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MASTER THESIS

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# INFLUENCE OF ROOTS ZONE CALCIUM ON POTATO (CV. RUSSET BURBANK) SHOOT CALCIUM AND PHYTOCHEMICAL CONTENT

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Master Thesis

Supervisor

Associate Professor Dr. Senay UGUR

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**Havva Eda Üstüntaş** tarafından **Doç. Dr. Şenay Uğur** danışmanlığında hazırlanan "**Influence of Roots Zone Calcium on Potato (Cv. Russet Burbank) Shoot Calcium and Phytochemical Content**" adlı bu çalışma jürimiz tarafından Niğde Ömer Halisdemir Üniversitesi Fen Bilimleri Enstitüsü **Bitkisel Üretim ve Teknolojileri** Ana Bilim Dalı'nda Yüksek Lisans tezi olarak kabul edilmiştir.

(The study titled "**Influence of Roots Zone Calcium on Potato (Cv. Russet Burbank) Shoot Calcium and Phytochemical Content**" and presented by **Havva Eda Üstüntaş** with the help of supervisor **Associate Professor Şenay Uğur,** has been found as Master thesis by the jury at the Department of **Plant Production and Technologies** of Nigde Ömer Halisdemir University Graduate School of Natural and Applied Sciences).

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## THESIS CERTIFICATION

I certify that the thesis has been written by me and that, to the best of my knowledge and belief. All information presented as part of this thesis is scientific and in accordance with the academic rules. Any help I have received in preparing the thesis, and all sources used, have been acknowledged in the thesis.

HAVVA EDA ÜSTÜNTAŞ

# ÖZET

KALSİYUM KONSANTRASYONUNUN PATATES BİTKİSİNİN (RUSSET BURBANK) YEŞİL AKSAMININ KALSİYUM VE FİTOKİMYASAL İÇERİKLERİ ÜZERİNE ETKİSİ

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Bitkisel Üretim ve Teknolojileri Bölümü

Danışman : Doç. Dr. Şenay UĞUR

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Kalsiyum bitki büyüme ve gelişmesindeki önemi yapılan çalışmalarla ortaya konmuştur. Bitki hücre duvarında ve zarında yapısal öneme sahip olan kalsiyum aynı zamanda hücre duvarının ve zarının arasında oluşan etkilerine bağlı olarak hücre bütünlüğünü sağlaması açısından da oldukça önemli görevleri bulunmaktadır. Sitosolik kalsiyum miktarındaki değişiklere bağlı olarak bitki bulunduğu koşullara tepki vererek kendini koruma altına almaktadır. Yapılan çalışmalar stres koşulları altında sitosolik kalsiyum miktarının değişimine bağlı olarak bitkinin ikincil metabolizma ürünlerini ürettiğini ve defense sistemini harekete geçirdiğini göstermektedir. Bitkide defense sistemi üzerine etkili olan bu bileşenlerin insan sağlığı acısından önemi de yapılan çalışmalarla ortaya konmuş durumdadır. Bu çalışmada kontrollü koşullar altında tek değişkenin kök bölgesi kalsiyum miktarı değiştirilerek bitki de üretilen insan sağlığına faydalı antioksidan değişimleri tespit edildi. Kök bölgesinin kalsiyum miktarı, bitki kalsiyum miktarı ve antioksidant bileşenler arasındaki korelasyon tespit edildi.

*Anahtar sözcükler:* TEAC, FRAP, troloks, sitosolik kalsiyum, fenolik, micro culture, Russet Burbank, toplam antioksidan

## SUMMARY

INFLUENCE OF ROOTS ZONE CALCIUM ON POTATO (CV. RUSSET BURBANK) SHOOT CALCIUM AND PHYTOCHEMICAL CONTENT



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The relationship between calcium and plant growth and development is well established. Calcium has roles on plant structure such as integrity of cell wall and cell membrane. Plant protects itself by increasing the cytosolic calcium concentration depending on the conditions. Studies shows that plant secondary metabolites are produced under stress conditions due to the change in the amount of cytosolic calcium and activates the plant defense system. These components, which are effective on the plant defense system, are also beneficial for human health. It has been found that these components are effective in protecting humans against diseases such as neurodegeneration and cancer, in which reactive oxygen species play major roles. In this study, antioxidant changes will be determined by changing the amount of calcium in the root region as a single variable under controlled conditions. The correlation between the amount of calcium in the root zone, concentratıon of calcium ın the plants and the biochemıcal components was determined ın the present study. The results showed that the root zone calcium concentration affects the tissue calcium content, phenolic level and antioxidant activity of the plants.



*Keywords:* TEAC, FRAP, trolox, cytosolic calcium, phenolic, micro culture, Russet Burbank, total antioxidant

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I am also grateful to my family (my father ***Yaşar,*** my mother ***Nebahar,*** and my brother ***Sait***) for their constant support, understanding, and encouragement.

*I love you all.*

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## SYMBOLS AND ABBREVIATIONS

| Abbreviation | Explanation |
|---|---|
| 1. Ca | Calcium |
| 2. HCl | Hydrochloric acid |
| 3. TP | Total Phenolic |
| 4. TAC | Total Antioxidant Activity |
| 5. TEAC | Trolox equivalent antioxidant capacity |
| 6. FRAP | Ferric reducing ability of plasma |

# CHAPTER I

## INTRODUCTION

Calcium plays a vital role in several biological processes and structures, such as cell membranes and cell walls, that may influence the development of calcium deficiency disorders (Palta and Schabow,2019). Plant cells can tolerate very high extracellular $Ca^{2+}$ concentration (Palta and Lee-Stadelmann, 1983) where the role of Ca at the cellular and whole plant level is well documented (Clarkson and Hanson, 1980; Tawfik, 1993; Marschner, 1995; Ozgen et al., 2006; Ozgen and Palta, 2005; Gunter et al., 2000; Ozgen et al., 2011; Busse, 2008; Hepler, 2005; Reddy and Reddy, 2004; Palta, 2013).

Ca contributes to the maintenance of cell membrane and cell wall structure by forming stable but, reversible, linkages between the polar head groups and in pectic acid fractions (Clarkson and Hanson, 1980; Marschner, 1995; Palta 1996), stabilizing the cell wall structure (Demarty et al., 1984). The natural pectic acids found in cell walls are most often in the form of $Ca^{2+}$ salts. Furthermore, extracellular $Ca^{2+}$ gives strength to bonds between cell wall and outer surface of cell membrane (Gomez-Lepe et al., 1979). Cell walls form stiff gels through $Ca^{2+}$-mediated crosslinking of its carboxyl groups through ionic and coordinate bonds with a pectin component called homogalacturonan or polygalacturonic acid (Cosgrove, 2005).

