes can be obtained if the cultivars were appropriately transplanted, well fertilized and the ideal environmental conditions were met during the growing season. As previously mentioned, bulb size will determine the potential market and uses.

Figure 15 displayed bulb size distribution in the different categories (small, medium, jumbo and colossal), according to bulb diameter.

For early- transplanted onions, the cv. Caramelo DS resulted in the highest production of small sizes bulb (14.7%) and the lowest in the cv. Don Victor T1 (1.7 %) as observed in Texas grown transplants. The cv. Lambada T1 displayed the greatest percentage of medium sized bulbs (50.7%) and the cv. Don Victor DS the lowest (20.1%). The 49.9% of the cv. Don Victor T1 total yield was attributed to jumbo bulbs. The greatest percentage of colossal was obtained by the cv. Caramelo T1 (5.7%). A similar result was observed by Leskovar et al. (2004), where 24% of transplants produced from one plant per cell had 36% of total marketable yield in the jumbo size category compared with 16% with direct seeding.

For mid-season transplants, the cv. Lambada T1 resulted in the highest production of small sized bulbs (18.5%) and the lowest with the cv. Don Victor T1 (2.42%). The cv. Caramelo DS displayed the greatest percentage of medium sized bulbs (43.2%) and the lowest with the cv. Don Victor DS (20.6%). The 46.7% of the cv. Don Victor T1 yield was attributed to jumbo bulbs. The greatest percentage of colossal bulbs was obtained by the cv. Don Victor DS (5.6%).

For late- transplanted onions, the cv. Lambada T1 resulted in the highest production of small sized bulbs (17.3%) and the lowest the cv. Don Victor T1 (2.1%). The cv. Caramelo DS displayed the greatest percentage of medium sized bulbs (37.7%) and the lowest the cv. Lambada T3 (11.3%). The 43.3% of the cv. Don Victor DS total yield was the jumbo size category. The greatest percentage of colossal bulbs was observed in the cv. Don Victor DS (34.4%). In late plantings, T1 and T3 did not produce bulbs in the colossal category. The cv. Lambada did not produce bulb in the colossal category in any on treatments or planting date. Caruso et al., (2014) observed the same tendency in onions transplanted in Italy in March rather than February; with late plantings locking the production of bulbs in the colossal category.

Moreover, the high percentage of small bulb sizes in T3 treatment during late transplanting could directly associated with high seed densities at nursery conditions. It has been reported that the delayed in bulb scale initiation contributes to the reduction in bulb scales weight, therefore, bulb size and yield (Mettananda and Fordham, 1999).

Arce (1995) observed that the percentage of medium bulb sizes increased as the jumbo size decreased as sowing date changed from September to October. Our results displayed a similar tendency from mid to late transplants. According to Coolong and Randle (2003), the temperature increase from 26.7°C to 32.2°C resulted in bulb size reduction, consequently lower yield reduction. They also reported that onions grown at 34°C reached maturity earlier than those grown at 12 to 21°C. Thus, bulb size reduction is influenced by climate and environment, primarily temperature. High temperatures will promote bulb formation and will reduce the time to bulb maturity (Khokhar, 2008). Furthermore, greater bulb sizes in DS and early transplants could be associated with longer growth season, which allowed plants to fully develop to their maximum potential by increasing the synthesis and assimilation of metabolites into the bulbs (Caruso et al., 2014).

As previously discussed, bulb size is vital to determine the potential market. For example, in Brazil the most valuable size for export is 50 – 70 mm. By contrast, the preferred size in the USA is colossal, 102 mm (Leskovar et al., 2004). Furthermore, bulb size controls the potential uses of a cultivar, for example, jumbo and colossal sizes are preferred for onion ring processing and fresh market production (Arce, 1995). If production of jumbo and colossal bulbs is desired, direct seeded and early and mid-season transplants are recommended. In terms, of medium sized late transplanting could be used as an alternative (Figure 18). It is also important to consider cultivars and the potential yield and bulb size according to the profile provided by the company. In our experiment, cvs. Don Victor and Caramelo had a greater number of bulbs in the jumbo category, while the cv. Lambada had the greatest bulb number in the medium sized category.

