CONTROL OF TRANSPLANT HEIGHT IN TOMATO AND CABBAGE USING PLANT GROWTH REGULATOR PROHEXADIONE-CALCIUM

Diyar Abdullah HASSAN

Master's Thesis

Department of Horticulture

Supervisor: Assist. Prof. Dr. Nusret ÖZBAY

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REPUBLIC OF TURKEY
BİNGÖL UNIVERSITY
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This master's thesis was accepted by the following committee on 17.05.2017 with the vote unity.

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PREFACE

I would like to express my sincere thanks and appreciation to my supervisor Dr. Nusret ÖZBAY, professor of vegetable crops, Department of Horticulture, Faculty of Agriculture, and University of Bingöl for his supervision, ingenious and kind guidance, encouragement, and positive criticism during the course of this investigation and writing of the thesis. Thanks for all people who taught and sustained me throughout my life.

Diyar Abdullah HASSAN

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LIST OF SYMBOLS AND ABBREVIATIONS

%	: Percentage
°C	: Celsius degree
ABA	: Abscisic Acid
BAP	: Benzyl Amino Purine
Cm	: Centimeter
cm^2	: Square Centimeter
DAP	: Day after planting
FAO	: United Nations Food and Agriculture Organization
FAOSTAT	: The Food and Agriculture Organization Corporate Statistical Database
g	: gram
GA	: Gibberellik Acid
ha	: Hectare
Kg	: Kilogram
L	: Liter
mg L^{-1}	: Milligram per liter
mm	: millimeter
P	: Significance
PBZ	: Paclobutrazol
ppm	: Parts per million
Pro-Ca	: Prohexadione-Calcium
SPAD	: The Soil Plant Analysis Development
TÜİK	: Turkish Statistical Institute
µM	: Micromolar

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BİTKİ BÜYÜME DÜZENLEYİCİSİ PROHEXADIONE-CALCIUM İLE DOMATES VE LAHANADA FİDE BOYUNUN KONTROLÜ

ÖZET

Kaliteli fidelerin üretiminde önemli olan fizyolojik olayların ayarlanmasında bitki büyüme düzenleyicilerinin önemi gederek artmaktadır. Bu tez çalışması Prohexadione-calcium (Pro-Ca)'un, farklı konsantrasyonları (0, 25, 50, 75 ve 100 mg L^{-1}) ve uygulama metotlarının (yapraktan ve topraktan), domates (*Lycopersicon esculentum* Mill) ve lahana (*Brassica oleracea* L. var. *capitata*) fidelerinin kalitesi ürerine etkilerini araştırmak amacıyla yürütülmüştür. Sonuçlar Pro-Ca uygulamalarının 25 günlük domates fidelerinde fide boyunu yapraktan uygulamada %14-44 oranında, topraktan uygulamada ise %33-53 oranında kısalttığını göstermiştir. 35 günlük domates fidelerinde bu oran yapraktan uygulamada %8-34 oranında, topraktan uygulamada ise %31-57 oranında gerçekleşmiştir. Pro-Ca uygulaması lahana fideleri üzerinde de de benzer etkide bulunmuş olup, yapraktan uygulamada %19-35 oranında, topraktan uygulamada ise %24-35 oranında kısalttığını göstermiştir. 35 günlük domates fidelerinde bu oran yapraktan uygulamada %26-41 oranında, topraktan uygulamada ise %37-46 oranında gerçekleşmiştir. Araştırmadan elde edilen bulgular düşük Pro-Ca konsantrasyonlarının verimde bir kayıp söz konusu olmadan biber fidelerinde aşırı boylanmanın kontrolü amacıyla kullanılabileceğini ortaya koymuştur.

Anahtar Kelimeler: Domates, lahana, priming, prohexadione-calcium, boy kontrolü.

CONTROL OF TRANSPLANT HEIGHT IN TOMATO AND CABBAGE USING PLANT GROWTH REGULATOR PROHEXADIONE CALCIUM

ABSTRACT

The role of plant growth regulators is become important in modulating physiological responses that will eventually lead to producing high quality seedlings. This thesis project was conducted to compare the effects of various Prohexadione-calcium (Pro-Ca) concentrations (0, 25, 50, 75 and 100 mg L^{-1}) and different application methods (foliar spray and soil drench), on the characteristics and quality of tomato *(Lycopersicon esculentum* Mill) variety (BT H2274) and cabbage (*Brassica oleracea* L. var. *capitata*) variety (Beyaz Lahana Bafra) transplants. The results showed that Pro-Ca reduced tomato seedling height by 14 – 44 % (foliar spray) and 33 – 53% (soil drench) for seedlings at age of 25 day-old plants. For tomato seedlings at age of 35 day-old plants the reduction was 8-33% (foliar spray) and 31-57% (soil drench). Applying Pro-Ca had similar effect of on cabbage seedling height; reduction by 19-35% (foliar spray), and 24-35% (soil drench) for 25 day-old seedlings. For 35 day-old cabbage seedlings the reduction was 26-41% and 37-46% for foliar spray and soil drench respectively. Pro-Ca applied either soil drench or foliar spray can be used in producing short seedlings of tomato and cabbage without affecting the productivity of the plant.

Keywords: Tomato, cabbage, priming, prohexadione-calcium, height control.

1. INTRODUCTION

Transplanting is a wide standard practice in commercial vegetable production. This practice provides the optimal growing conditions for the plants that might not be available in the field such as water and nutrient availability and, protection from insects and diseases. The production of transplants under field conditions reduces the number of acceptable and healthy seedlings because of the high temperature and humidity with low light intensity in spring and winter, while serious seedling damage occurs in summer or in high temperature conditions (Ozgur 2011). The efficient uses of hybrid seeds, which are usually expensive, are important. Moreover, seedling enhances cropping characteristics, earliness, quality, and quantity.

One major reason for vegetable seedling production for transplanting is to produce a plant that is relatively short, with a compact shoot and well-developed strong root system that provide a better chance of survival when it is transferred to the field from the protected environment. These transplants are commonly grown in a high plant density in trays in order to reduce production costs. These trays can encourage extreme elongation of the stem because of the shade avoidance responses. Hence, produced transplants will be unsuitable for shipping and transplanting (Agehara and Leskovar 2014). This high plant density causes changes in the seedling morphology because of the shade each transplant produce one to another (Garner and Björkman 1999). Shade develops weak, taller stems and produce less dry matter as they compete on less amount of light in the plant canopy; this phenomenon is referred to as shade-avoidance response (Casal 2013). These seedlings are also less compatible with transplanting machines due to their height compare to moderately-sized plants, as mechanical planters are generally designed for seedling with specific size (Bozokalfa 2008; Ozbay and Ergun 2015).

Stretching and legginess in vegetable transplants such as tomatoes and cabbages become a problem when field planting in the spring is delayed due to bad weather conditions.

High light intensities at which the transplants are grown, together with either low natural radiation levels during the rainy season and winter or high greenhouse temperature in summer, often result in badly stretched seedlings being produced (Kim et al. 2008). Therefore, eliminating this undesirable trait, excessive height of seedlings, is essential in the production process of transplants. The increasing demand on horticultural products with high quality, leads to developing various methods and techniques for achieving these goals. One possible and successful method is using plant growth regulators. Plant growth regulators have the ability to improve and control a number of physiological traits that contribute to the development in vegetative and reproductive stages (Kang *et al.* 2010). Plant growth regulators are artificial and natural compounds which are used on plants to regulate growth or development and they have various benefits. They can regulate the plant chemical composition, initiate or terminate seed, controlling growth and development, buds and tubers dormancy, root size and spreading, control plant or organ size and many more (Nickell 1994).

Berova and Zlatev (2000) had studied the physiological effect of paclobutrazol (PBZ) and how it affects the yield of 'Precador' tomato variety. The seedlings were treated at the time of pricking out with medium and foliar spray applications of PBZ at concentrations of 1.0 and 25.0 mg L^{-1} respectively. They reported that treatment with paclobutrazol resulted in height reduction and the increased thickness of the young tomato plant stems as well as the accelerated root formation. Brigard et al. (2006) reported that paclobutrazol seed soak treatment at 100 mg L^{-1} for 1 hour prevented early stretch of tomato seedlings and had limited long term effect on plant growth. The research revealed that this treatment was effective in preventing excessive seedling elongation when seeds were germinated under low light conditions.

Prohexadione-calcium (Calcium3, 5-dioxo-4 propionylcyclohexanecarboxylate) is a new plant growth regulator with low toxicity a limited persistence that inhibits the biosynthesis of growth active gibberellins, thus reducing shoot elongation (Kamiya et al. 1991; Smit et al. 2005). Its structure is similar to that of 2-oxoglutaric acid, which is the co-substrate for dioxygenases catalyzing hydroxylations involved in late stages of gibberellins biosynthesis. Prohexadione-calcium (Pro-Ca) targets mainly 3ß hydroxylation, hence its application decreases levels of GA1 and accumulates of its

immediate precursor, GA20 (Evans et al. 1999). This type of plant growth regulator can be applied in many ways; direct foliar spraying, as soil drench around seedlings or tree trunks, in paint applied to the trunk, injection of trunk (Petracek et al. 2003). It has the ability to reduce the growth of the vegetative parts of apple, sweet cherry and trees. It inhibits the biosynthesis of growth active gibberellins thus suppressing the shoot elongation (Sugar et al. 2004). In some tress like pear; the application of prohexadione-calcium has the ability to inhibit shoot elongation at early elongation stage (Elfving et al. 2003; Rademacher et al. 2004). Pro-Ca reduces shoot growth of top and side shoots (Greene 2007; Guak 2013).