Cytosolic $Ca^{2+}$ provides a cellular signal that regulates metabolism and mediates plant responses to environmental and biotic stress (Gondwe et al., 2019). Plant growth and development have been regulated by cyotosolic free $Ca^{2+}$ level (Trewavas and Gilroy, 1991) and high $Ca^{2+}$ concentration in $Ca^{2+}$ storing organelles such as apoplast, mitochondria (Marschner, 1995). Changes in cytosolic $Ca^{2+}$ by extra- or intra- cellular signals activates $Ca^{2+}$-modulated proteins and their targets regulate variety of cellular processes (Bush, 1995; Reddy and Reddy, 2004; Harper et al., 2004).

There are many studies that demonstrate variety of stimuli alter cytosolic free $Ca^{2+}$ leading to a response in plants. One such response is flavonoid synthesis in parsley cell cultures (Frohnmeyer et al., 1999). Phenolic concentration in plants has been associated with biotic and abiotic stresses. Many studies showed that under stress conditions the

application of extracellular $Ca^{2+}$ increases the resistance of plants by elevating the phenolics in plants (Ngadze et al., 2014; Lister et al., 1996; Zhang et al., 2008; Kumar et al., 1991).

Calcium is reported to impart a major role in physiology and many plant cell signaling pathways including a transitory increase in cytosolic $Ca^{2+}$ levels (Koda et al., 1992; Raices et al., 2001, 2003; Reddy et al., 2004; Du and Poovaiah, 2005). Calcium signaling can be activated within seconds or minutes in response to quite diverse sets of stimuli (Harper et al., 2005). Increase in free cytosolic $Ca^{2+}$ concentration is one of the first events in the transduction of signals which may change various biochemical processes in plants by altering the activity of particular enzyme (Upadhyaya et al., 2013).

Calcium plays an important role of plant response to biotic and abiotic stress (Palta, 2013). Endoplasmic reticulum signals are transduced by membrane bound transcription factors which are activated and mobilized under environmental stress conditions (Liu and Howell, 2010). Sodium content decreases slightly at higher $Ca^{2+}$ levels. Nevertheless, there is variability between plant species in terms of their response to supplemental calcium under salinity (Cramer, 2004) Calcium has a role in senescence ( Poovaiah, 1979) and several plant tissue and organs have been shown to be retarded by calcium (Pooviah, 979, Ferguson et al., 1983). Calcium regulates senescence by regulating antioxidant enzyme systems (Sairam et al., 2011). Calcium also has a role in biotic stress signaling. The calcium-mediated pathogen defense have been recently reviewed. The plant defense responses are triggered by increased cytosolic $Ca^{2+}$. Recent reports have found conserved and unique response of calcium regulated genes to biotic and abiotic stresses (Narsai et al., 2013).

Calcium signals regulate a large number of abiotic stress responses (McAinsh et al., 2009), as well as stomatal aperture (Ng et al., 2001), self-incompatibility during fertilization (Franklin- Tang et al., 1993), interactions with pathogenic and symbiotic microorganisms. Calcium signals also participate in light and circadian signaling.

Calcium signals are core regulators of stomatal aperture (Ng et al., 2001). Information encoded in $[Ca^{2+}]$cyt oscillations alone can program stomatal aperture because

artificially imposed oscillations in guard cell $[Ca^{2+}]$cyt close stomata, and the $[Ca^{2+}]$cyt oscillation frequency and amplitude determine steady-state aperture (Allen et al., 2001).

Phenolic compounds are secondary plant metabolites that have a diffuse structure based on an aromatic ring with one or more hydroxyl substituents (Beckman 2000; Parr et al., 2000; Valcarcel et al., 2015). These compounds include flavonoids, phenolic acids, tannins, stilbenes, coumarins, and lignans (Ignat et al., 2011; Lemos et al., 2015) and affect the sensory qualities of plant-derived processed foods, including taste, color, and texture ( Alasalyar et al., 2001; Kroon et al., 1999; Rytel et al., 2014).

Potatoes are good sources of phenolic compounds, with total phenolic content higher than other widespread fruits and vegetables like carrots, onions, or tomatoes because of their high consumption rates (Chun et al., 2005). Nowadays, potatoes have received substantial interest as a valuable source of antioxidants because they contain a variety of secondary metabolites including phenols and are consumed in relatively high amounts (Wegener and Jansen, 2013). Phenols have been related with certain health benefits such as prevention of high cholesterol, decrease of the risk of heart disease, prevention of some types of cancer, and retardation of macular degeneration (Kita et al., 2013). The germplasm of the potato has a stunning variety in terms of the phenolic compounds (Andre et al., 2007). The phenolic compounds are present in the potato peel and flesh. The peel is reported to have the highest amount of these compounds (Ezekiel et al., 2013). Phenolic compounds in potatoes are mainly phenolic acids and flavonoids including flavonols, flavanols, and anthocyanins (DeuBer et al., 2012).

Phenolic acids are the most abundant phenolic compounds in potatoes (Habeebullah et al., 2012; Mader et al., 2009; Schieber et al., 2009; Singh et al., 2011). Between all these phenolic acids, chlorogenic acid, which is the ester of caffeic acid and quinic acid, has been substantially reported in potatoes (Mohdaly et al., 2010; Al- Weshahy et al., 2009; Amado et al., 2014; Finotti et al., 2006; Külen et al., 2013).

Chlorogenic acid, caffeic acid and ferrulic acid are the main phenolics in potato tubers (Ngadze et al., 2014). These acids have antibacterial effects and reduce growth of soft rot (Ghanekar et al., 1984; Kumar et al., 1991). Chlorogenic acid produced by plant is for defense against infection or injury (Ghanekar et al., 1984). It has been found that

caffeic acid inhibit the growth of soft rot bacteria (Kumar et al., 1991). Furthermore, combination of these two acid could reduce infection of soft rot pathogen in potatoes (Ghanekar et al., 1984; Kumar et al., 1991). Addition of calcium in soil amendment increased concentration of phenylalanine (PAL), polyphenol oxidase (PPO) and peroxidase (POD) enzymes that are involved in plant defense mechanisms (Ngadze et al., 2014).