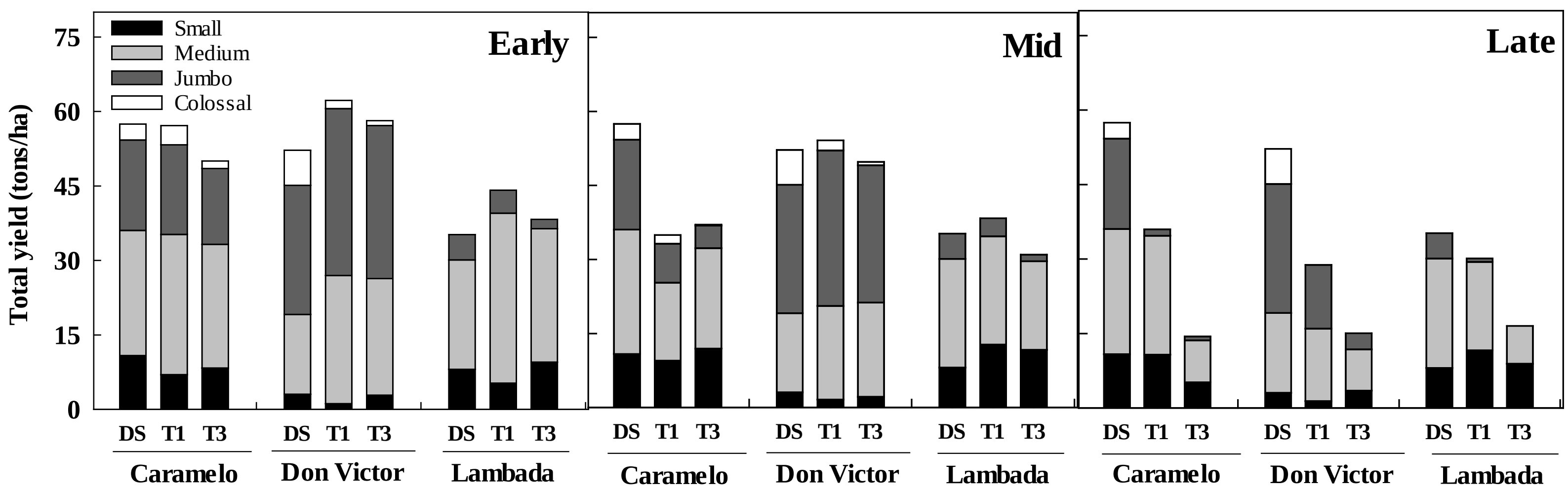

Figure 15. Effect of planting date (Early, Nov 14; mid, Dec 8; late, Jan 9) and plant density (Direct seeding, DS; one plant per cell, T1; three plants per cell, T3) of marketable grade bulbs of three onion short day cultivars (Caramelo, Don Victor and Lambada).

Onion Quality

Shape Index (SI)

SI was significantly affected by planting date × onion cultivar interaction (Table 13). The cvs. Don Victor and Lambada tended to form into a flatter shape when transplanted late. The cv. Caramelo had a flat shape as was expected due to its granex phenotype. Longbrake (1987) observed intermediate onion types planted in November and December were poorly shaped and elongated bulbs. In our study none of the treatments indicated a tendency towards elongated bulbs.

Table 13. Onion shape index from Ruskin, FL transplants.

Planting date	Cultivar	Shape index	
Early	Caramelo	1.6	Aa
	Don Victor	1.0	Ac
	Lambada	1.1	Ab
Mid	Caramelo	1.6	Aa
	Don Victor	1.0	Ac
	Lambada	1.1	Ab
Late	Caramelo	1.5	Aa
	Don Victor	1.1	Ac
	Lambada	1.2	Ab

Results that are significantly different at the $P = 0.05$ level of significance determined by LSD. Upper caser letters represent multiple comparisons of the means between planting dates. Lower case letters represent multiple comparisons for the effects due to plant density.

Total Soluble Solids (TSS) and Pyruvic Acid (PyA)

TSS was significantly affected by cultivar ($P < 0.001$). Among all cultivars, the cv. Lambada displayed the highest TSS content, 7.95°Brix, followed by Caramelo, 6.41°Brix and Don Victor 6.08°Brix. Lee et al., (2015) reported values of 7.3ºBrix for the cv. Legend, a yellow type onion, while red onion cvs. D40, D42 and Leylon had values above 10ºBrix. Our TSS levels were lower than previously reported in both yellow and red varieties. Thus, we concluded TSS is related to the genetics of the cultivar rather than the skin color.

PyA concentrations were significantly affected by cultivar and planting date. The cv. Lambada displayed the highest PyA concentration (4.39 µmol/mL) followed by cvs. Don Victor (4.20 µmol/mL) and Caramelo (3.57 µmol/mL) (Figure 16 A, B). The range of PyA ranges from 3.57 to 4.39 µmol/mL, which is considered mild according to Lee et al. (2009). PyA levels appear to be higher in red onions. Similar results were found by Lee et al. (2015), three of the red genotypes resulted in PyA concentrations above 5 µmol/mL, however there was not a distinctive relationship between bulb color and pungency in the tested genotypes.

PyA content did not show a clear tendency of increasing or decreasing with timing. Early plantings showed the highest PyA content (4.14 µmol/mL) followed by late (4.02 µg/mL) and mid-season (4.00 µg/mL). Caruso et al., (2014) observed the opposite tendency, higher levels of PyA were observed in early transplanted onions in February (8.6µmolg^{-1} f.wt.) compared to March plantings (7.8µmolg^{-1} f.wt).

Correlation between TSS and PyA was positive and relatively low (R^2 =0.02) as observed by Lee et al. (2015).

Anthocyanin Content

Onion cultivar and planting date showed a significant effect on anthocyanin content. As expected, the cv. Lambada displayed the greatest anthocyanin content 21.19 µg/mL as compared to cvs. Caramelo (3.60 µg/mL) and Don Victor (3.52 µg/mL). Among all planting dates the cv. Lambada had the highest levels of anthocyanin in early planting (26.58 µg/mL) and the lowest levels in onions transplanted late (16.64 µg/mL). Contrary, cvs. Caramelo and Don Victor had increased anthocyanin levels when transplanted late (Figure 16 C, D).

The correlation between anthocyanin content and AOA in the three cultivars is presented in Figure 16C, D. The AOA levels determine by the F-C assay showed a moderate and positive correlation coefficient of 0.41. While AOA levels determined by DPPH method displayed a correlation coefficient of 0.39. However, Lee et al., (2015) did observe an opposite tendency response, with a 0.07 positive correlation with the F-C and .65 with DPPH and concluded that anthocyanin content is more sensitive to the DPPH method. Furthermore, anthocyanin might not be the major determinant of AOA levels assessed by the F-C assay. Our results suggest anthocyanins might influence AOA levels in onion. Finally we can conclude anthocyanin and AOA levels content vary by cultivars and methods of evaluation.

Folin-Ciocalteu, (F-C) Assay and DPPH Assay

The AOA levels determined by the F-C assay showed an effect due to onion cultivar. Overall, among the three planting dates cv. Lambada had the highest AOA levels 347.06 GAE µg/mL, then cv. Don Victor and lastly cv. Caramelo.