Similar to trees, this plant growth regulator can regulate vegetable seedlings through suppressing plant height hence producing short seedlings with appropriate characters. In the study conducted by Ramírez et al. (2010), the effects of prohexadione-calcium as growth retardant on vegetative and productive growth as well as on the chemical properties of husk tomato (*Physalis philadelphica*) fruits were investigated. Pro-Ca was applied twice; first was done when first flower buds appeared and the other application was 20 days later at concentrations of 0, 125, 175 and 200 mg L^{-1} for both applications. The results showed suppression of stem elongation by 19.84%. Ozbay and Ergun (2015) evaluated the effects of prohexadione calcium concentrations of 0, 50, 100, or 150 mg L^{-1}on seedling growth parameters of eggplant in a greenhouse experiment. All Pro-Ca concentrations significantly reduced internode length and shoot height compared to the control treatment. Researchers reported that the treatments of 100 and 150 mg L^{-1}Pro-Ca significantly reduced yield per plant and total fruit yield, whereas the treatment of 50 mg L^{-1} did not cause any change in yield compared to the control.

Tomato (*Lycopersicon esculentum*) and Cabbage (*Brassica oleracea* var. *capitata*) are two important cash crops and produced in wide areas all around the globe. Their production was more than 161 million tons fresh fruit from 4.8 million ha, and 55 million tons of fresh heads from 2.6 million ha, respectively (FAOSTAT 2013).

The objective of the present thesis experiment was to the effects of Prohexadione-Calcium (Pro-Ca) concentrations (0, 25, 50, 75, and 100 mg L^{-1}) and application methods (foliar spray and soil drench), on growth and quality of tomato and cabbage seedlings.

2. LITERATURE REVIEW

Seedling height control is essential in vegetable production. Seedlings with longer internodes and weaker stems can be challenging. They might get damaged when transferring to the field using planting machines. Furthermore, seedlings might need more regular irrigation, especially with bare-root transplants, than sturdy ones. Hence, these disadvantages cause delayed and decreased total yield.

Two primary methods of plant height control, environmental and chemical, have been used in commercial crop production (Passian and Bennett 2001). The environmental factors can be regulated to control height in plugs by modifying light quality and quantity, temperature, water and nutrition levels (Dole and Wilkins 1999). While the environmental factors can be regulated to control plant height, some problems arise out if good environmental control is unavailable.

Chemical plant growth regulators, especially plant growth retardants can also control seedling height. The plant growth retardants can delay cell division and elongation of aerial parts the plant as well inhibit gibberellins biosynthesis, resulting in reduced internodes and vegetative growth (Magnitskiy et al. (2006).

Berova and Zlatev (2000) had studied the physiological effect of paclobutrazol (PBZ) and how it affects the yield of 'Precador' tomato variety. The seedlings were treated at the time of pricking out with medium and foliar spray applications of PBZ at concentrations of 1.0 and 25.0 mg L^{-1} respectively. They reported that treatment with paclobutrazol resulted in height reduction and the increased thickness of the young tomato plant stems as well as the accelerated root formation.

Pasian and Bennett (2001) documented that seedling height may be controlled by soaking their seeds in paclobutrazol solution. In their experiment, seeds of marigold (*Tagetes patula* L.), geranium (*Pelargonium hortorum* L.H. Bailey), and tomato (*Lycopersicon*

esculentum Mill.) were soaked for 6, 16, or 24 hours in paclobutrazol solutions of 0, 500, or 1000 mg L^{-1}. When tomato seeds were imbibed 6, 16, or 24 hours, tomato seedling heights were decreased by 61%, 37% and 76%, respectively.

In the study evaluating effects of gibberellins and prohexadione calcium (Pro-Ca) on flowering and stem elongation in cabbage (*Brassica oleracea* var. *capitata*) varieties, GA1, GA4 and Pro-Ca, a gibberellin biosynthesis inhibitor, were applied to the shoot tip of cabbage with or without cold treatment. Without any cold treatment, stem elongation was increased by gibberellins and suppressed by Pro-Ca in cabbage plants. Flowering was not induced by gibberellin or Pro-Ca without cold treatment (Hamanoa et al. 2002).

Reekie and Hicklenton (2002) reported that strawberry plant growth can be influenced by Pro-Ca, and among the different concentrations tested 62.5 mg L^{-1} sprayed to run off was effective in controlling plant height for more than 3 weeks.

Black (2004) tested Pro-Ca for suppressing runners of cold-stored dormant plants of the day-neutral strawberry cultivars 'Seascape' and 'Selva' in a spring-planted production system. Pro-Ca was applied as a foliar spray at concentration of 240 mg L^{-1}, either as a single application, or twice at a one-week interval just after pre-initiated flower bloom. Significant reduction (57 – 93%) in fall runners was observed when the prohexadione-calcium applied in high concentrations (120 and 240 mg L^{-1}).

Reekie et al. (2005) demonstrated that strawberry plants treated with prohexadione-calcium reduced shoot elongation. They foliar sprayed 70 strawberry plants of Camarosa and Sweet Charlie varieties with 62.5 mg L^{-1} prohexadione-calcium and 70 plants of the same varieties were sprayed with water as control. The height, leaf number, leaf area, of daughter plants and the total dry weights of their stems, leaves, and roots were all affected by Pro-Ca treatments. They discovered that Pro-Ca treated plants were half as tall as water treated plants.

Tsegaw et al. (2005) reported that potato treated with 0, 45.0, 67.5 and 90.0 mg paclobutrazol per plant as a foliar spray resulted in short and compact plants having dark green and thicker leaves, and increased stem and root diameters by about 52%.

Grossi *et al.* (2005) conducted experiments to determine effects of paclobutrazol on growth and fruiting responses of ornamental pepper (*Capsicum chinense*) grown in containers. In the first experiment, paclobutrazol was sprayed on the canopy at concentrations of 0, 30, 60, 90, 120 or 150 mg L^{-1}. In the second experiment, paclobutrazol were applied as medium drenches at 0, 5, 10, 15, 30 and 60 150 mg L^{-1}. In both experiments, it was reported that increasing paclobutrazol concentration decreased plant height, plant diameter, and plant dry mass but increased leaf chlorophyll content of ornamental pepper. All growth retardant treatments reduced plant height by at least 10% compared to the control plants.

The effects of 0, 250, 500, 750, or 1000 mg L^{-1} paclobutrazol seed soak and soaking times from 1 to 12 hours on tomato seed germination, seedling growth, and plant growth were tested by Brigard et al. (2006). The results of the study show that paclobutrazol seed soak treatment at 100 mg L^{-1} for 1 hour prevented early stretch of tomato seedlings and had limited long term effect on plant growth. The research revealed that this treatment was effective in preventing excessive seedling elongation when seeds were germinated under low light conditions.

Different irradiances and Pro-Ca at 0, 100, 200, or 300 mg L^{-1} were evaluated to investigate their effects on the growth and physiological parameters of tomatoes. Application of Pro-Ca was done 5 weeks after germination. Main stem length of tomatoes plants decreased in a quadratic pattern as the concentration of Pro-Ca increased. High concentration (300 mg L^{-1}) resulted in shorter plants compared to the control plants. The leaf chlorophyll concentrations were also affected by the application of Pro-Ca. The growth retardant resulted in a significant decline of the leaf chlorophyll concentration of the plants grown under the effect of various concentrations. However, significant decrease of leaf chlorophyll concentrations was found with 300 mg L^{-1} application and not in lower concentrations (Giannakoula and Ilias 2007).

Ilias et al. (2007) conducted an experiment under greenhouse conditions to identify the response of three cultivars of okra (*Abelmoschus esculentus* L.) to exogenous hormones gibberellic acid (GA3) and prohexadione calcium (Pro-Ca) applied as foliar spray. The okra transplants were sprayed with 100 μM concentration of both plant growth regulators.

Researchers reported that stem length, stem and leaf dry masses were significantly enhanced by the application of exogenous GA3, but Pro-Ca inhibited stem elongation of okra. However, application of Pro-Ca resulted in important increase of seed dry mass in Pileas and Psalidati cultivars.

Kofidis et al. (2008) investigated the effects of the two plant growth inhibitors (daminozide, prohexadione-calcium) on some growth and anatomical characteristics of coriander plant (*Coriandrum sativum* L.). Prohexadione-calcium at 100, or 200 mg L^{-1} and daminozide (Alar) at 500, or 1000 mg L^{-1} were evaluated in the study. Both daminozide, prohexadione-calcium were found effective in reducing stem elongation. Coriander plants treated with daminozide were shorter up to 25% than the control plants. Pro-Ca affected plant height even more noticeable. The response varied with the concentration used. Pro-Ca at 200 mg L^{-1} reduced stem height by 38%. They also reported that the growth retardant-treated coriander plants possessed thicker leaves and wider stems.

Under greenhouse conditions, three plant growth retardants (ethephon, uniconazole and daminozide) were evaluated on two tomato varieties ('Seokwang' and 'Seogeon') in an experiment to determine the suitable concentration and soaking duration to suppress height and hypocotyl length of seedlings. The experiment showed that seedling height and hypocotyl length were suppressed by uniconazole treatments in both tomato varieties. Of the three plant growth regulators tested, uniconazole was shown to be the most effective growth retardant followed by ethephon and daminozide. The growth of tomato seedlings was efficiently regulated by 100 mg L^{-1} uniconazole (1 day soaking) treatment (Shin et al. 2009).

Ouzounidou et al. (2010) evaluated pre- and post-harvest physiology and quality responses of green pepper (*Capsicum annuum* L. cultivar 'Standar p.13/0211003-01-Agris') to exogenous gibberellic acid (100 μM), prohexadione-Ca (100 mg L^{-1}), Cycocel (100 mg L^{-1}), and Ethephon (100 mg L^{-1}) applied as foliar sprays. Plant growth regulators were applied when pepper seedlings had nine to ten leaves. Researchers reported that 100μM GA3 was effective in promoting flowering and better for vegetative characteristics. They also reported that the above indices were significantly reduced

under Cycocel, Ethephon and Prohexadione-Calcium. Under Cycocel and Ethephon treatments significant reduction was observed by 33 and 42% respectively.