Flavonoids represent the most common group of plant phenolic compounds and their presence influences the flavor and color of fruits and vegetables. The six significant subclasses of flavonoids are the flavones, flavonols, flavanones, flavan-3-ols, anthocyanidins, and isoflavones. Occasionally, they can be found as aglycones but most flavonoids are attached to sugars (glycosides). In potatoes, one of the most abundant flavonoids is catechin, ranging between 0 and 204 mg/100 g dry weight (Mader et al.,2009; Blessington et al., 2010; Brown et al., 2005; Leo et al., 2008; Reddivari et al., 2007). Flavonols like quercetin and kaempferol rutinose are also present in potato tubers (DeuBer et al., 2012; Andre et al., 2009; Sanchez et al., 2014; Andre et al., 2007; Navarre et al., 2011).

Glycoalkaloids are the major group of bioactive compounds in *Solanum* species. Glycoalkaloids are derivatives of solanidine (aglycone) and differ from each other in the number, type and binding site of the sugar moiety (glucose, galactose, rhamnose. High levels of glycoalkaloids in leaves are the first line of defense against pathogens and herbivores (McCue, 2009).

Potato tubers have some important characteristics that make potato a good model to study the role of calcium in plants. Since potato tubers are under ground, their transpiration is low. Therefore, the movement of water and calcium into potato tubers is low. (Palta, 1996). Potatoes are rich in potassium and phosphorus but are rather poor sources of sodium and calcium (Lampitt and Goldenberg 1940). Calcium taken up by the main root system is transported into the plant leafage and only the calcium taken in by tuber roots and stolon roots is transported into the tuber. (Kratzke and Palta 1986)

The origin of Russet Burbank was reported by Luther Burbank in 1914 as being a chimera selected from the variety Burbank by Lou Sweet. The potato cultivar 'Russet

Burbank' is used extensively around the world for french fry production, and is becoming increasingly important in the potato processing industry in New Zealand, because of the preference of a multi-national fast food restaurant chain (Martin et al., 1992).

Our objective in this study was to define the root zone $Ca^{2+}$ levels that influence the tissue $Ca^{2+}$ concentration and phytochemical content of micro culture potato shoots.

# CHAPTER II

# MATERIALS AND METHODS

## 2.1 Plant Material, Medium and Growth Conditions

The culture media was supplemented with calcium chloride (1, 5, 10, 25, 125, 200 and 400 ppm Ca) to vary the $Ca^{+2}$ concentration in the root zone (Figure 2.1). 0.5 mM myoinositol was added and pH adjusted to 5.6±0.02. Agar (0.7%) was added prior to autoclaving at (132°C) for 15 minutes. The 2nd and 3rd nodes of one month of old micropropagated potato (*Solanum tuberosum* L. cv. Russet Burbank) plantlets were grown on MS medium (Murashige and Skoog, 1962) as single node cuttings in culture tubes (Figure 2.2). The culture media was supplemented with calcium chloride (1, 5, 10, 25, 125, 200 and 400 ppm Ca) to vary the $Ca^{+2}$ concentration in the root zone (Figure 2.1). 0.5 mM myoinositol was added and pH adjusted to 5.6±0.02. Agar (0.7%) was added prior to autoclaving at (132°C) for 15 minutes. Cultures were placed, shown in Figure 2.3 under continuous light with about 60 µmol $m^{-2}s^{-1}$ photosynthetic photon flux (PPF) from cool white fluorescent lamps (Ozgen et al., 2011). Each treatment had 9 replications. Plantlets were harvested after 4 of weeks of growth in the media. Shoots from each tube were separated from their roots. Plantlets were placed in freeze dryer after grinding for further analyses.

## 2.2 Methods

**Figure 2. 1.** Preparation of MSmedium different Ca concentration

**Figure 2.2.** Micropropagation of 2nd and 3rd nodes from 4 weeks old Russet Burbank plantlets

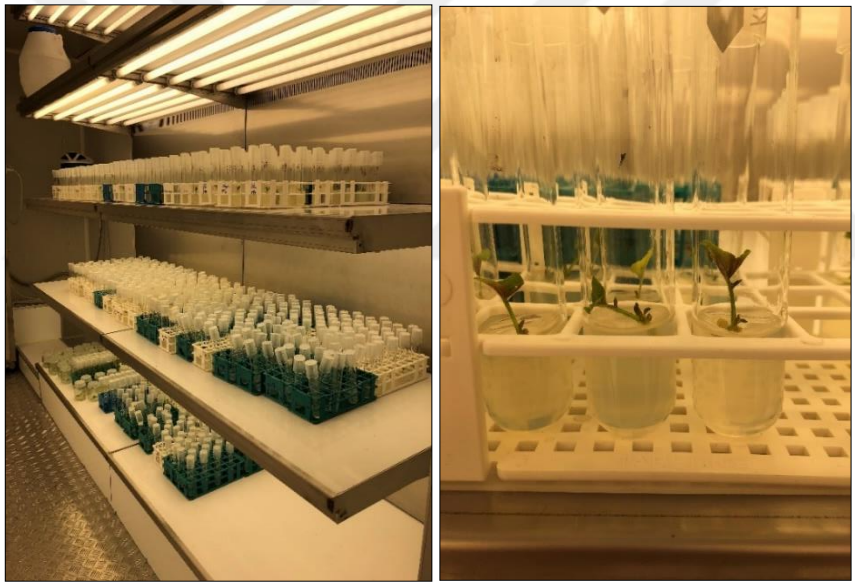

**Figure 2.3.** Single node culture placed under continuous light in the growth chamber

### 2.2.1 Total antioxidant activity (TAC)

TAC was assessed using two standard actions; FRAP (The ferric reducing ability of plasma) and TEAC (Trolox equivalent antioxidant capacity) assays.

**FRAP assay**; 0.05 grams of freeze dry potato plantlets were taken and mixed with 10ml methanol and HCl (99:1 v/v) solution and incubated for 24 h in the dark at 4˚C. After centrifugation (Figure 2.4), 0.2 ml of sample was taken into 2.8 ml FRAP solution and

FRAP procedure was applied ( Benzie and Strain 1996) described. Buffers were prepared by mixing 0.1 mol/L acetate (pH 3.6), 10 mmol/L TPTZ, and 20 mmol/L ferric chloride solutions (10:1:1). Aliquots from sample were mixed with FRAP reagent prior to analysis (Figure 2.5). After 30 min, the absorbance of the reaction mixture was determined spectrophotometrically at 593 nm (Figure 2.6).