AOA levels obtained with the DPPH assay displayed lower values than the F-C assay. In DPPH assay, the general levels of AOA were mainly affected by onion cultivar and planting date. The cv. Caramelo was the lowest in AOA, 15.87 GAE µg/mL; the cv. Don Victor had 19.41 GAE µg/mL; and cv. Lambada displayed the highest levels, 35.36 GAE µg/mL. The highest levels of AOA were obtained during early planting- 25.48 GAE µg/mL; late planting showed 23.16 GAE µg/m; and low levels were seen in mid planting 22.00 GAE µg/mL. These results indicate there were substantial variations between cultivars.

Correlation between F-C and DPPH was moderately good- R^2 =0.35 (Figure 16E), AOA levels will vary based on the methodology used.

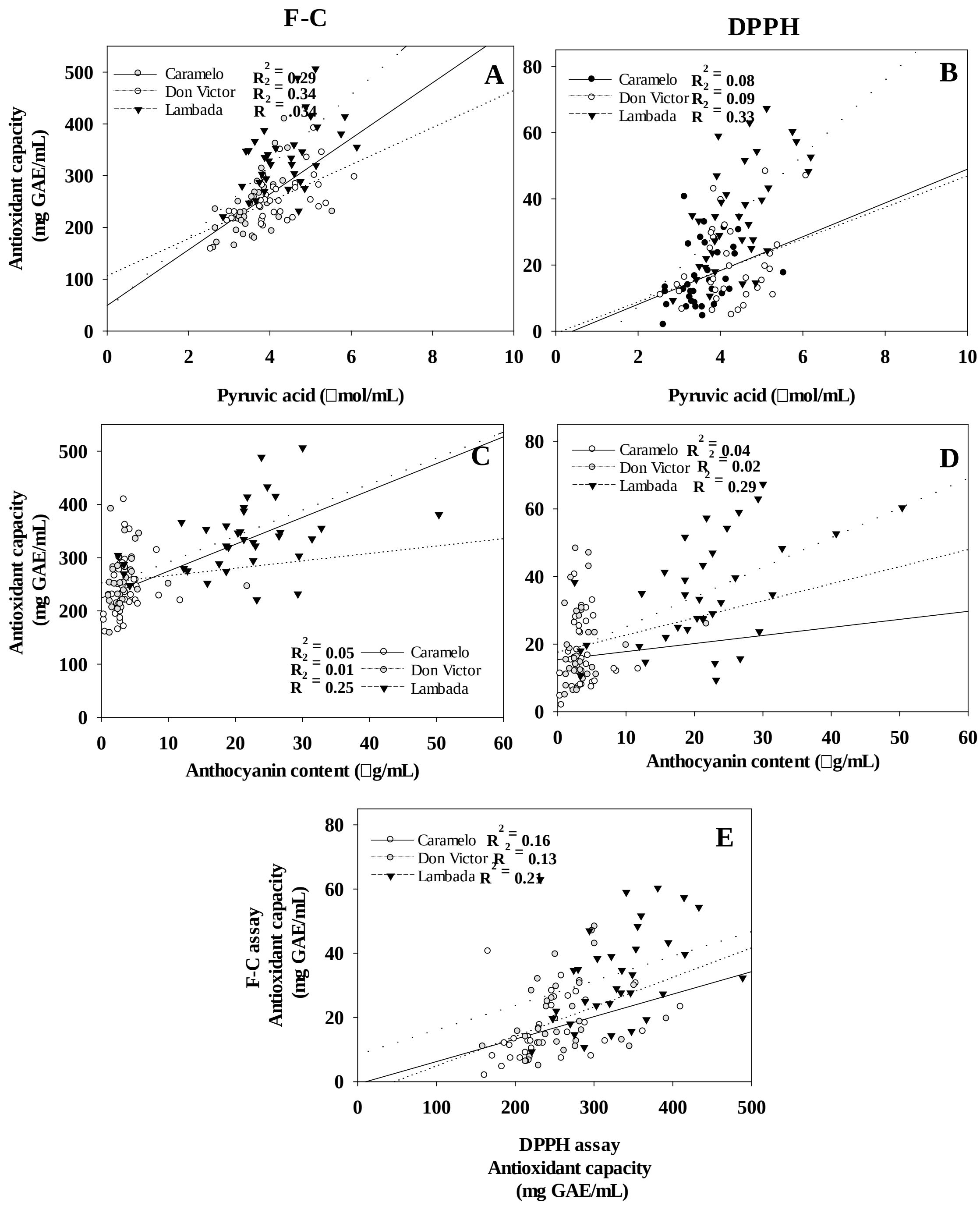

Figure 16. Correlation between the pyruvic acid content and antioxidant capacity by the F-C (A) and DPPH (B) method; the anthocyanin content and antioxidant activity by the F-C (C) and DPPH (D): and the correlation between the F-C and DPPH assays in two yellow and one red onion cultivars The regressions were y =27.2+62.7x (R^2=0.39) for (A) y =-15.8+9.7x (R^2=0.26) for (B) y =233.2+5.15x (R^2=0.41) for (C) y =14.8+.91x (R^2=0.38) for (D) y =205.4+3.26x (R^2=0.35) for (E).

Conclusion

In this study, we have investigated growth, yield and quality of three onion cultivars (Caramelo, Don Victor and Lambada) base on seedling density (DS, T1 and T3), planting dates (early, mid and late) and transplant growing location (Alamo, TX and Ruskin, FL). Direct seeding (October) and early season (November) extended the number of days to reach maturity. Conversely, mid (December) and late (January) transplanted onions reduced the number of days required to reach maturity, due to temperature and daylength increases. Time of planting is a critical strategy to regulate bulb category distribution; with early planting increasing the number of bulbs in the category of jumbo and colossal (most valuable) while mid to late plantings increase the number of the bulbs in medium size. Furthermore, determining the proper transplanting date for short-day onions is important to minimize overwinter losses and bolting susceptibility, while maximizing yield and quality.