In the study conducted by Ramírez et al. (2010), the effects of prohexadione-calcium as growth retardant on vegetative and productive growth as well as on the chemical properties of husk tomato (*Physalis philadelphica*) fruits were investigated. Pro-Ca was applied twice; first was done when first flower buds appeared and the other application was 20 days later at concentrations of 0, 125, 175 and 200 mg L^{-1} for both applications. The results showed suppression of stem elongation by 19.84%.

Altintas (2011) investigated the effects of prohexadione-calcium (100 mg L^{-1}) and chlormequat chloride (2000 mg L^{-1}) on vegetative and generative growth of tomato plant. Pro-Ca was applied to plants either as a spray or medium drench, while chlormequat chloride was applied only as a spray. Results of the study showed that inhibiting effect of Pro-Ca and chlormequat chloride applications on seedling height were statistically significant and both growth retardants produced shorter seedlings compared to the control. The height reduction with chlormequat chloride treatment was 56%, while it varied from 46 to 55% with Pro-Ca treatments.

Ozgur (2011) has treated cucumber cv. 'Maraton F1' seeds with paclobutrazol (500 and 1000 mg L^{-1}), daminozide (7500 and 15000 mg L^{-1}) and chlormequat chloride (7500 and 1500 mg L^{-1}), each for 12 and 24 hours, in order to study their effect on control of elongation in cucumber seedlings. Paclobutrazol treatment caused significant height decrease at all dosages in both hypocotyl and epicotyl. Seedling inhibition ratio of height was 63.4% and 74.9% at concentration of 500 mg/L and 1000 mg/L paclobutrazol respectively.

Another experiment was established by Ramírez et al. (2012) to explain the effect of prohexadione-calcium on final plant height, yield and fruit quality in some *Solanaceae* vegetables (tested wild pepper, jalapeño pepper, tomato and husk tomato). In this trial they tested wild pepper, jalapeño pepper, tomato and husk tomato. These crops were sprayed with prohexadione-calcium at concentrations of 0 (control), 125, 175 and 200 mg L^{-1} at growth stages of 10 true leaves, 50% blossom and/or 20 days later. They confirmed that Pro-Ca was a strong retardant and reduced final plant height of the evaluated

vegetable crops.

To optimize abscisic acid (ABA) application timing for effective height control, Agehara and Leskovar (2014) examined age-dependent sensitivity of various growth variables to ABA in two water melon varieties (one diploid and one triploid) The seedlings were sprayed once with 1.9 mM ABA at 11, 18, or 25 days before transplanting or twice with 0.95 mM ABA at 25 and 18 days before transplanting. They reported that only the single-spray treatment at 25 days before transplanting (cotyledon stage) suppressed plant height by inhibiting stem elongation. This effect was similar in both watermelon cultivars with 13% to 14% reductions at the transplanting stage compared with the untreated control.

The influence of prohexadione calcium on vegetative growth and storage root yield of sweet potato (*Ipomoea batatas* Lam.) was evaluated in a study conducted by Njiti et al. (2013). Sweet potato plants were sprayed with 0 and 810 mg L^{-1} Pro-Ca twice. The first spray was after 2 weeks and the second spray was after 6 weeks of transplanting. Pro-Ca significantly decreased the length and yield of vine but the total root yield was increased compared to the control.

In the study conducted by Ramirez et al. (2015a), the effect of prohexadione-calcium was examined on jalapeño chili ('Orozco' hybrid variety). Three levels of prohexadione-calcium; 100, 125 and 175 mg/L and water as control were applied at the stage of 10 true leaves. Their result showed reduction in stem height by 30 % in the pepper plants treated with 75 mg L^{-1} Pro-Ca. This inhibitory effect disappeared at the final stage of the growing process. Stem diameter of peppers increased by 20 % with Pro-Ca at mg L^{-1}.

Gholampour et al. (2015) investigated the effects of concentration and application method of chlormequat on plant height and some other parameters ornamental cabbage and. The plants (40 days after transplanting) were sprayed and medium drenched with 500, 1000 and 1500 mg L^{-1} chlormequat (cycocel). Application of 1500 mg L^{-1} cycocel resulted in about 50% and 20% shorter plants than control plants, 60 and 90 days after transplanting, respectively.

Transplants of jalapeño pepper 'Grande hybrid' were foliar sprayed twice with prohexadione-calcium; at 10 true leaves stage and 15 days later from first application.

The concentrations were 0 (water as control), 100, 200 and 300 mg L^{-1}. The Pro-Ca significantly reduced the rate of main stem growth and diameter of pepper plants. Height control was observed due to the inhibition of gibberellins biosynthesis (Ramírez et al. 2015b).

Ozbay and Ergun (2015) evaluated the effects of prohexadione calcium concentrations of 0, 50, 100, or 150 mg L^{-1} on seedling growth parameters of eggplant in a greenhouse experiment. All Pro-Ca concentrations significantly reduced internode length and shoot height compared to the control treatment. The concentrations of 50, 100, and 150 mg L^{-1} Pro-Ca reduced shoot height by 27, 32, and 38%, respectively. Researchers also reported that the treatments of 100 and 150 mg L^{-1} Pro-Ca significantly reduced yield per plant and total fruit yield, whereas the treatment of 50 mg L^{-1} did not cause any change in yield compared to the control.

Sergiev et al. (2016) documented that prohexadione-calcium decreases shoots elongation. They showed that the length of shoots was reduced when pea seedlings with two fully developed leaves were sprayed with prohexadione-calcium at concentration of 0.01 mM.

Ramírez et al. (2016a) have used 'Jaguar' habanero pepper variety to investigate the effect of prohexadione-calcium on the vegetative growth, yield, gibberellins in the stem apex, and antioxidants content in ripen fruits. The seedling of pepper was sprayed with Pro-Ca once 10 days after transplanting or twice after 10 and 31 days of transplanting. The dosages of Pro-Ca were 0, 100, 175 and 250 mg L^{-1}. Their results indicated that Pro-Ca has temporary reduction effect on pepper seedling height stem diameter. This effect was related with a reduction in the synthesis of gibberellins GA-1, GA-4 and GA7 in the apex.

Ramírez et al. (2016b) conducted a research to determine tomato responses to plant growth regulators under greenhouse conditions. 'Raptor F1' hybrid tomato seedlings were foliar sprayed when first floral primordia appeared and they were sprayed for the second time after 15 days after first application. Treatments were; GA4/7 (50 and 100 mg L^{-1}), GA4/7 (50 and 100 mg L^{-1}), 6-BAP (50 and 100 mg L^{-1}), GA4/7 (50 mg L^{-1}) + 6-BAP (50 mg L^{-1}) and GA4/7 (100 mg L^{-1}) + 6-BAP (100 mg L^{-1}), Pro-Ca (50 mg L^{-1}), and control (water). They have reported that Pro-Ca inhibited the synthesis of gibberellins

A1, A4 and A7 thus reducing stem elongation. The researchers also indicated that the Pro-Ca increased stem diameter, number of flowers, leaves, fruits.

3. MATERIAL AND METHODS

3.1. Location

The greenhouse experiments were conducted from April 2016 to July 2016 at the Horticultural Sciences Research Center of Bingöl University, in Bingöl, Turkey (38°53'N, 40°29'E, at 1139 m altitude) (Figure 3.1).

Figure 3.1. The research center where the experiments were conducted.

3.2. Climate in Bingöl

Bingöl's climate is classified as warm and temperate. The rain in Bingöl falls mostly in the winter, with relatively little rain in the summer. The average temperature in Bingöl is 12.5 °C. Precipitation averages 823 mm. The least amount of rainfall occurs in August with an average of 5 mm. In March, the precipitation reaches its peak, with an average of

126 mm. The temperatures are highest on average in July, at around 26.8 °C. January is the coldest month of the year at -2.4 °C on average (Table 3.1, Table 3.2).

Table 3.1. Extreme maximum and minimum, average maximum and minimum, and average temperatures measured in long period (1950-2015) for Bingöl (MGM, 2016)

Months	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Extreme Maximum Temperature (°C)	13.3	16.2	22.3	30.3	33.4	39	42	41.3	37.8	32.1	25.5	22.8
Extreme Minimum Temperature (°C)	-23.2	-21.6	-20.3	-9.2	1	3.5	8.8	7.8	4.2	-2.4	-15	-25.1
Average Temperature (°C)	-2.4	-1.4	3.9	10.7	16.3	22.1	26.8	26.4	21.2	14	6.6	0.5
Average Maximum Temperature (°C)	2.1	3.6	9.2	16.4	22.8	29.3	34.5	34.5	29.7	21.5	12.5	5.0
Average Minimum Temperature (°C)	-6.1	-5.2	-0.4	5.7	10.1	14.6	19.0	18.6	13.5	8.2	2.2	-2.9
Average Sunshine Duration (h)	3.2	4.2	5.6	5.4	7.3	9.4	9.5	9.2	8.3	6.1	4.3	3.1
Precipitation (mm)	122	114	126	108	71	19	6	5	9	55	87	101

Table 3.2**.** Maximum, minimum, average temperature, and relative humidty outside and inside of the greenhouse during the experiments period in 2016

Months	**April**		**May**		**June**		**July**	
	Outside	Inside	Outside	Inside	Outside	Inside	Outside	Inside
Average Temp. (°C)	13.91	21.0	16.33	24.7	22.2	27.5	26.9	29.4
Average Max. Temp. (°C)	21.28	29.5	23.35	37.3	29.4	38.5	34.6	38.2
Average Min. Temp. (°C)	7.28	12.5	10.2	13.7	15.35	16.5	19.6	22.6
Relative Humidity (%)	48.4	52.2	57.4	60.1	43.6	52.3	33.4	50.1

3.3. Vegetal Material

Tomato seeds *(Lycopersicon esculentum* Mill. variety BT H2274, Bursa Seed Company, Turkey) and cabbage seeds (*Brassica oleracea* L. var. *capitata*, variety Beyaz Lahana Bafra, Bursa Seed Company, Turkey) were used for the experiments (Figure 3.2 and Figure 3.3).