**TEAC assay**; samples were prepared by taking 0.05 grams of potato plantlets and were added into 10 ml methanol and HCl (99:1 v/v) solution and incubated for 24 h in the dark at 4˚C and followed TEAC essay (Ozgen and Sekerci 2013). ABTS (2,2-Aniso-bis 3-ethylbenzothiazoline-6-sulfonic acid) was mixed with potassium bisulfate and allowed to stand in the dark for 12-16 hours and aliquots from the sample were mixed and incubated. After 24 h, 2.8 mL buffer was added (Figure 2.7) to 0.2 mL of the sample extract, followed by spectrophotometric measurement of absorbance at 734 nm (Figure 2.8). The antioxidant capacities of the samples were calculated using Trolox standard graph and Trolox equivalent/g as an example.

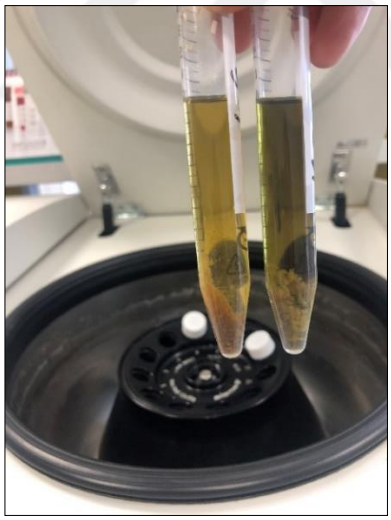

**Figure 2.4.** Centrifugation of incubated samples

**Figure 2.5.** Addition of FRAP reagent to the samples

**Figure 2.6.** Samples for the measurement of FRAP values through a spectrophotometric absorbance at 593 nm

**Figure 2.7.** Addition of TEAC buffer solution to the samples

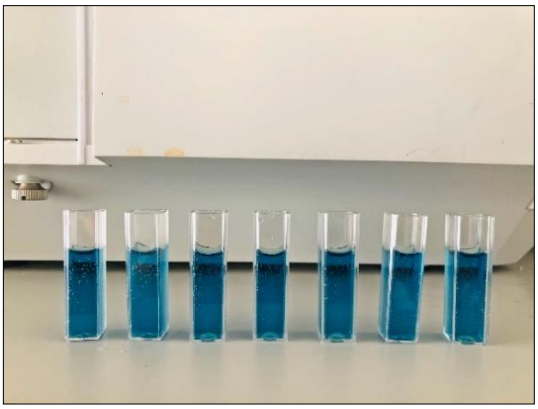

**Figure 2.8.** Samples for the measurement of TEAC values through a spectrophotometric absorbance at 734 nm. .

### 2.2.2 Total phenolic analysis (TP)

Total phenolic content was measured according to Ozgen and Sekerci (2013). 0,05 grams of potato plantlets were taken and were taken 10 ml buffer solution. Incubated for 24 h by buffer containing acetone, water and acetic acid (Figure 2.9). Samples were incubated in the dark at 4˚C. Then 0.2 ml of the sample extract was taken and Folin-Ciocalteus, phenol reagent with 3,5 ml of distilled water mixture was incubated for 8 min followed by adding 2,5 ml sodium carbonate (Figure 2.10). Then samples were incubated for two hours (Figure 2.11). The absorbance was measured at 750 nm by UV-vis spectrophotometer (Figure 2.12). The outcomes were stated as µg garlic acid equivalent fresh weight basis.

**Figure 2.9.** Incubated samples for total phenolic analysis

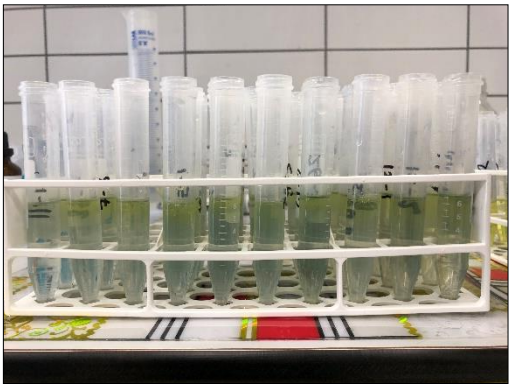

**Figure 2.10.** Samples mixed with Folin-Ciocalteus, distilled water and sodium carbonate

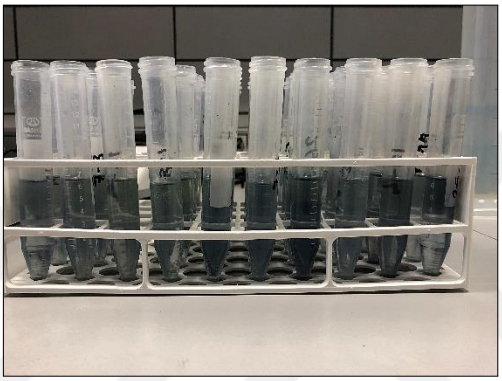

**Figure 2.11.** Samples after two hours incubation

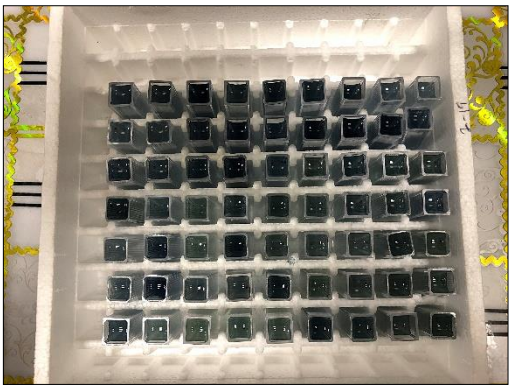

**Figure 2.12.** Samples for the measurement of phenolic values through a spectrophotometric absorbance at 750 nm.

### 2.2.3 Calcium analysis

Ca analysis has been done following the modified procedure of Jones (2001); For this purpose, 0.1 g ground samples were weighed (Figure 2.13) and ashed at 550ºC about 5h (Figure . The ash was then dissolved in 0.1 M HCl (hydrochloric acid) solution (Figure

2.15). Samples were filtred by using filter paper (Figure 2.16). Then, Calcium concentration was determined using an atomic absorption spectrophotometer (Figure 2.17).