Moreover, transplants can reduce the field growing period reducing the time of exposure to environmental and biological stresses. Transplanting gives growers early harvests and is more efficient when considered the high cost of hybrid seeds. Containerized transplants grown from three plants per cavity transplanted in early November can be an alternative and cost effective system that could increase yield over direct seeding system.

In terms of quality, overall AOA levels, anthocyanin levels, pyruvic acid and TSS content appear to be affected by planting date and seedling density, and major differences were primarily due to cultivar. Overall the red onions had higher AOA and anthocyanin content than yellow onions.

CHAPTER V

SUMMARY AND CONCLUSIONS

Effects of Plant Growth Regulators on Seed Germination and Root Growth Traits of Red and Yellow Onion

- Hydro – priming is a simple and practical method to improve seed germination and root traits in onion seedlings. Although it was impossible to differentiate whether germination enhancement resulting from hydro – priming and/or hormonal treatment, lack of response could be attributed to seeds grown in optimal conditions, a follow up experiment should be conducted to assess the PGR effects on seeds grown under hostile conditions
- Exogenously *trans*-zeatin applications could increase onion primary root growth at low concentrations. However, there has not been reported in the literature root length increase with the exogenously CTKs application; this might indicate that onion has a different mechanism of action compared to other crops.
- Ethephon priming treatments can increase root surface area at moderate concentrations. Even though, root hair development was not quantified in these studies, onion roots treated with Ethephon at 30 and 100 μM displayed a higher root surface area, which could be attributed to the increased root hair development (Figure 2, 4).
- Addition of IAA, tZ and ACC to ethephon treatments could be an alternative to overcome the detrimental effects of ethephon on primary root growth.

- Nevertheless, further experiments need to be conducted in the field to reinforce the hypothesis that PGRs can modify seed germination and root architecture to deliver in better plant stand establishments and higher yields.

Evaluation of Growth, Yield and Quality of Onion Based on Seedling Density, Planting Date and Nursery Growing Environments

- Cell-grown transplants significantly decreased the onion growing time in the field compared with direct seeding, thus reducing the potential for biotic and abiotic stresses affecting growth, development and yield. In addition, the technique of establishing onions from single and multi-seeded transplants provides a practical and economical alternative to produce earlier crops. Nonetheless, interplant competition during early development in the nursery could reduce transplant size and influence post-transplant development.
- Cell-grown transplants are known for the high cost of production. However, a further economic analysis needs to be conducted to consider savings in seeds and inputs such as water fertilizer, pesticides, fungicides applications and labor.
- Mid transplanted onions displayed similar yield to DS and early transplants, while delaying time of harvest for 15 days. Thus, mid-season transplants should be considered for those situations where growers can obtain higher prices for late harvests.
- According to our results, onions could be transplanted until early-December reducing days to maturity and delaying harvesting time from direct seeding season without significantly reducing yields. Planting in January is not

recommended as it increases the risk of freeze injury and significantly reduces plant survival, bulb size and final yield.

- Selecting appropriate cultivars is vital to estimate final yield and bulb size distribution. The cv. Don Victor showed the highest potential to produce a greater number of bulbs in the jumbo and colossal categories, thus higher yield. Conversely, the cv. Lambada leans toward medium bulb size category, therefore lower yield when compared to cv. Don Victor. Ultimately, the desired bulb size is determined by the market. For example, onions exported to South America (e.g. Brazil) should fit into the medium size category while high value onion sizes in the USA are jumbo or colossal.
- Pyruvic acid and TSS content were independent of planting date and plant density treatments. Levels of these compounds are attributed to cultivar selection rather than performance in the field. Therefore, it is highly important that onion cultivars are screened for the pungency and sweetness.
- Anthocyanins and AOA in DPPH and F-C assays were higher in the red onion cv. Lambada. The high levels of AOA could be due to high anthocyanin content in red onion compared to the yellow cvs. Don victor and Caramelo.

REFERENCES

Aikman, D.P., 1989. Potential increase in photosynthetic efficiency from the redistribution of solar radiation in a crop. Journal of Experimental Botany 40:855-864

Allen, E., Jones, J., and Salter, P., 1978. Effects of date of sowing on overwintered onion varieties in pembrokeshire. The Journal of Agricultural Science 90:237-239.

Aloni, R., Aloni, E., Langhans, M., and Ullrich, C., 2006. Role of cytokinin and auxin in shaping root architecture: regulating vascular differentiation, lateral root initiation, root apical dominance and root gravitropism. Annals of botany 97:883-893.

Arce, J.P., 1995. Effect of planting date, nitrogen rate, and in-row plant spacing on the maturity, diasease infection severity, pungency, bolting and doubling incidence, and yield of selected short-day onions. Texas A&M University, Doctor of phylosophy.

Arteca, R.N. and Arteca, J.M., 2008. Effects of brassinosteroid, auxin, and cytokinin on ethylene production in *Arabidopsis thaliana* plants. Journal of Experimental Botany 59:3019-3026.

Asano, M., Suzuki, S., Kawai, M., Miwa, T., and Shibai, H., 1999. Characterization of novel cysteine proteases from germinating cotyledons of soybean [Glycine max (L.) Merrill]. The Journal of Biochemistry 126:296-301.

Ascard, J. and Fogelberg, F., 2008. Mechanical in-row weed control in transplanted and direct-sown bulb onions. Biological Agriculture & Horticulture 25:235-251.

Benkeblia, N., 2005. Free-radical scavenging capacity and antioxidant properties of some selected onions (*Allium cepa* L.) and garlic (*Allium sativum* L.) extracts. Brazilian Archives of Biology and Technology 48:753-759.