Figure 3.2. BT H2274 tomato variety

BT H2274 is a bush type tomato. It is mid-seasonal and it can preserve its hardness when it ripens. It can be grown in outdoor within all regions in Turkey. It is Mid-early variety and harvested in 80 days. Fruits are roundish, red, and fleshy with thick skin. It is suitable for the transportation. The weight of fruit is about 160-180 g. Average yield is 60-80 tons/ha.

Figure 3.3. Beyaz Lahana Bafra cabbage variety

Beyaz Lahana Bafra is an open pollinated cabbage variety. It has high adaptation ability; it can be grown in any region. It has thin leaves with very few veins. Heads are slightly flat on top and on bottom and loose with an average of 4-6 kg. The variety needs 95-100 days after transplanting for maturity. It is mostly suitable for stuffing and pickling.

3.4. Growing Media

The mixture of peat moss and perlite [4:1 (v/v)] was used in the experiments. Peat moss is the most commonly used soilless medium. It is widely available and relatively inexpensive (Kueper 2010). Peat moss is formed from sphagnum mosses in very acid bog conditions which preserve most of the plant fiber structure. It is lightweight, relatively pathogen free, and has a high water holding capacity (Meche 2017). Perlite is a volcanic rock that is heated and expanded to become a lightweight material. Because it is heated to 760-1090 °C, it is sterile. Perlite improves drainage and aeration by creating tiny air tunnels that allow water and air to flow freely to the roots. Perlite can hold 3-4 times its weight in water, yet will not become soggy (Kueper 2010; Meche 2017). All growing media materials were purchased from E-Tartes Company (İzmir, Turkey). No pre-plant fertilization was included in the media.

Peat Moss

Perlite

Figure 3.4. Two growing media used in the experiments

3.5. The Greenhouse Experiment

The experiment was carried out at an automated and heated polycarbonate-covered greenhouse with natural daylight conditions at average day/night temperature of 29/18°C and relative humidity of 55%. The greenhouse experiment investigated the effects of prohexadione calcium (Pro-Ca) concentrations on the growth and quality of tomato and cabbage seedlings. Tomato and cabbage seeds were sown (two-seed/cell) into 45-cell plastic trays (cell volume 75 cm^3) filled with growing media filled with a media consisting of peat and perlite in the ratio of 4:1 and placed on a greenhouse bench (Figure 3.5.).

Figure 3.5. Seedlings trays placed on greenhouse benches

Irrigation of seedlings was manually performed using a sprinkler nozzle connected to a hose and performed daily or twice a day according to the environmental conditions using enough water to avoid stress in the cultivated seedlings. Seedlings were fertilized with 18-18-18 N-P-K soluble fertilizer at a rate of 100 mg L^{-1} N once a week. At the emergence of the third true leaf, tomato and cabbage seedlings were sprayed one time with 0, 25, 50, 75 or 100 mg L^{-1} Pro-Ca solutions containing 0.1% Tween 20 as a wetting agent. A solution containing 0.1% Tween 20 was also applied to the control plants.

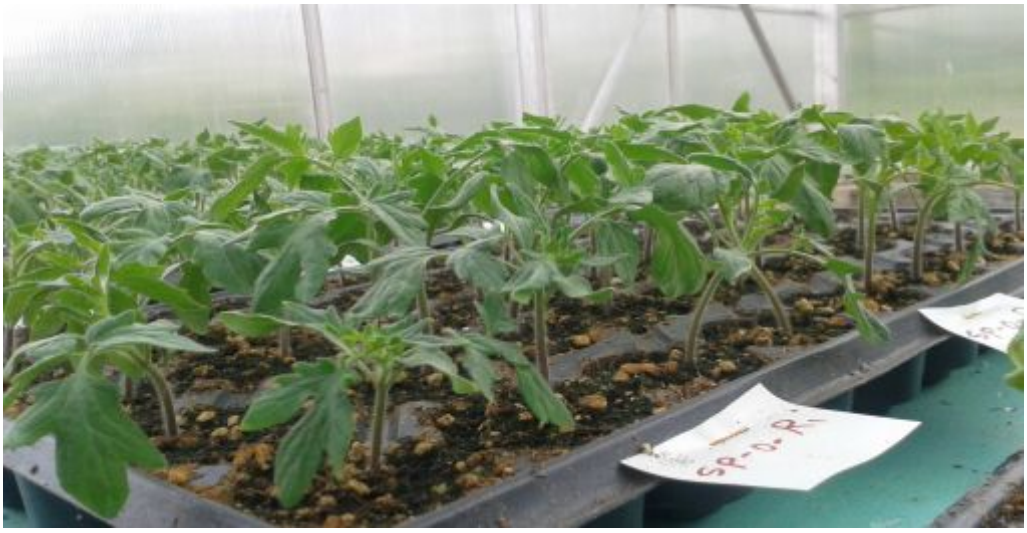

Figure 3.6. Five-week old tomato seedlings

Figure 3.7. Five-week old cabbage seedlings

As second application method, Pro-Ca (0, 25, 50, 75 or 100 mg L^{-1}) was applied as a soil drench to the growing medium (20 mL per cell) at emergence of the third true leaf. The growth period of the seedlings in the nursery was 5 weeks, until reaching commercial transplanting size (Figure 3.6. and Figure 3.7.).

3.6. Variables Determined In the Seedlings

Ten plants per treatment were randomly chosen from each replicate to determine seedling growth parameters. Plant growth measurements of the 5-week-old tomato and cabbage transplants included seedling height (at 25 and 35 day DAP), internode length (at 25 and 35 day DAP), stem diameter (measured below cotyledons), number of true leaves, root length, relative leaf chlorophyll content with a chlorophyll meter (Figure 3.8) SPAD-502 (Konica Minolta Sensing, Inc., Sakai, Osaka, Japan), leaf area using LI-3100C portable area meter (LI-COR Biosciences, Lincoln, Nebraska, USA). The growing medium was then separated from the roots, and plant organs (shoots and roots) were separately weighted to determine fresh weights. The samples were then dried in a forced-air oven (105 °C) for 24 h and recorded as dry weights.

Figure 3.8. Measurements of the relative leaf chlorophyll content tomato seedlings with a chlorophyll meter (Figure 3.8) SPAD-502

Figure 3.9. Leaf area measurement with LI-3100C portable area meter

3.7. Experimental Design and Statistics

The experiment was a 5 x 2 factorial in randomized complete design with three replications (15 plants per replication) on benches within the greenhouse. A rotation of the trays within blocks was performed daily to avoid positional bias. Mean values of the seedlings parameters were statistically analyzed by SAS ANOVA procedure to evaluate the significant effect of the Pro-Ca doses and application methods. Means were separated by using Tukey HSD Test at a significance level of $P \leq 0.05$.

4. RESULTS

The effects of Pro-Ca were determined in greenhouse via applying different concentrations of Pro-Ca with different application methods on tomato and cabbage seedlings through measuring some physiological traits.

4.1.1. Height of 25 and 35 day-old Tomato and Cabbage Seedlings

The results of the analysis of variance carried out on the relevant data of tomato and cabbage seedlings are presented in summarized form in table 4.1 and 4.2. Tomato seedling height ranged from 3.43 to 7.65 cm in 25 day-old seedlings and from 5.04 to 12.84 cm in 35 day-old seedlings (Table 4.1). There were significant differences in application methods, Pro-Ca concentrations and their interaction for 25 and 35 day-old tomato seedlings height.

Table 4.1. Effects of Pro-Ca doses and application methods on seedling heights of 25 and 35 day-old tomato seedlings in the greenhouse

	Seedling height 25 DAP (cm)			Seedling height 35 DAP (cm)		
Pro-Ca ($mg\ L^{-1}$)	Application methods			Application methods		
	Foliar Spray	Soil Drench	Mean	Foliar Spray	Soil Drench	Mean
0	7.65 a	7.27 a	7.46 a	12.84 a	11.63 ab	12.23 a
25	6.61 a	4.89 b	5.75 b	11.82 ab	8.09 d	9.95 b
50	6.54 a	4.48 bc	5.51 b	10.72 b	7.44 de	9.08 bc
75	5.00 b	3.46 c	4.23 c	10.24 bc	5.73 ef	7.98 c
100	4.31 bc	3.43 c	3.87 c	8.62 d	5.04 f	6.83 d
Mean	6.02 a	4.71 b		10.85 a	7.59 b	
Significance	ProCa =*** Method =*** ProCa x Method =**			ProCa =*** Method =*** ProCa x Method =**		

** Significant at P<0.01, *** Significant at P<0.001, Means followed by the same letter are not significantly different at the 0.05 level, using Tukey test.

The results of the study showed that all Pro-Ca concentrations significantly reduced tomato seedling height as compared to the control at both seedling ages (Figure 4.1). The higher concentrations of Pro-Ca exhibited significant reduction in 25 day-old tomato seedlings height as compared to control. The treatments of 75 and 100 mg L^{-1} Pro-Ca produced the shortest tomato seedlings (4.23 and 3.87 cm, respectively) while the control treatment (0 mg L^{-1} Pro-Ca) produced the tallest tomato seedlings (7.46 cm). With the application of 75 and 100 mg L^{-1} Pro-Ca tomato seedling height was suppressed by 43 and 48%, respectively compared to the control (Table 4.1).