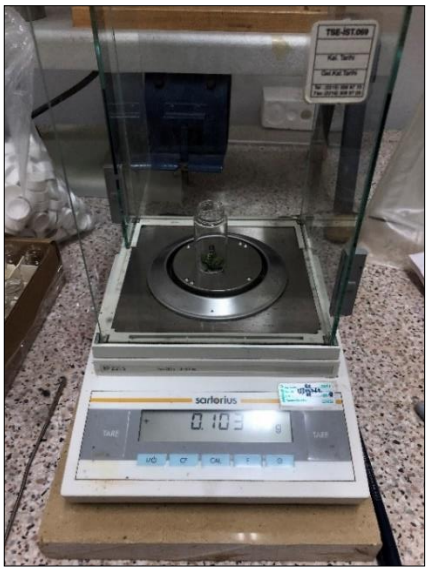


**Figure 2.13.** Weighing the samples

**Figure 2.14.** Placement of samples into the ashing oven


**Figure 2.15**. (a) Addition of HCL into ashed sample (b) ashed samples that is dissolved in HCL


**Figure 2.16.** Filtering samples dissolved in HCL solution


**Figure 2.17.** Atomic absorption spectrophotometry

# CHAPTER III

## RESULTS

Results of the study showed that shoot tip growth are dramatically affected by root zone calcium concentration (Figure 3.1 A-G). The injury occurred due to lack of a critical in vivo concentration of root zone Ca. Plantlets grown on medium with insufficient Ca concentration had an altered morphology (Figure 3.1 A-E). Plantlets with insufficient Ca concentration resulted necrosis at the shoot tips and subsequent loss of apical dominance with axillary shoot development. Plantlets with sufficient Ca concentration had shoot apex without necrosis and leaf-internode expansion occurred coordinated as a single shoot (Figure 3.1 F-G). Our results show that under the micro culture conditions a concentration of minimum 200 ppm is needed to prevent the development of lateral branching and loss of apical dominance. Furthermore, optimum growth of the shoot apex occurred at root zone Ca concentration of 200 ppm and above (Figure 3.1 F-G).


**Figure 3.1.** Pattern of Russet Burbank shoot growth at 30 days after transfer. Plantlets from 1 ppm (A), 5 ppm (B), 10 ppm (C), 25 ppm (D), 125 ppm (E), 200 ppm (F), 440 ppm (G) Ca in the root zone.

### 3.1 Total Antioxidant Activity (TAC)

Total antioxidant capacity of the plantlets were measured with the ferric reducing ability of plasma (FRAP) and trolox equivalent antioxidant power (TEAC) methods. The data of FRAP values are shown in Table 3.1 and Figure 3.2. TEAC values can be seen in Table 3.2 and Figure 3.3.

#### 3.1.1 FRAP (The ferric reducing ability of plasma)

The results showed that FRAP values of the plantlets were increased when root zone Ca increased (Table 3.1). FRAP values varied from 100.9 to 427.4 mg Trolox/g DW among the treatments. While 1, 5 and 10 ppm root zone Ca had lowest FRAP values, the highest FRAP values were recorded in 125, 200 and 400 ppm root zone Ca. The maximum value of FRAP was observed in 440 ppm (427.4 mg Trolox/g DW) whereas the lowest value was observed in 1 ppm (100.9 mg Trolox/g DW).

**Table 3.1.** Effect of medium Ca concentration on antioxidant capacity (FRAP) of plantlets foliage. The value shown are mean of nine replications. Means were compared by LSD ($p \leq 0.05$) within each column; means with the same letter do not differ significantly.

| **Ca in Media (ppm)** | **FRAP** ***(mg Trolox/g DW)*** |
|---|---|
| ***1*** | 100.9 c |
| ***5*** | 102.6 c |
| ***10*** | 121.0 c |
| ***25*** | 257.8 *b* |
| ***125*** | 414.5 a |
| ***200*** | 419.4 a |
| ***440*** | 427.4 a |

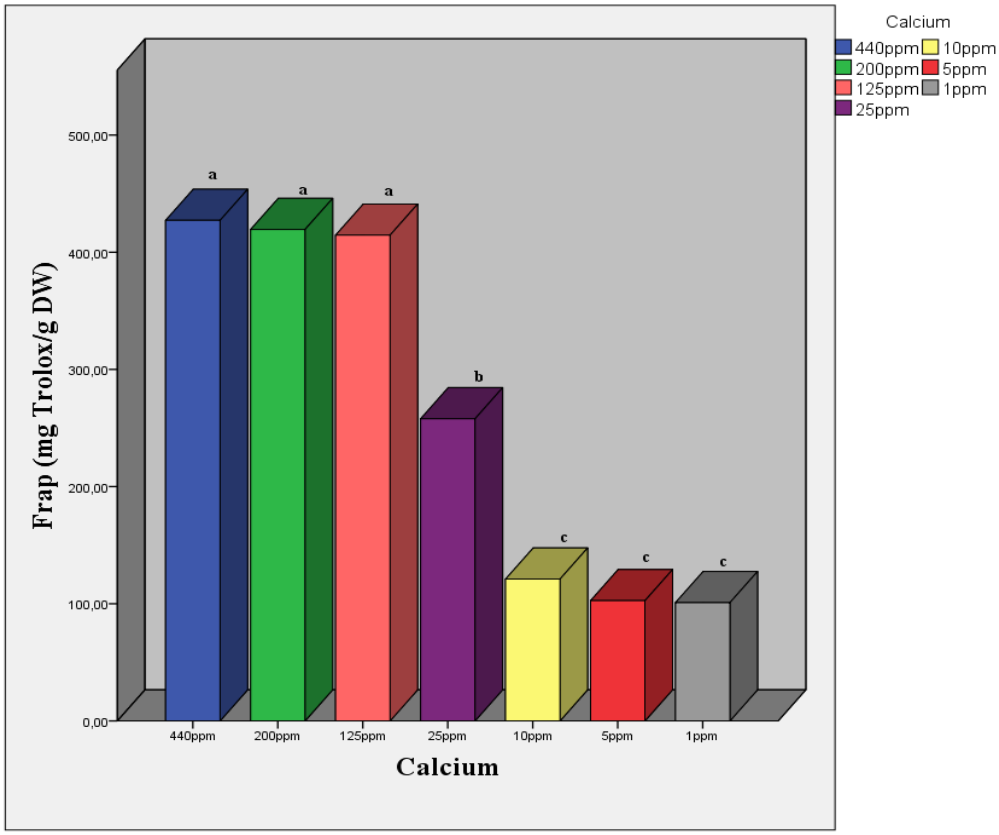


**Figure 3.2.** Relationship between root zone Ca and FRAP content of potato shoot after 4 weeks of growth in media containing varying concentration of Ca. Means were compared by LSD ($p \leq 0.05$) within each column; means with the same letter do not differ significantly.