Benjamin, L., 1990. Variation in time of seedling emergence within populations: a feature that determines individual growth and development. Advances in Agronomy 44:1-25.

Bertell, G. and Eliasson, L., 1992. Cytokinin effects on root growth and possible interactions with ethylene and indole-3-acetic acid. Physiologia Plantarum 84:255-261.

Bialek, K., Michalczuk, L., and Cohen, J.D., 1992. Auxin biosynthesis during seed germination in *Phaseolus vulgaris*. Plant Physiology 100:509-517.

Black, M., Bewley, J.D., and Fountain, D., 1974. Lettuce seed germination and cytokinins: their entry and formation. Planta 117:145-152.

Borghetti, F., Noda, F.N., and Sá, C.M.d., 2002. Possible involvement of proteasome activity in ethylene-induced germination of dormant sunflower embryos. Brazilian Journal of Plant Physiology 14:125-131.

Bourquin, M. and Pilet, P.E., 1990. Effect of zeatin on the growth and indolyl-3-acetic acid and abscisic acid levels in maize roots. Physiologia Plantarum 80:342-349.

Boyhan, G.E., Torrance, R.L., Cook, J., Riner, C., and Hill, C.R., 2009. Sowing date, transplanting date, and variety effect on transplanted short-day onion production. HortTechnology 19:66-71.

BrewsterJ., Lakes, W., and Whitlock, A.J., 1987. The phenology of onion bulb development at different sites and its revelavnce to incomplete bulbing ('thick-necking'). Journal of Horticultural Science 62:371-378.

Brewster, J., 1977. The physiology of the onion. I. Horticultural Abstracts 47:17.

Brewster, J., 1994. Onion and other vegetable *Alliums*, Cab International, WallingtonUK

Brewster, J.L., 1990. Onions and allied crops: agronomy, biotic interactions, pathology and crop protection. In: D. Rabinowitch, J. L. B. (ed.), Cultural system and agronomic practices in temperate climates. CRC Press, Florida,USA.

Brewster, J.L., 1990. Onion and allied crops: botany, physiology and genetics. CRC Press, Boca Raton, FL.

Brewster, J.L., 2008. Onion and other vegetable *Alliums*, Wallingford, UK.

Brocklehurst, P. and Dearman, J., 1983. Interactions between seed priming treatments and nine seed lots of carrot, celery and onion. Annals of Applied Biology 102:577-584.

Cao, G., Sofic, E., and Prior, R.L., 1997. Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. Free Radical Biology and Medicine 22:749-760.

Caruso, G., Conti, S., Villari, G., Borrelli, C., Melchionna, G., Minutolo, M., Russo, G., and Amalfitano, C., 2014. Effects of transplanting time and plant density on yield, quality and antioxidant content of onion (*Allium cepa* L.) in southern Italy. Scientia Horticulturae 166:111-120.

Casimiro, I., Beeckman, T., Graham, N., Bhalerao, R., Zhang, H., Casero, P., Sandberg, G., and Bennett, M.J., 2003. Dissecting *Arabidopsis* lateral root development. Trends in Plant Science 8:165-171.

Chang, L., Ramireddy, E., and Schmulling, T., 2015. Cytokinin as a positional cue regulating lateral root spacing in *Arabidopsis*. Journal of Experimental Botany 66:4759-4768.

Coolong, T.W. and Randle, W.M., 2003. Temperature influences flavor intensity and quality in granex 33'onion.Journal of the American Society for Horticultural Science 128:176-181.

Corgan, J., 1988. 'NuMex Sundial'and'NuMex Suntop'onion. HortScience 23:421-422.

Corgan, J.N. and Kedar, N., 1990. Onion cultivation in subtropical climates, p. 31-47. In: Rabinowitch, H. D. and Brewster, J. L. (eds.), Onions and allied crops CRC Press, Boca Raton, FL.

Corgan, J.N., Wall, M., Cramer, C., Sammis, T., Lewis, B., and Schroeder, J., 2000. Bulb onion culture and management. Cooperative Extension Service, College of Agriculture and Home Economics, New Mexico State University New Mexico.

Cramer, C.S., 2003. Performance of fall-sown onion cultivars using four seeding dates. Journal of the American Society for Horticultural Science 128:472-478.

Currah, L. and Proctor, F.J., 1990. Onions in tropical regions. Natural Resources Institute, Overseas Development Administration, Kent, UK.

Díez, J., López-Sáez, J., and González-Bernáldez, F., 1970. Growth components in *Allium* roots. Planta 91:87-95.

Dowker, B. and Fennell, J., 1974. Some responses to agronomic treatments of different genotypes of bulb onions, *Allium cepa* L. Journal of Horticultural Science 49:1-14.

Dubrovsky, J.G., Sauer, M., Napsucialy-Mendivil, S., Ivanchenko, M.G., Friml, J., Shishkova, S., Celenza, J., and Benková, E., 2008. Auxin acts as a local morphogenetic trigger to specify lateral root founder cells. Proceedings of the National Academy of Sciences 105:8790-8794.

Duclos, D.V., Ray, D.T., Johnson, D.J., and Taylor, A.G., 2013. Investigating seed dormancy in switchgrass (*Panicum virgatum* L.): understanding the physiology and mechanisms of coat-imposed seed dormancy. Industrial Crops and Products 45:377-387.

El-Rehim, G., Ahmed, F., Shalaby, G., and Waly, A., 1997. Effect of transplanting date and planting density on bulb yield and quality of Giza 20 onion in upper Egypt. Egyptian J. Agr. Res. 75:681-695.