Figure 4.1. Pro-Ca applied tomato seedlings

In 35 old-day tomato seedlings, the treatment of 100 mg L^{-1} Pro-Ca produced the shortest seedlings (6.83 cm). As for application methods, there were significant differences between the methods in terms of the seedling height at both seedling ages (25 and 35 day-old). Soil drench method produced shorter tomato seedlings than the foliar application method.

In 25 day-old tomato seedlings, applied as soil drench, 75 and 100 mg L^{-1} Pro-Ca treatments resulted in shortest tomato seedlings (3.46 and 3.43 cm, respectively)

compared to other treatments especially the control treatment (7.65 cm). Similarly, applied as soil drench, 100 mg L^{-1} Pro-Ca treatment produced shortest tomato seedlings (5.04 cm) compared to other treatments especially the control treatment (12.84 cm) in 35 day-old tomato seedlings (Table 4.1).

Table 4.2. Effects of Pro-Ca doses and application methods on seedling heights of 25 and 35 day-old cabbage seedlings in the greenhouse

	Seedling height 25 DAP (cm)			Seedling height 35 DAP (cm)		
Pro-Ca (mg L^{-1})	Application methods			Application methods		
	Foliar Spray	Soil Drench	Mean	Foliar Spray	Soil Drench	Mean
0	5.60	5.80	5.70 a	9.86	10.24	10.05 a
25	4.54	4.37	4.46 b	7.32	6.45	6.89 b
50	3.98	4.44	4.21 bc	6.58	6.32	6.45 bc
75	4.12	3.77	3.94 bc	6.36	5.88	6.12 cd
100	3.66	3.79	3.73 c	5.79	5.54	5.67 d
Mean	4.38	4.43		7.18	6.89	
Significance	ProCa =*** Method =^{NS} ProCa x Method =^{NS}			ProCa =*** Method =^{NS} ProCa x Method =^{NS}		

*** Significant at P<0.001, ^{NS} Non significant, Means followed by the same letter are not significantly different at the 0.05 level, using Tukey test.

Cabbage seedling height ranged from 3.66 to 5.80 cm in 25 day-old seedlings and from 5.54 to 10.24 cm in 35 day-old seedlings (Table 4.2). Significant differences were observed for the Pro-Ca levels, but the methods of application and the interaction of Pro-Ca levels and method were non-significant at both seedling ages of cabbage.

Pro-Ca concentrations significantly reduced cabbage seedling height as compared to control at both seedling ages in cabbage. When cabbage seedling height was measured at age of 25 day-old, the treatment of 100 mg L^{-1} Pro-Ca produced the shortest seedlings (3.73 cm), while the control treatment (0 mg L^{-1} Pro-Ca) produced the tallest cabbage seedlings (5.70 cm). The same result was obtained when cabbage seedlings were measured at 35 day-old. Treatment of 100 mg L^{-1} Pro-Ca produced the shortest seedlings (5.67 cm) while the control treatment (0 mg L^{-1} Pro-Ca) produced the tallest cabbage seedlings (10.05 cm).

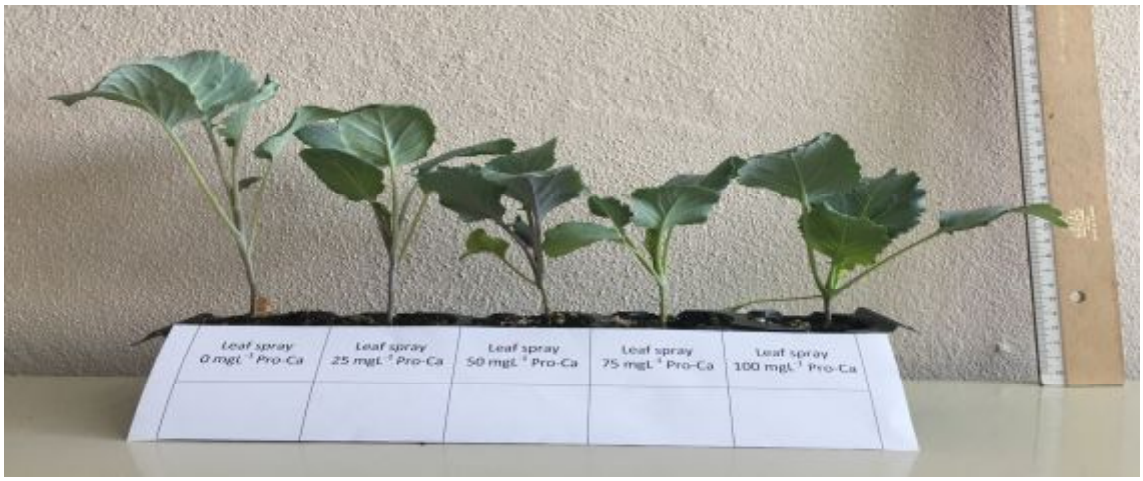

Figure 4.2. Pro-Ca applied cabbage seedlings

4.1.2. Internode Length of 25 and 35 day-old Tomato and Cabbage Seedlings

The results of the analysis of variance carried out on the relevant data of tomato and cabbage seedlings are presented in summarized form in table 4.3 and 4.4. Tomato internode lengths ranged from 0.37 to 1.62 cm in 25 day-old seedlings and from 0.66 to 2.54 cm in 35 day-old seedlings (Table 4.3).

Table 4.3. Effects of Pro-Ca doses and application methods on internode length of 25 and 35 day-old tomato seedlings in the greenhouse

	Internode length 25 DAP (cm)			Internode length 35 DAP (cm)		
Pro-Ca ($mg\ L^{-1}$)	Application methods			Application methods		
	Foliar Spray	Soil Drench	Mean	Foliar Spray	Soil Drench	Mean
0	1.62 a	1.49 a	1.56 a	2.54 a	2.22 ab	2.38 a
25	1.57 a	0.80 cd	1.18 b	2.45 ab	1.36 cd	1.90 b
50	1.14 b	0.57 ef	0.86 c	2.34 ab	1.16 d	1.75 b
75	0.97 bc	0.42 f	0.69 d	2.17 b	0.81 e	1.49 c
100	0.71 de	0.37 f	0.54 e	1.56 c	0.66 e	1.11 d
Mean	1.20 a	0.73 b		2.21 a	1.24 b	
Significance	ProCa =*** Method =*** ProCa x Method =***			ProCa =*** Method =*** ProCa x Method =***		

*** Significant at P<0.001, Means followed by the same letter are not significantly different at the 0.05 level, using Tukey test.

There were significant differences in application methods, Pro-Ca concentrations and their interaction for 25 and 35 day-old tomato internode length. The results of the study showed that all Pro-Ca concentrations significantly reduced internode length as compared to the control at both seedling ages. The higher concentrations of Pro-Ca exhibited significant reduction in 25 day-old tomato internode length as compared to control. The treatment of 100 mg L^{-1} Pro-Ca produced the shortest tomato internode (0.54 cm) while the control treatment (0 mg L^{-1} Pro-Ca) produced seedlings with tallest internodes (1.56 cm).

At 35 DAP, the treatment of 100 mg L^{-1} Pro-Ca produced shortest internode (1.11 cm) compared to control (2.38 cm). As for application methods, there were significant differences between the methods in term of the internode length at both ages (25 and 35 day-old). Soil drench method produced shorter internode than the foliar application method. In 25 day-old tomato seedlings, applied as soil drench 75 and 100 mg L^{-1} Pro-Ca treatments resulted in shorter internodes (0.42 and 0.37 cm, respectively) compared to the other treatments especially control treatment (1.62 cm). Similarly, applied as soil drench, 75 and 100 mg L^{-1} Pro-Ca treatments produced shortest internode (0.81 and 0.66 cm, respectively) compared to the other treatments especially control treatment (2.54 cm) in 35 day-old tomato seedlings (Table 4.3).

Table 4.4. Effects of Pro-Ca doses and application methods on internode length of 25 and 35 day-old cabbage seedlings in the greenhouse

	Internode length 25 DAP (cm)			Internode length 35 DAP (cm)		
Pro-Ca ($mg\ L^{-1}$)	Application methods			Application methods		
	Foliar Spray	Soil Drench	Mean	Foliar Spray	Soil Drench	Mean
0	1.80	1.81	1.80 a	1.92	1.85	1.88 a
25	1.33	1.03	1.18 b	1.45	1.19	1.32 b
50	0.98	1.08	1.03 bc	1.17	1.18	1.17 b
75	0.94	0.80	0.87 c	1.06	0.83	0.95 c
100	0.94	0.77	0.86 c	1.04	0.85	0.94 c
Mean	1.20	1.10		1.33 a	1.18 b	
Significance	ProCa =*** Method =^{NS} ProCa x Method = ^{NS}			ProCa =*** Method =** ProCa x Method =^{NS}		

** Significant at P<0.01, *** Significant at P<0.001, ^{NS} Non significant, Means followed by the same letter are not significantly different at the 0.05 level, using Tukey test.

Cabbage internode lengths ranged from 0.77 to 1.81 cm in 25 day-old seedlings and from 0.83 to 1.92 cm in 35 day-old seedlings (Table 4.4). Significant differences were observed for the Pro-Ca levels, but the methods of application and the interaction of Pro-Ca levels and method were non-significant at both seedling ages of cabbage.