### 3.1.2 TEAC (Trolox equivalent antioxidant capacity)

Medium with 1 ppm Ca had the highest TEAC values (84.3 mg Trolox/g DW) compared to the rest of the treatments. Lowest TEAC value (43.9 mg Trolox/g DW) was observed in plantlets grown in 440 ppm root zone Ca. There was almost two fold increase between 1 ppm Ca and 440 ppm Ca. There was no significant difference between 125 ppm and 200 ppm. According to the results, FRAP and TEAC values of plantlets were adversely related to root zone Ca. While FRAP value was the highest in 440 ppm Ca, TEAC value was the lowest in 440 ppm Ca.

**Table3.2.** Effect of medium Ca concentration on antioxidant capacity (TEAC) of plantlets foliage. The value shown are mean of nine replications. Means were compared by LSD ($p \leq 0.05$) within each column; means with the same letter do not differ significantly.

| Ca in Media (ppm) | TEAC (mg Trolox/g DW) |
|---|---|
| ***1*** | 84.3 *a* |
| ***5*** | 79.8 *b* |
| ***10*** | *74.4 c* |
| ***25*** | *61.6 d* |
| ***125*** | *56.7 e* |
| ***200*** | 54.5 e |
| ***440*** | 43.9 f |

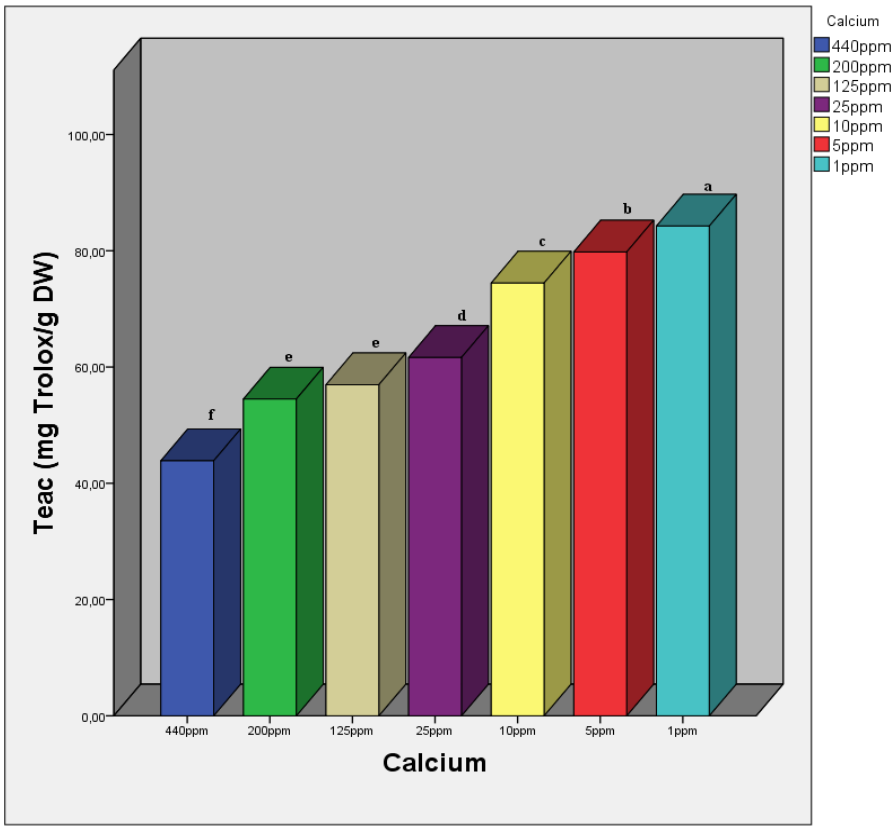


**Figure 3.3.** Relationship between root zone Ca and TEAC content of potato shoot after 4 weeks of growth in media containing varying concentration of Ca. Means were compared by LSD ($p \leq 0.05$) within each column; means with the same letter do not differ significantly.

### 3.2 Determination of Total Phenolic (TP)

Total phenolic content of the plantlets from different root zone Ca showed that low root zone Ca increased the phenolic content of the plantlets foliage (Table 3.3). Phenolic content values were 928.4, 864.6, 716.9, 700.3, 671.6, 666.4, 587.4 µg GAE/g DW at 1, 5, 10, 25, 125, 200, 400 ppm root zone Ca, respectively. Although,-10, 25, 125 and 200 ppm root zone Ca did not cause a statistically significant difference on plant phenolic content. The highest phenolic content (928.4 µg GAE/g DW) was observed in 1 ppm root zone Ca medium. The lowest phenolic content (587.5 µg GAE/g DW) was seen at 440 ppm root zone Ca medium. Our results showed that when root zone Ca concentration increased, total phenolic content of the plantlets were decreased.

**Table 3.3.** Effect of medium Ca concentration on total phenolic content of plantlets foliage. The value shown are mean of nine replications. Means were compared by LSD ($p \leq 0.05$) within each column; means with the same letter do not differ significantly.

| **Ca in Media (ppm)** | **Total Phenolic *(µg GAE/g DW)*** |
|---|---|
| ***1*** | 928.4 a |
| ***5*** | 864.6 ab |
| ***10*** | 716.9 b |
| ***25*** | 700.3 b |
| ***125*** | 671.6 b |
| ***200*** | 666.4 b |
| ***440*** | 587.5 c |