Ellis, R. and Roberts, E., 1981. The quantification of ageing and survival in orthodox seeds. Seed Science and Technology (Netherlands). 9:373-409

FAOSTAT, 2013. Food and agriculture organization of the United States: Statistics Division. FAO. 10 July 2016. http://faostat.org/

Finch-Savage, W.E. and Leubner-Metzger, G., 2006. Seed dormancy and the control of germination. New Phytologist 171:501-523.

Finkelstein, R., Reeves, W., Ariizumi, T., and Steber, C., 2008. Molecular aspects of seed dormancy. Annual Review of Plant Biology 59:387-415.

Finlayson, S.A., Liu, J.H., and Reid, D.M., 1996. Localization of ethylene biosynthesis in roots of sunflower (*Helianthus annuus*) seedlings. Physiologia Plantarum 96:36-42.

Fossen, T. and Andersen, Ø.M., 2003. Anthocyanins from red onion, *Allium cepa*, with novel aglycone. Phytochemistry 62:1217-1220.

Fuqua, M.C., 1975. Variety and planting-date trials of spring and fall field-seeded onions in the Rolling Plains of Texas. Texas Agricultural Experiment Station. Prog. Rpt. No. PR-3330.

Geetha, M., Ponmozhi, P., and Suganyadevi, P., 2016. Extraction of anthocyanin and analyzing its antioxidant properties from different onion (*Allium cepa*) varieties. International Journal of Research in Pharmaceutical Sciences 2:497-506.

Gray, D. and Steckel, J.R., 1993. Effect of the plant raising environment, transplant weight and date of transplanting on the subsequent growth and development of leeks (*Allium porrum* L.) grown for early production. Journal of Horticultural Science 68:955-965.

Halmer, P., 2004. Methods to improve seed performance in the field, p. 125-165, In: R. L Benech Amold and R.A. Sanchez (eds). Handbook of seed physiology: application to agriculture. The Haworth Press, New York, NY.

Hamilton, B.K, Pike L.M., and Yoo, K.S., 1997. Clonal variations of pungency, sugar content and bulb weight of onions due to sulfur nutrition. Scientia Horticulturae 71:131-136.

Hasegawa, N., Yamaji, Y., Minoda, M., and Kubo, M., 2003. Effects of D-methionine or L-methionine on root hair of *Brassica rapa*. Journal of Bioscience and Bioengineering 95:419-420.

Hatridge-Esh, K. and Bennett, J., 1980. Effects of seed weight, plant density and spacing on yield responses of onion. Journal of Horticultural Science 55:247-252.

Herison, C., Masabni, J.G., and Zandstra, B.H., 1993. Increasing seedling density, age, and nitrogen fertilization increases onion yield. HortScience 28:23-25.

Hodge, A., Berta, G., Doussan, C., Merchan, F., and Crespi, M., 2009. Plant root growth, architecture and function. Plant and Soil 321:153-187.

Irfan, A., Shahzadm, A.B., and Amir, I., 2005. The effects of seed soaking with plant growth regulators on seedling vigor of wheat under salinity stress. Journal of Stress Physiology & Biochemistry 1:6-14.

Ivanchenko, M.G., Muday, G.K., and Dubrovsky, J.G., 2008. Ethylene–auxin interactions regulate lateral root initiation and emergence in *Arabidopsis thaliana*. The Plant Journal 55:335-347.

Jackson, K., Duff, A.A., and O'Donnell, W.E. 1997. Development of a suite of onion varieties for the subtropical region of the Lockyer Valley in SE Queensland, Australia. Acta Horticulturae 555:239-242

Kakei, Y., Mochida, K., Sakurai, T., Yoshida, T., Shinozaki, K., and Shimada, Y., 2015. Transcriptome analysis of hormone-induced gene expression in Brachypodium distachyon. Nature 5:1- 14.

Kamula, S.A., Peterson, C.A., and Mayfield, C.I., 1994. The plasmalemma surface area exposed to the soil solution is markedly reduced by maturation of the exodermis and death of the epidermis in onion roots. Plant, Cell and Environment 17:1183 - 1193.

Kandil, A.A., Sharief, A.E., and Fathalla, F.H., 2013. Effect of transplanting dates of some onion cultivars on vegetative growth, bulb yield and its quality. Crop Production 2:72-82.

Kappler, R. and Kristen, U., 1986. Exogenous cytokinins cause cell separation and cell expansion in the root tip cortex of *Zea mays*. Botanical Gazette 147:247-251.

KeÇpczyński, J. and KeÇpczyńska, E., 1997. Ethylene in seed dormancy and germination. Physiologia Plantarum 101:720-726.

Khokhar, K.M., 2008. Effect of temperature and photoperiod on the incidence of bulbing and bolting in seedlings of onion cultivars of diverse origin. The Journal of Horticultural Science and Biotechnology 83:488-496.

Kolářová, P., Bezděčková, L., and Procházková, Z., 2010. Effect of gibberellic acid and ethephon on the germination of European beech dormant and chilled beechnuts. Journal of Forest Science 56:389-396.

Kucera, B., Cohn, M.A., and Leubner-Metzger, G., 2005. Plant hormone interactions during seed dormancy release and germination. Seed Science Research 15:281-307.

Lancaster, J.E. and Boland, M.J., 1990. Flavor biochemestry, p. 33-72. In: Brewster, J. L. and Rabinowitch, H. D. (eds.), Onion and Allied Crop. CRC Press, Boca Raton, FL, USA.

Langhans, R.W. and Tibbitts, T., 1997. Plant growth chamber handbook. Iowa Agricultural and Home Economics Experiment Station, Ames.

Lee, J.E., Patil, B.S., and Yoo, K.S., 2015. Antioxidant of 15 onions white, yellow, red colors and their relationship with pungency and quercetin. Food Science and Technology 63:108-114.