The Pro-Ca concentrations significantly reduced internode length at both seedling ages of cabbage compared to control. Applying the concentration of 75 and 100 mg L^{-1} Pro-Ca produced the shortest internodes (0.87 and 0.86 cm respectively) while the control treatment (0 mg L^{-1} Pro-Ca) produced the tallest internodes with 1.80 cm (Table 4.4). However, there was no significant difference between the application methods; and also no significant interaction was observed between methods and Pro-Ca dosages in 25 day-old cabbage seedlings. When cabbage seedling measured at age of 35 day-old, the Pro-Ca concentrations and methods were significantly differed but not their interaction. 75 and 100 mg L^{-1} Pro-Ca concentration produced the shortest internodes (0.95 and 0.94 cm, respectively) while the control treatment (0 mg L^{-1} Pro-Ca) produced the tallest internode (1.88 cm). As for application methods, soil drench method produced shorter internode with 1.18 cm than foliar spray application method which is 1.33 cm.

4.1.3. Leaf Number and Leaf Area

The results of the analysis of variance carried out on the relevant data of tomato and cabbage seedlings are presented in summarized form in table 4.5 and 4.6. Tomato leaf numbers ranged from 3.91 to 4.57 in 35 day-old seedlings (Table 4.5). Significant differences were observed for the Pro-Ca levels, but the methods of application and the interaction of Pro-Ca levels and method were non-significant.

When Pro-Ca applied, the leaf number and leaf area of tomato seedlings were decreased in all Pro-Ca dosages compared to the control treatment. However, there were no significant differences among the Pro-Ca dose levels in terms of leaf number of tomato seedlings.

As for leaf area, tomato seedlings leaf area ranged from 49.95 to 88.92 cm^2 in 35 day-old seedlings (Table 4.5). There were significant differences in application methods, Pro-Ca

concentrations and their interaction in leaf area of 35 day-old tomato seedlings. The treatment of 75 and 100 mg L^{-1} Pro-Ca produced the lowest leaf area values (60.59 and 56.14 cm^2, respectively) compared to control treatment (0 mg L^{-1} Pro-Ca) which had a leaf area (87.03 cm^2). As for application methods there were significant differences between the methods in terms of seedling leaf area. Foliar spray method produced higher leaf area (77.21 cm^2) than soil drench application method (60.41 cm^2). Applied as soil drench, 100 mg L^{-1} Pro-Ca treatment resulted in the lower tomato leaf area (49.95 cm^2) compared to other treatments especially control treatment (88.92 cm^2).

Table 4.5. Effects of Pro-Ca doses and application methods on leaf number and leaf area of tomato seedlings in the greenhouse

	Leaf Number			Leaf Area (cm^2)		
Pro-Ca	Application methods			Application methods		
(mg L^{-1})	Foliar Spray	Soil Drench	Mean	Foliar Spray	Soil Drench	Mean
0	4.35	4.57	4.46 a	88.92 a	85.13 ab	87.03 a
25	4.31	4.46	4.38 a	84.47 ab	61.59 cd	73.03 b
50	4.28	4.32	4.30 ab	81.40 ab	53.15 cd	67.27 bc
75	4.00	4.20	4.10 ab	68.94 bc	52.24 d	60.59 cd
100	3.91	4.00	3.95 b	62.33 cd	49.95 d	56.14 d
Mean	4.17	4.31		77.21 a	60.41 b	
Significance	ProCa =* Method =^{NS} ProCa x Method =^{NS}			ProCa = *** Method =*** ProCa x Method =*		

* Significant at P<0.05, *** Significant at P<0.001, ^{NS} Non significant, Means followed by the same letter are not significantly different at the 0.05 level, using Tukey test.

Cabbage leaf numbers ranged from 4.28 to 5.11 cm in 35 day-old seedlings (Table 4.6). Significant differences were observed for the Pro-Ca levels and the methods of application in terms of leaf numbers, but the interaction of Pro-Ca levels and method were non-significant.

The treatment of 75 and 100 mg L^{-1} Pro-Ca produced cabbage seedlings with more leaves (4. 92 and 5.03, respectively) compare to the other treatments especially control treatment (4.37). As for application methods, there were significant differences between the methods in term of the leaf number. Soil drench method produced cabbage seedling with more leaves (4.77) than the foliar spray application method (4.61).

As for leaf area, cabbage seedlings leaf area ranged from 83.52 to 117.04 cm^2 in 35 day-old seedlings (Table 4.6). There were significant differences in Pro-Ca concentrations, application methods, and but the interaction of Pro-Ca levels and method were non-significant in leaf area of 35 day-old cabbage seedlings.

Table 4.6. Effects of Pro-Ca doses and application methods on leaf number and leaf area of cabbage seedlings in the greenhouse

	Leaf Number			Leaf Area (cm^2)		
Pro-Ca ($mg\ L^{-1}$)	Application methods			Application methods		
	Foliar Spray	Soil Drench	Mean	Foliar Spray	Soil Drench	Mean
0	4.28	4.46	4.37 d	89.07	95.03	92.05 bc
25	4.46	4.42	4.44 cd	86.12	95.94	91.03 bc
50	4.57	4.80	4.68 bc	83.52	89.60	86.56 c
75	4.77	5.06	4.92 ab	88.96	99.46	94.21 b
100	4.95	5.11	5.03 a	107.18	117.04	112.11 a
Mean	4.61 b	4.77 a		90.97 b	99.41 a	
Significance	ProCa =*** Method =* ProCa x Method = ^{NS}			ProCa =*** Method =*** ProCa x Method =^{NS}		

* Significant at P<0.05, *** Significant at P<0.001, ^{NS} Non significant, Means followed by the same letter are not significantly different at the 0.05 level, using Tukey test.

Leaf area of cabbage seedlings was increased 100 mg L^{-1} Pro-Ca application compared to the other Pro-Ca treatments. 100 mg L^{-1} Pro-Ca application Gave the highest leaf area value (112.11 cm^2), as for application methods, soil drench method produced leaves with large area (99.41 cm^2) than the foliar spray application method (90.97 cm^2).

4.1.4. Relative Leaf Chlorophyll Content

The results of the analysis of variance carried out on the relevant data of tomato and cabbage seedlings are presented in summarized form in table 4.7 and 4.8. Tomato relative leaf chlorophyll contents (SPAD) ranged from 43.29 to 49.94 SPAD in 35 day-old seedlings (Table 4.7). There were significant differences in application methods, Pro-Ca concentrations and their interaction for 35 day-old tomato chlorophyll contents.

Tomato seedlings treated with 50, 75 and 100 mg L^{-1} Pro-Ca had the highest chlorophyll contents values (46.89, 47.53 and 48.40 SPAD, respectively). The lowest chlorophyll contents values were obtained from control and 25 mg L^{-1} Pro-Ca treatments (43.45 and 43.85 SPAD, respectively). As for application methods, there were significant differences between the methods in term of relative leaf chlorophyll content of tomato seedlings. Soil drench method produced tomato seedling with higher leaf chlorophyll content (47.06 SPAD) than the foliar spray application method (44.99 SPAD). As for interaction, applying soil drench, 75 and 100 mg L^{-1} Pro-Ca treatments resulted in higher tomato seedlings leaf chlorophyll concentration (49.94 and 49.93 SPAD, respectively) compared to other treatments especially the control treatment (0 mg L^{-1} Pro-Ca) that produced lowest leaf chlorophyll content (43.29 SPAD).

Table 4.7. Effects of Pro-Ca doses and application methods on chlorophyll of tomato seedlings in the greenhouse

Pro-Ca (mg L^{-1})	Relative leaf chlorophyll content (SPAD)		
	Application methods		
	Foliar Spray	Soil Drench	Mean
0	43.62 c	43.29 c	43.45 b
25	43.77 c	43.94 c	43.85 b
50	45.58 bc	48.20 ab	46.89 a
75	45.11 bc	49.94 a	47.53 a
100	46.88 abc	49.93 a	48.40 a
Mean	44.99 b	47.06 a	
Significance	ProCa =***	Method =***	ProCa x Method =*

* Significant at P<0.05, *** Significant at P<0.001, Means followed by the same letter are not significantly different at the 0.05 level, using Tukey test.

Cabbage relative leaf chlorophyll contents (SPAD) ranged from 42.80 to 54.52 SPAD in 35 day-old seedlings (Table 4.8). There were significant differences in application methods, Pro-Ca concentrations and their interaction for 35 day-old cabbage chlorophyll contents. Cabbage seedlings treated with 75 and 100 mg L^{-1} Pro-Ca had the highest chlorophyll contents values (50.75 and 51.97 SPAD, respectively). The lowest chlorophyll contents values were obtained from control treatment (43.05 SPAD). As for application methods, there were significant differences between the methods in term of relative leaf chlorophyll content of cabbage seedlings. Soil drench method produced

cabbage seedlings with higher leaf chlorophyll content (49.88 SPAD) than the foliar spray application method (46.09 SPAD).

As for interaction, applying soil drench, 75 and 100 mg L^{-1} Pro-Ca treatments resulted in higher tomato seedlings leaf chlorophyll concentration (54.52 and 54.22 SPAD, respectively) compared to other treatments especially the control treatment (0 mg L^{-1} Pro-Ca) that produced lowest leaf chlorophyll content (42.80 SPAD).

Table 4.8. Effects of Pro-Ca doses and application methods on chlorophyll of cabbage seedlings in the greenhouse

	Relative leaf chlorophyll content (SPAD)		
Pro-Ca (mg L^{-1})	Application methods		
	Foliar Spray	Soil Drench	Mean
0	43.31 e	42.80 e	43.05 c
25	44.64 de	47.89 bc	46.26 b
50	45.81 cde	49.95 b	47.88 b
75	46.97 bcd	54.52 a	50.75 a
100	49.72 b	54.22 a	51.97 a
Mean	46.09 b	49.88 a	
Significance	ProCa =*** Method =*** ProCa x Method =***		

*** Significant at P<0.001, Means followed by the same letter are not significantly different at the 0.05 level, using Tukey test.