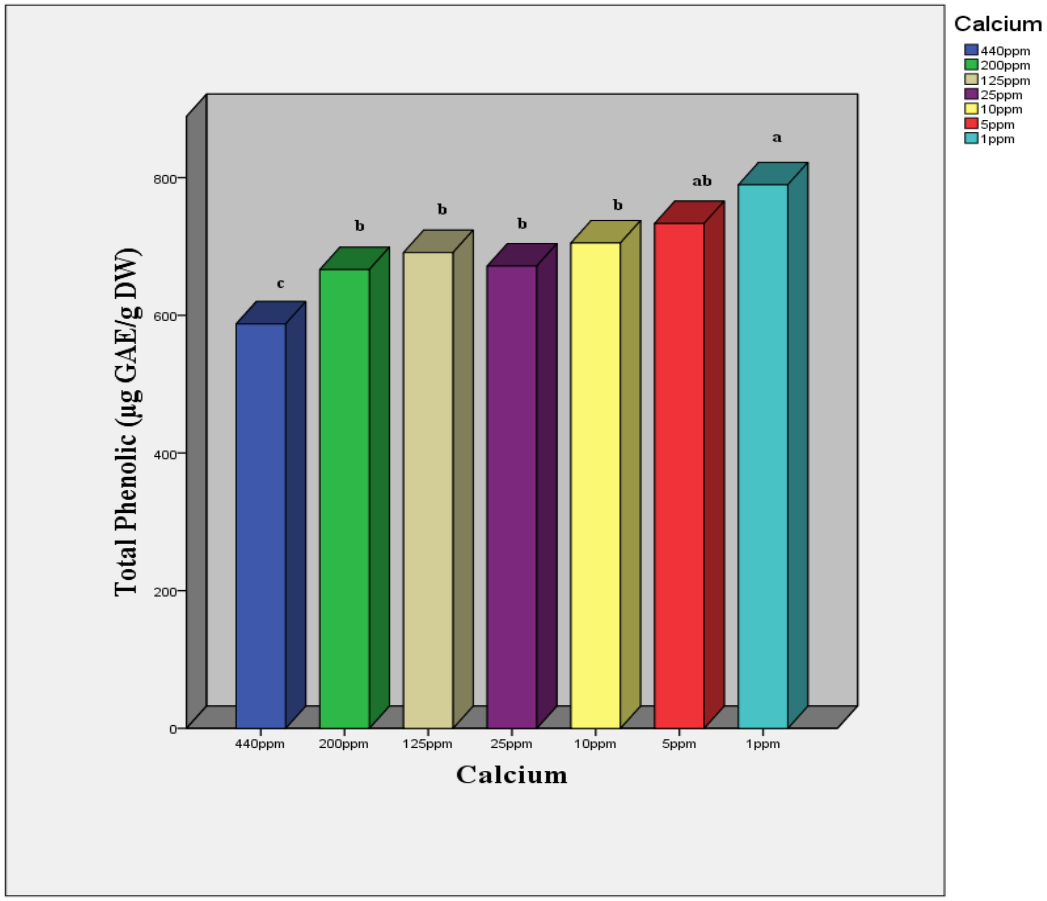


**Figure 3.4.** Relationship between root zone Ca and total phenolic content of potato shoot after 4 weeks of growth in media containing varying concentration of Ca. Means were compared by LSD ($p \leq 0.05$) within each column; means with the same letter do not differ significantly.

### 3.3 Shoot Calcium Concentration

High Ca in media resulted in an increase in shoot Ca concentrations (Table 3.4 and Figure 3.4). Mean shoot Ca concentration increased from 153.6 mg/L to 1950.5 mg/L ~~as~~ when Ca concentration in media increased from 1 ppm to 440 ppm. There was no significant difference between 125 ppm Ca and 200 ppm root zone Ca treatment. Shoot Ca concentrations were 0.017, 0.034, 0.043, 0.048, 0.073, 0.082, 0.195 at 1, 5, 10, 25, 125, 200, and 400 ppm root zone Ca concentration, respectively.

**Table3.4.** Effect of medium Ca concentration on percent tissue Ca and tissue Ca concentration as mg/L of plantlets foliage. The value shown are mean of nine replications. Means were compared by LSD ($p \leq 0.05$) within each column; means with the same letter do not differ significantly.

| Ca in Media (ppm) | Tissue Ca (%) | Tissue Ca concentration (mg / L) |
|---|---|---|
| ***1*** | 0.017 | 153.6 e |
| ***5*** | 0.034 | 326.1 d |
| ***10*** | 0.043 | 393.0 dc |
| ***25*** | 0.048 | 480.2 c |
| ***125*** | 0.073 | 726.6 b |
| ***200*** | 0.082 | 817.0 b |
| ***440*** | 0.195 | 1950.5 a |

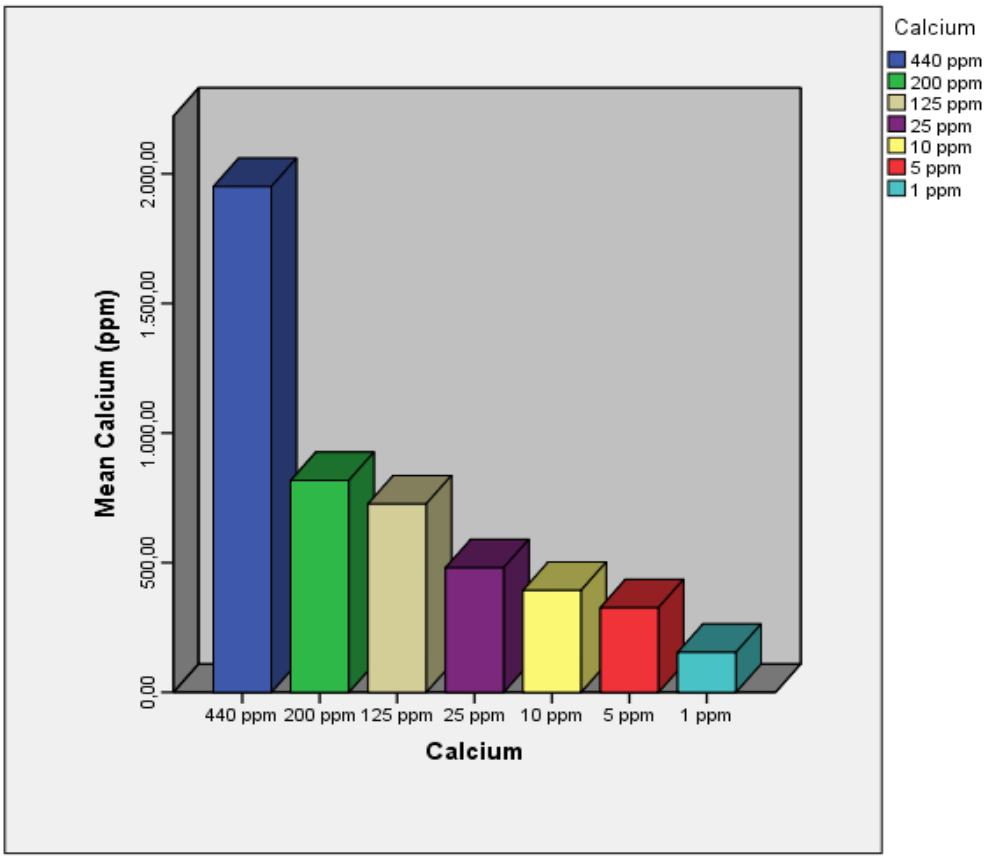


**Figure 3.5.** Relationship between root zone Ca of potato shoot after 4 weeks of growth in media containing varying concentration of Ca. Means were compared by LSD ($p \leq 0.05$) within each column; means with the same letter do not differ significantly.