Lee, E.J., Yoo, K.S., Jifon, J., and Patil, B.S., 2009. Characterization of shortday onion cultivars of 3 pungency levels with flavor precursor, free amino acid, sulfur, and sugar contents. Journal of Food Science 74:475-480

Leskovar, D.I., Cantamutto, M., Marinangelli, P., and Gaido, E., 2004. Comparison of direct-seeded, bareroot, and various tray seedling densities on growth dynamics and yield of long-day onion. Agronomie 24: 35-40.

Leskovar, D.I., Kolenda, K., and Pena, J., 2002. Containerized onion transplants: III. Yield response to increased seedling density. Texas a &M Agricultural Research and Extension at Uvalde.

Leskovar, D.I. and Vavrina, S.C., 1999. Onion growth and yield are influenced by transplant tray cell size and age. Scientia Horticulturae 80:133-143

Linkies, A., Müller, K., Morris, K., Turečková, V., Wenk, M., Cadman, C.S., Corbineau, F., Strnad, M., Lynn, J.R., and Finch-Savage, W.E., 2009. Ethylene interacts with abscisic acid to regulate endosperm rupture during germination: a comparative approach using Lepidium sativum and *Arabidopsis thaliana*. The Plant Cell 21:3803-3822.

Lloret, P.G. and Pulgarín, A., 1992. Effect of naphthaleneacetic acid on the formation of lateral roots in the adventitious root of *Allium cepa*: number and arrangement of laterals along the parent root. Canadian Journal of Botany 70:1891-1896.

Locke, J.M., Bryce, J.H., and Morris, P.C., 2000. Contrasting effects of ethylene perception and biosynthesis inhibitors on germination and seedling growth of barley (*Hordeum vulgare* L.). Journal of experimental botany 51:1843-1849.

Longbrake, T., 1972. The effect of seeding date (daylength) on yield, size and quality of 20 onion cultivar in South Texas. Texas A&M University, College Station, TX, MSc.

Longbrake, T., Hartz, T., Cotner, S., Roberts, R., Parsons, J., Stockton, A., Cartwright, B., and Amador, J., 1987. Keys to Profitable Onion Production in Texas. Texas FARMER Collection.

Lopes, J.F., 1987. Effects of planting and spacing on several characteristics of short day onion varieties grown in South Texas. Texas A&M, College Station, TX, Doctor of philosophy.

Matilla, A.J., 2000. Ethylene in seed formation and germination. Seed Science Research 10:111-126.

Mazza, G. and Miniati, E., 1993. Anthocyanins in fruits, vegetables, and grains. CRC press. Boca Raton, FL.

McGeary, D.J., 1985. The effects of plant density on the shape, size, uniformity, soluble solids content and yield of onions suitable for pickling. Journal of Horticultural Science 60:83-87.

Mergemann, H. and Sauter, M., 2000. Ethylene induces epidermal cell death at the site of adventitious root emergence in rice. Plant Physiology 124:609-614.

Mettananda, S.A. and Fordham, Sr., 1999. The effects of plant size and leaf number on the bulbing of tropical short-day onion cultivars (*Allium cepa* L.) under controlled environments in the United Kingdom and tropical field conditions in Sri Lanka. The Journal of Horticultural Science and Biotechnology 74:622-631.

Middleton, E., Kandaswami, C., and Theoharides, T.C., 2000. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacological Reviews 52:673-751.

Miransari, M. and Smith, D., 2014. Plant hormones and seed germination. Environmental and Experimental Botany 99:110-121.

Mohan, S. and Howard, F., 1995. Compendium of onion and garlic diseases. The American Phytopathological Society. St. Paul, MN.

Negi, S., Ivanchenko, M.G., and Muday, G.K., 2008. Ethylene regulates lateral root formation and auxin transport in *Arabidopsis thaliana*. The Plant Journal 55:175-187.

Patil, D., Dhake, A., Sane, P., and Subramaniam, V. 2012. Studies on different genotypes and transplanting dates on bulb yield of high solid white onion (*Allium cepa* L.) under short-day conditions. Acta Horticulturae 969:143-148.

Peffley, E.B., Ortiz, M., and Corgan, J.N., 1981. A technique for onion cold hardiness evaluation: effects of plant age and size on hardiness. HortScience 16:773.

Penning De Vries, F.W.T, Jansen D.M. ten Berge H.F and Bakena, A. 1989. Stimulation of echophysiological processes of growth in several annual crops. Simulation Monographs. PUDOC, Wageningen, The Netherlands.

Petruzzelli, L., Coraggio, I., and Leubner-Metzger, G., 2000. Ethylene promotes ethylene biosynthesis during pea seed germination by positive feedback regulation of 1-aminocyclo-propane-1-carboxylic acid oxidase. Planta 211:144-149.

Petruzzelli, L., Sturaro, M., Mainieri, D., and Leubner-Metzger, 2003. Calcium requirement for ethylene-dependent responses involving 1-aminocyclopropane-1-carboxylic acid oxidase in radicle tissues of germinated pea seeds. Plant, Cell & Environment 26:661-671.

Pike, L.M., 1986. Onion Breeding, p. 357-394. In: M.J., B. (ed.), Breeding vegetables. AVI, Wesport, CO.

Platenius, H. and Knott, J., 1941. Factors affecting onion pungency. Journal of Agricultural Research 35:681-684.

Poupart, J., Rashotte, A.M., Muday, G.K., and Waddell, C.S., 2005. The rib1 mutant of Arabidopsis has alterations in indole-3-butyric acid transport, hypocotyl elongation, and root architecture. Plant Physiology 139:1460-1471.

Prior, R.L., Wu, X., and Schaich, K., 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry 53:4290-4302.