4.1.5. Stem Diameter and Root Length

The results of the analysis of variance carried out on the relevant data of tomato and cabbage seedlings are presented in summarized form in table 4.9 and 4.10. Tomato stem diameter values ranged from 2.82 to 3.47 mm in 35 day-old seedlings (Table 4.9). Tomato root length values ranged from 13.55 to 16.11cm in 35 day-old seedlings (Table 4.9).

There were no significant differences in Pro-Ca concentrations and Pro-Ca*method interaction for 35 day-old tomato seedlings stem diameter and root lengths. However the application method was found important for both parameters.

Table 4.9. Effects of Pro-Ca doses and application methods on stem diameter and root length of tomato seedlings in the greenhouse

	Stem Diameter (mm)			Root Length (cm)		
Pro-Ca ($mg\ L^{-1}$)	Application methods			Application methods		
	Foliar Spray	Soil Drench	Mean	Foliar Spray	Soil Drench	Mean
0	3.12	3.47	3.29	13.61	15.50	14.55
25	3.14	3.31	3.23	13.73	14.21	13.97
50	3.15	3.40	3.27	13.55	15.75	14.65
75	3.08	3.30	3.15	14.65	16.11	15.38
100	2.82	3.34	3.08	14.23	14.36	13.80
Mean	3.05 b	3.36 a		13.75 b	15.19 a	
Significance	ProCa $=^{NS}$ Method =*** ProCa x Method $=^{NS}$			ProCa $=^{NS}$ Method =** ProCa x Method $=^{NS}$		

** Significant at P<0.01, *** Significant at P<0.001, ^{NS} Non significant, Means followed by the same letter are not significantly different at the 0.05 level, using Tukey test.

There were no significant differences in Pro-Ca concentrations and Pro-Ca*method interaction for 35 day-old tomato seedlings stem diameter and root lengths. However the application method was found important for both parameters.

Soil drench method produced tomato seedlings with thicker stem (3.36 mm) than the foliar spray method which had stem diameter of 3.05 mm (Table 4.9). The longest roots were obtained with Pro-Ca applied as soil drench method (15.19 cm) while the shortest roots were produced at foliar sprays application method (13.75 cm).

Cabbage stem diameter values ranged from 2.11 to 2.42 mm in 35 day-old seedlings (Table 4.10). Cabbage root length values ranged from 14.93 to 16.15 cm in 35 day-old seedlings (Table 4.10). There were no significant differences among Pro-Ca concentrations, application methods and Pro-Ca*method interactions for 35 day-old tomato stem diameter and root lengths (Table 4.10).

Table 4.10. Effects of Pro-Ca doses and application methods on stem diameter and root length of cabbage seedlings in the greenhouse

	Stem Diameter (mm)			**Root Length (cm)**		
Pro-Ca ($mg\ L^{-1}$)	Application methods			Application methods		
	Foliar Spray	Soil Drench	Mean	Foliar Spray	Soil Drench	Mean
0	2.18	2.22	2.20	15.06	14.40	14.73
25	2.19	2.11	2.15	15.90	16.15	16.02
50	2.17	2.16	2.16	15.55	15.81	15.68
75	2.27	2.18	2.23	15.18	15.93	15.55
100	2.42	2.20	2.31	14.93	15.61	15.27
Mean	2.25	2.17		15.32	15.58	
Significance	ProCa $=^{NS}$ Method $=^{NS}$ ProCa x Method $=^{NS}$			ProCa $=^{NS}$ Method $=^{NS}$ ProCa x Method $=^{NS}$		

^{NS} Non significant, Means followed by the same letter are not significantly different at the 0.05 level, using Tukey test.

4.1.6. Shoot Fresh and Dry Weight

The results of the analysis of variance carried out on the relevant data of tomato and cabbage seedlings are presented in summarized form in table 4.11 and 4.12. Tomato shoot fresh weight values ranged from 2.78 to 4.39 g in 35 day-old seedlings (Table 4.11). Significant differences were observed for the Pro-Ca levels and the methods of application in terms of shoot fresh weights of tomato seedlings, but the interaction of Pro-Ca levels and method was non-significant.

Applying the treatment of 75 and 100 mg L^{-1} Pro-Ca produced the lowest shoot fresh weight values (3.11 and 2.94 g, respectively) and they decreased the shoot fresh weight of tomato seedlings compared to the control treatment. Rest of the Pro-Ca treatments gave the similar shoot fresh weights and there were statistically in the same group. As for application methods, there were significant difference between methods; foliar spray method produced highest shoot fresh weight (3.75 g) than soil drench application method (3.21 g).

Table 4.11. Effects of Pro-Ca doses and application methods on shoot fresh weight and shoot dry weight of tomato seedlings in the greenhouse

	Shoot Fresh Weight (g)			Shoot Dry Weight (g)		
Pro-Ca	Application methods			Application methods		
(mg L^{-1})	Foliar Spray	Soil Drench	Mean	Foliar Spray	Soil Drench	Mean
0	3.92	3.90	3.91 a	0.62 ab	0.57 abc	0.60 a
25	4.07	3.19	3.60 ab	0.69 a	0.43 cd	0.56 ab
50	4.39	3.32	3.85 a	0.70 a	0.55 abcd	0.63 a
75	3.44	2.78	3.11 bc	0.53 bcd	0.42 cd	0.48 bc
100	3.03	2.89	2.94 c	0.43 cd	0.40 d	0.41 c
Mean	3.75 a	3.21 b		0.60 a	0.47 b	
Significance	ProCa =*** Method =*** ProCa x Method =^{NS}			ProCa =*** Method =*** ProCa x Method =**		

** Significant at P<0.01, *** Significant at P<0.001, ^{NS} Non significant, Means followed by the same letter are not significantly different at the 0.05 level, using Tukey test.

Tomato shoot dry weight values ranged from 0.40 to 0.70 g in 35 day-old seedlings (Table 4.11). There were significant differences in Pro-Ca concentrations, application methods, and their interaction in 35 day-old tomato shoot dry weight values. Pro-Ca treatments did not have any positive effects on shoot dry weight of tomato seedlings, even some of treatments such as 75 and 100 mg L^{-1} decreased shoot fresh weight compared to the control. Statistically, the highest shoot dry weight values were obtained from the treatments of 0, 25 and 50 mg L^{-1} Pro-Ca (0.60, 0.56 and 0.63 g, respectively). On the other hand, the lowest shoot dry weight values were obtained from 75 and 100 mg L^{-1} Pro-Ca treatments (0.48 and 0.41 g, respectively) (Table 4.11). As for application methods, foliar spray method produced the higher shoot dry weight (0.60 g) than the soil drench method (0.47 g). As for interaction, applied as foliar spray, 25 and 50 mg L^{-1} Pro-Ca concentration resulted in highest shoot dry weight values (0.69 and 0.70 g, respectively).

Cabbage shoot fresh weight values ranged from 3.29 to 4.04 g in 35 day-old seedlings

(Table 4.12). Cabbage shoot dry weight values ranged from 0.49 to 0.59 g in 35 day-old seedlings (Table 4.12). There were no significant differences among Pro-Ca concentrations, application methods and Pro-Ca*method interactions for 35 day-old tomato shoot fresh and dry weight (Table 4.12).

Table 4.12. Effects of Pro-Ca doses and application methods on shoot fresh weight and shoot dry weight of cabbage seedlings in the greenhouse

	Shoot Fresh Weight (g)			Shoot Dry Weight (g)		
Pro-Ca ($mg\ L^{-1}$)	Application methods			Application methods		
	Foliar Spray	Soil Drench	Mean	Foliar Spray	Soil Drench	Mean
0	3.70	4.04	3.87	0.50	0.57	0.54
25	3.98	3.38	3.68	0.57	0.53	0.55
50	3.54	3.88	3.71	0.51	0.58	0.54
75	3.71	3.57	3.64	0.58	0.51	0.54
100	4.04	3.29	3.67	0.59	0.49	0.54
Mean	3.79	3.63		0.55	0.54	
Significance	ProCa = ^{NS} Method = ^{NS} ProCa x Method = ^{NS}			ProCa = ^{NS} Method = ^{NS} ProCa x Method = ^{NS}		

^{NS} Non significant, Means followed by the same letter are not significantly different at the 0.05 level, using Tukey test.

4.1.7. Root Fresh and Dry Weight

The results of the analysis of variance carried out on the relevant data of tomato and cabbage seedlings are presented in summarized form in table 4.13 and 4.14. Tomato root fresh weight values ranged from 1.21 to 1.79 g in 35 day-old seedlings (Table 4.13). The interaction between Pro-Ca doses and the application methods was significant. However, there were no significant differences among the Pro-Ca doses and between the application methods in terms of root fresh weight (Table 4.13).

Pro-Ca treatments did not have any positive effects on root fresh weight of tomato seedlings, even some of interaction treatments such as 100 mg L^{-1} Pro-Ca applied as foliar spray decreased root fresh weight compared to the control.

Tomato root dry weight values ranged from 0.11 to 0.15 g in 35 day-old seedlings (Table 4.13). An application method was found to be significantly important. The interaction

between Pro-Ca doses and the application methods was also significant. However, there were no significant differences among the Pro-Ca doses in terms of root dry weight of tomato seedlings (Table 4.13). For application methods, foliar spray method produced the higher root dry weight (0.13 g) than the soil drench application method (0.11 g).