# CHAPTER IV

# DISCUSSION

Results of our study showed that plantlets growth was dramatically affected by root zone Ca concentration. Our results are consistent with previous reports on Dark Red Norland potato shoot cultures (Ozgen et al., 2006; Busse et al., 2008) in which They found out that plantlets cultured in sufficient amount of Ca in medium had single shoot without apical meristem necrosis. This study and ours concluded that 200 ppm Ca in root zone was sufficient for plant to maintain apical dominance. In our study, 200 ppm Ca in root zone was given coordinated leaf and internode expansion below apical meristem resulted unbranched shoot (Figure 3.1 F).

Injury of potato sprout coming from tuber has been studied in relation to Ca deficiency (Dostal 1943, Dyson and Digby 1975a and 1975b). Results of these studies indicated that sub-apical necrosis could be prevented by Ca application at the stage of sprout emergence. We concluded that sub-apical necrosis of potato sprout was dependent on Ca deficiency in that region.

The role of Ca in plant structure and function is well documented with previous studies (Clarkson and Hanson 1980; Tawfik 1993; Marschner 1995). Calcium is a key element for maintenance of cell membrane and cell wall structure by forming stable, but reversible linkages between the polar head groups and pectic acid fractions in the cell wall (Palta 1996). It also has bridging effect of divalent Ca ions to the outer surface of the plasma membrane phospholipids to maintain membrane stability and cell integrity (Hanson 1984, Hirschi 2004). In addition, Ca known as a secondary messenger coupling stimuli in abiotic (environmental factors) and biotic (pest and disease pressure) stresses (McAinsh 2003; Sanders et al., 1999; Perombelon and Kelman 1980; McGuire and Kelman 1986; Schöber et al., 1999).

When Ca is taken by roots, it is released into the xylem and translocated from roots to the rest of the plant by cation exchange reactions (Bell and Biddulph 1963). Calcium is known as an immobile element in phloem and is distributed with water in the transpiration system (White and Broadley 2003). So, lack of Ca has an impact at tissues

with low transpiration rate, such as young leaves where rapid cell division and enlargement occur, fruits, underground tubers and portion of the plant supported by phloem rather than xylem (White and Broadley 2003).

The result of present study indicated that low Ca increased the phenolic content of the plantlets (Table 3.1). On the other hand, shoot Ca concentration was the lowest plantlets grown in low root zone Ca (Table 3.4). Thus, present data indicates that phenolic content of the plantlets could be influenced by root zone Ca concentration. It has been clearly shown that Ca is a primary nutrient associated with plant defense. Kulbat 2016 indicated that phenolic compounds has key roles in plant defense to certain stresses. Thus, it is not surprising that the plantlets Ca concentration is closely linked to plantlets phenolic content. Ngadze et al. 2014 designed a field study to show the effect of Ca on phenolic content (ferrulic acid, chlorogenic acid and caffeic acid) of potato leaves and tubers. As a result, they found a positive and significant correlation of Ca application to increase chlorogenic and caffeic acid concentration in two seasons of potato growth.

Original MS medium has 440 ppm Ca concentration in final growth medium. This amount is clearly sufficient for plantlets to grow without any anatomical and morphological changes. Manipulation of Ca concentration in MS medium, especially at low amounts, made plant to feel under stress condition. Plant have developed diverse defense mechanisms against stress factors during the evolution process. The first defense mechanisms activated is to increase in the concentration of reactive oxygen species (ROS) that have very high chemical reactivity (Bartosz 2004). Indeed, all living cells naturally contains ROS at low level due to their role in regulation of cell metabolism. For example, hydrogen peroxide being capable of diffusing into nucleus where is directly involved in transcriptional regulation of specific defense genes (Czajka 2006; De Gara et al., 2003).

Root zone Ca concentration also influenced the capacity of the plantlets (Table 3.2 and Table 3.3). Low root zone Ca increased antioxidant capacity (TEAC) in plantlets shoots compare to the high root zone Ca. In addition, plant in low root zone Ca lost the apical maintenance due to cell death (Figure 3.1 A). Phenolic compounds also have antioxidant properties and ability to quenching of free radical reactions (Foti, 2007; Bozin, 2008). Antioxidant properties of phenolics have ability to inhibit membrane lipid

peroxidation by catching alkoxyl radicals (Kulbat, 2016). Phenols, especially flavonoids, stabilize to limit the diffusion of free radicals with reducing the peroxidation of membrane lipids by binding some of integral membrane proteins and phospholipids (Michalak ,2006).

High root zone Ca increased FRAP power of the plantlets compare to the low root zone Ca. As it is expected, free antioxidant power of phenolics in high root zone Ca was able to scavenge harmful component in plantlets cell.

# CHAPTER V

## CONCLUSION

Present study is the first systematic study that relates root zone Ca concentration, shoot Ca concentration and phenolic content of the shoot under *in vitro* system. There is a strong evidence that Ca signals can modulate growth and development of plants by Ca/calmodulin regulated protein kinases (Poovaiah 1985). Evidence has been presented to show that cytoplasmic Ca concentration can regulate plant response to hormones such as auxin and gibberellins (Hepler and Wayne 1985). Since plant growth mechanisms are under hormonal control, it is possible that Ca is influencing both phenolic content and apical dominance via Ca/calmodulin type of system. Further studies could be conducted to understand the mechanism(s) of Ca regulation on total and specific plant secondary metabolites by using the system in the present study.

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## CURRICULUM VITAE

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