Rahman, A., Bannigan, A., Sulaman, W., Pechter, P., Blancaflor, E.B., and Baskin, T.I., 2007. Auxin, actin and growth of the *Arabidopsis thaliana* primary root. The Plant Journal 50:514-528.

Richwine, P., 1990. Effect of Fall Planting Date on Bulb Yield of Sweet Winter'Onion in the Rolling Plains of Texas. HortScience 25:981-981.

Rubatzky, V. and Yamaguchi, M., 1997. World vegetables principles, production, and nutritive values. AVI, Wesport, CT.

Saleh, A.N., 1981. The effect of kinetin on the indoleacetic acid level and indoleacetic acid oxidase activity in roots of young plants. Physiologia Plantarum 51:399-401.

Sanderson, J., McLauchlan, W.R., and Williamson, G., 1999. Quercetin inhibits hydrogen peroxide-induced oxidation of the rat lens. Free Radical Biology and Medicine 26:639-645.

Satoh, S. and Esashi, Y., 1983. Ethylene production, 1-aminocyclopropane-l-carboxylic acid content and its conversion to ethylene in axial segments of dormant and nondormant cocklebur seeds. Plant and cell physiology 24:883-887.

Schwimmer, S. and Weston, W.J., 1961. Enzymatic development of pyruvic acid in onion as a mesuare of pungency. Journal of Agricultural and Food Chemistry 9:301-304.

Shinohara, T., Martin, E., and Leskovar, D., 2016. Ethylene regulators influence germination and root growth of globe artichoke seedlings exposed to heat stress conditions. Seed Science and Technology 45:1-12.

Silverstone, A.L., Jung, H.-S., Dill, A., Kawaide, H., Kamiya, Y., and Sun, T.-p., 2001. Repressing a repressor gibberellin-induced rapid reduction of the RGA protein in *Arabidopsis.* The Plant Cell 13:1555-1566.

Slimestad, R., Fossen, T., and Vågen, I.M., 2007. Onions: a source of unique dietary flavonoids. Journal of agricultural and food chemistry 55:10067-10080.

Stajner, D. and Varga, I.S., 2003. An evaluation of the antioxidant abilities of *Allium* species. Acta Biologica Szegediensis 47:103-106.

Stenlid, G., 1982. Cytokinins as inhibitors of root growth. Physiologia Plantarum 56:500-506.

Stoffella, P., 1996. Planting arrangement and density of transplants influence sweet spanish onion yield and bulb size. HortScience 31:1126-1130.

Sullivan, D.M., Brown, B.D., Shock, C.C., Horneck, D.A., Stevens, R.G., Pelter, G.Q., and Feibert, E.B.G., 2001. Nutrient management for onions in the pacific northwest. Pacific Northwest Extension. Ext. Publi. 546.

Taiz, L., Zeger, E., Moller, I., and Murphy, A., 2010. Plant Physiology. Sinauer Associates, Santa Cruz, CA.

Tei, F., Scaife, A. and Aikman, D.P 1996. Growth of letucce, onion and red bet. 1. Growth analysis, light interception, and radiation use efficiency. Annals of Botany 78:633-643.

Tanimoto, E., 2005. Regulation of root growth by plant hormones—Roles for auxin and gibberellin. Critical Review of Plant Science 24:249-265.

Trademap, 2016. Import & export values, volumes, growth rates, market shares, etc. International Trade Center.

USDA. 2016. Vegetables and pulses production yearbook data (online). USDA-National Agriculture Statistics Service. 10 July 2016. https://www.usda.gov/

Wall, M.M. and Corgan, J.N., 1992. Relationship between pyruvate analysis and flavor perception for onion pungency determination. HortScience 27:1029-1030.

Wareing, P., 2016. Endogenous cytokinins as growth regulators. Perspectives in Experimental Biology 2:103-109.

Weerasinghe, S. and Fordham, R. 1993. The effects of plant density on onions established from multiseeded transplants. Acta Horticulturae 371:97-104.

Werner, T., Motyka, V., Laucou, V., Smets, R., Van Onckelen, H., and Schmülling, T., 2003. Cytokinin-deficient transgenic *Arabidopsis* plants show multiple developmental alterations indicating opposite functions of cytokinins in the regulation of shoot and root meristem activity. The Plant Cell 15:2532-2550.

Werner, T., Motyka, V., Strnad, M., and Schmlling, T., 2001. Regulation of Plant Growth by Cytokinin. Proceedings of the National Academy of Sciences of the United States of America 98:10487-10492.

Whalen, M.C. and Feldman, L.J., 1988. The effect of ethylene on root growth of *Zea mays* seedlings. Canadian journal of botany 66:719-723.

Yoo, K.S., Lee, E.J., and Patil, B.S., 2011. Underestimation of pyruvic acid concentrations by fructose and cysteine in 2,4-dinitrophenylhydrazine-mediated onion pungency test. Journal of Food Science 76:1136-1142.

Yoo, K., Pike, L.M., Crosby, K., Jones, R.L., and Leskovar, D.I., 2006. Differences in onion pungency due to cultivars, growth environment and bulb sizes. Scientia Horticulturae 110:144-149.

Yoo, K.S. and Pike, L.M., 1998. Determination of flavor precursos compound S-alk(en)yi-cysteine sulfoxides by a method and their distribution in *Allium* species. Scientia Horticulturae 75:1-10.

Yoo, K.S., Pike, L.M., and Hamilton, B.K., 1995. A simplified pyruvic acid analysis suitable for onion breeding programs. HortScience 3:1306.

Zhang, J., Gao, W.-Y., Wang, J., and Li, X.-l., 2012. Effects of sucrose concentration and exogenous hormones on growth and periplocin accumulation in adventitious roots of *Periploca sepium* Bunge. Acta Physiologiae Plantarum 34:1345-1351.