Table 4.13. Effects of Pro-Ca doses and application methods on root fresh weight and root dry weight of tomato seedlings in the greenhouse

	Root Fresh Weight (g)			Root Dry Weight (g)		
Pro-Ca	Application methods			Application methods		
(mg L^{-1})	Foliar Spray	Soil Drench	Mean	Foliar Spray	Soil Drench	Mean
0	1.70 a	1.54 abc	1.62	0.14 abc	0.12 abcde	0.13
25	1.66 a	1.21 c	1.43	0.15 a	0.11 bcde	0.13
50	1.75 a	1.54 abc	1.65	0.14 ab	0.11 bcde	0.13
75	1.44 abc	1.59 ab	1.51	0.12 abcd	0.11 bcde	0.11
100	1.27 bc	1.79 a	1.53	0.10 de	0.12 abcd	0.11
Mean	1.56	1.53		0.13 a	0.11 b	
Significance	ProCa $=^{NS}$ Method $=^{NS}$ ProCa x Method =***			ProCa $=^{NS}$ Method =*** ProCa x Method =***		

*** Significant at P<0.001, ^{NS} Non significant, Means followed by the same letter are not significantly different at the 0.05 level, using Tukey test.

Cabbage root fresh weight values ranged from 1.12 to 1.52 g in 35 day-old seedlings (Table 4.14). There were no significant differences among the Pro-Ca doses in terms of root fresh weight of cabbage seedlings (Table 4.14). On the other hand, there were no significant differences between the application methods and interaction also was significant. The highest root fresh weight values were obtained from the treatments of 50 and 75 mg L^{-1} Pro-Ca (1.51 and 1.38 g, respectively), and the lowest root fresh weight (1.13 g) was obtained from of 100 mg L^{-1} Pro-Ca treatment in cabbage seedlings (Table 4.14).

Cabbage root dry weight values ranged from 0.11 to 0.15 g in 35 day-old seedlings (Table 4.14). The effect of Pro-Ca doses was found to be significantly important. The interaction between Pro-Ca doses and the application methods were not significant. However, there were no significant differences among the Pro-Ca doses in terms of root

dry weight of tomato seedlings (Table 4.14).

Table 4.14. Effects of Pro-Ca doses and application methods on root fresh weight and root dry weight of cabbage seedlings in the greenhouse

	Root Fresh Weight (g)			**Root Dry Weight (g)**		
Pro-Ca ($mg\ L^{-1}$)	Application methods			Application methods		
	Foliar Spray	Soil Drench	Mean	Foliar Spray	Soil Drench	Mean
0	1.35	1.15	1.25 bc	0.12	0.12	0.12 ab
25	1.27	1.12	1.19 bc	0.12	0.12	0.12 ab
50	1.50	1.52	1.51 a	0.13	0.14	0.14 a
75	1.13	1.12	1.38 ab	0.11	0.11	0.13 a
100	1.42	1.35	1.13 c	0.15	0.12	0.11 b
Mean	1.33	1.25		0.12	0.12	
Significance	ProCa = *** Method = ^{NS} ProCa x Method = ^{NS}			ProCa =* Method =^{NS} ProCa x Method =^{NS}		

* Significant at P<0.05, *** Significant at P<0.001, ^{NS} Non significant, Means followed by the same letter are not significantly different at the 0.05 level, using Tukey's test

Pro-Ca doses did not have any positive effect on cabbage seedling dry weight compared to the control, even 100 mg L^{-1} Pro-Ca treatment decreased the root dry weight of the seedlings. The lowest root dry weight value (0.11 g) was obtained from the treatment of 100 mg L^{-1} Pro-Ca.

5. DISCUSSION

The results of the study showed that all Pro-Ca concentrations significantly reduced tomato seedling height as compared to the control at both seedling ages. Pro-Ca caused reduction of tomato seedlings height by 14 - 44 % when applied as foliar spray and 33 – 53% as soil drench application for seedlings at age of 25 day-old plants. The Pro-Ca application on seedlings at age of 35 day-old plant reduced height by 8 - 33% and 31-57% for foliar spray and soil drench respectively. Our findings on tomato seedling height are close to what some other researchers found (Giannakoula and Ilias (2007; Ramírez et al. 2010, 2012, 2016; Altintas 2011). Soaking of faba bean seeds in solutions of Prohexadione-Ca caused significant reduction in shoot height and shoot fresh weight. The reduction corresponded with increasing Prohexadione-Ca concentration (Bekheta et al. 2009). Similarly, Ouzounidou et al. (2011) reported that significant inhibition of the shoot height in onion and garlic with application of Prohexadione-Ca was observed. The inhibitory effect of Prohexadione-Ca on seedling height is further supported by other researchers on petunia and impatient (Ilias and Rajapakse, 2005), tomato (Giannakoula and Ilias 2007), okra (Ilias et al. 2007), cucumber (Ergun et al. 2007), and eggplant (Ozbay and Ergun).

Pro-Ca application had similar effect on cabbage seedlings at age of 25 and 35 day-old. The tested plant growth regulator caused in an obvious reduction in seedling height in both application methods (foliar spray and soil drench). Pro-Ca caused reduction of cabbage seedlings height by 19-35 % when applied as foliar spray and 24-35% as soil drench application for seedlings at age of 25 day-old plants. The Pro-Ca application on seedlings at age of 35 day-old plant reduced height by 26 - 41% and 37-46% for foliar spray and soil drench respectively. These results are in agreement with some previous studies conducted by Hamanoa et al. (2002) and Gholampour et al. (2015) on cabbage seedling height control, which Pro-Ca caused reduction in height. Reekie et al. (2005) demonstrated that strawberry plants treated with prohexadione-calcium reduced shoot

elongation. The Pro-Ca application suppress shoot elongation without lowering plant productivity as a structural mimic of 2-oxoglutorale, thus inhibiting dioxygenases that catalyze distinct steps in gibberellin GA biosynthesis (Rademacher 2000).

The results of the study revealed that all Pro-Ca concentrations significantly reduced internode length as compared to the control at both seedling ages. These results are in agreement with some previous studies conducted on some other vegetable and fruit crops (Davis and Curry 1991; Rossini Pinto et al. 2005) PBZ reduced shoot growth by decreasing the internode length; however Cycocel decreased it by decreasing internode number. Ozbay and Ergun (2015) evaluated the effects of prohexadione calcium concentrations of 0, 50, 100, or 150 mg L^{-1} on seedling growth parameters of eggplant in a greenhouse experiment. All Pro-Ca concentrations significantly reduced internode length and shoot height compared to the control treatment.

Leaf chlorophyll concentration increased by application of Pro-Ca, thus boosting the photosynthesis rate of the leaves. Using foliar application method increases chlorophyll concentration by 7.5 % and 15 % for soil drench method for tomato seedlings. Pro-Ca applications had the same effect on cabbage seedlings, an increase in chlorophyll concentration was observed for both application methods; 15% and 27 % for foliar pray and soil drench method respectively. Similar results were reported by Gemici et al. (2000) who reported that when tomato seedlings were treated with plant growth retardant chlorophyll increased by 78-93%. However, our findings were in contrast for what Giannakoula and Ilias (2007) suggested the application of Pro-Ca caused reduction in leaf chlorophyll concentration. Significant decrease of leaf chlorophyll concentrations was found with 300 mg L^{-1} application and not in lower concentrations (Giannakoula and Ilias 2007).

Leaf number and leaf area of tomato seedlings were decreased when Pro-Ca was applied using both methods, while a significant increase in the number of the leaves and leaf area occurred when Pro-Ca applied on cabbage seedlings. These results are in agreement with some previous studies conducted on some other vegetable and fruit crops (Shin et al. 2009) leaf number and leaf area were not reduced when the seeds were treated with ethephon, but significantly reduced by uniconazole treatment. (Shin and Jeong 2002; S

till and pill 2003) they reported that leaf number and leaf area of tomato are increased as soaking period in plant growth regulator.
Our result did not showed any increasing in stem diameter and root length of tomato seedlings at any concentrations of Pro-Ca. This finding was against what Berova and Zlatev (2000), and Ramírez et al. (2016b) concluded in their experiment, as the application of plant growth regulator increased the stem thickness.

Applying the treatment of 75 and 100 mg L^{-1} Pro-Ca produced the lowest shoot fresh weight values (3.11 and 2.94 g, respectively) and they decreased the shoot fresh weight of tomato seedlings compared to the control treatment. These results are in agreement with some previous studies conducted on some other vegetable and fruit crops. (Sergive et al. 2016) shoot fresh weight slightly decreased were applied Pro-Ca. (Shin et al. 2009) shoot fresh and dry weight significantly decreased when seeds were soaking. (Grossi et al. 2000) plant dry weight decreased linearly in both sprayed and drenched.

Pro-Ca treatments did not have any positive effects on root fresh weight of tomato seedlings, even some of interaction treatments such as 100 mg L^{-1} Pro-Ca applied as foliar spray decreased root fresh weight compared to the control. These results are in agreement with some previous studies conducted on some other vegetable and fruit crops. (Shin et al. 2009) root fresh weight did not affect when compared to the control, but root dry weight reduced significantly.

6. CONCLUSION

Transplants height is important in vegetable production. Transplants with longer internodes and weaker stems can be challenging. Transplants might be damaged when establishing a field using planting machines. They also need more frequent irrigation, especially with bare-root transplants, than sturdy ones. These disadvantages mentioned above result in delayed harvest and decreased total yield, which is important for conventional production. From the viewpoint of the results of this experiment, it seems that Pro-Ca having an inhibiting effect on stem elongation on tomato and cabbage transplants would be sufficient to control stem elongation. The response varies with the applied concentration. The lower Pro-Ca (25 and 50 mg L^{-1}) with soil drench method can be used to control excessive elongation of tomato and cabbage seedlings without any major loss in transplant quality.

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RESUME

Diyar Abdullah Hassan was born in 1976 in Sulaymaneyah, Iraq. He finished primary, secondary and high school in Sulaymaneyah. In 2000 he graduated from Horticulture department-College of Agriculture, University of Slemani. He started working for the Sulaymaneyah Directorate of Agriculture Research-Ministry of Agriculture and Water Resources in 2002 as a researcher on vegetable crops. He has been working as a researcher at the same directorate since then.