<u>Şerife Evrim ARICI<sup>2</sup></u>

Meryem SARI<sup>2</sup>

ÖZ

## Farklı fusarik asit ve bor konsantrasyonları altında *in vitro* da kültüre alınan patates (*Solanum tuberosum* cv. Agria) sürgünlerinin gelişimi ve antioksidan tepkileri

Bu çalışmada, in vitro da kültüre alınan patates çeşidine (Solanum tuberosum cv Agria) farklı konsantrasyonlarda uygulanan bor (B), fusarik asit (FA), B X FA interaksiyonuna karşı prolin ve peroksidaz enzim miktarı araştırılmıştır. Sürgün uçları ayrı ayrı B (0, 1, 3, 5, 10 mM), FA (0, 0.1, 0.3, 0.5 mM), 0.1 mM FA X 3 mM B iceren MS ortami üzerinde 4 hafta süreyle kültüre alınmıştır. Yapılan değerlendirmeler sonucunda 0-1 mM B, 0-0.1 mM FA içeren ortam üzerinde kültüre alınan sürgün uçlarında, sürgün uzunluğu, kök uzunluğu ve bitki yaş ağırlığı açısından gelişmenin diğer uygulamalara göre daha yüksek olduğu belirlenmiştir. 3-5 mM B, 0.3-0.5 mM FA içeren ortam üzerinde kültüre alınan sürgün uçlarında sararmalar, kök uzunluğunda azalmalar tespit edilmiştir. 0.1 mM FA X 1 mM B ve 0.1 mM FA X 3 mM B, içeren ortam üzerinde gelişen sürgünlerde kök gelişimi gözlenmemiş, fakat 0.1 mM FA X 1 mM B ortamında gelişen bitkilerde bitki boyu uzunluğu 0.1 mM FA X 3 mM B' da ki bitki boy uzunluğundan daha fazla olmuştur. Farklı konsantrasyonlarda FA, B ve FA X B uygulamasının prolin miktarını arttırdığı tespit edilmistir. 0.1, 0.3 ve 0.5 mM FA uygulamasında protein miktarının kontrol ve diğer uygulamalardan daha düşük seviyede olduğu belirlenmiştir. Bunun yanı sıra peroksidaz enzim miktarı (POD) 0.3 ve 0.5 mM FA uygulamasında, kontrol ve diğer uygulamalara kıyasla daha düşük seviyede olmuştur.

Anahtar kelimeler: Fusarium spp., cv Agria, boron, fusarik asit, peroksidaz, prolin.



<sup>&</sup>lt;sup>1</sup> This work is made from a part of the Master's Thesis

<sup>&</sup>lt;sup>2</sup> University of Suleyman Demirel, Agricultural Faculty, Department of Plant Protection, Isparta, Turkey

Corresponding author e-mail: evrimarici@sdu.edu.tr

Alınış (Received): 20.10.2016, Kabul ediliş (Accepted): 11.03.2017

## ABSTRACT

In this study, the effect of different concentrations of boron (B), fusaric acid (FA), and interaction of B X FA was investigated on the amount of proline and peroxidase enzyme of potato plants (*Solanum tuberosum* cv. Agria) cultured *in vitro*. Shoot tips of Agria were cultured into MS medium containing separately B (0, 1, 3, 5, 10 mM), FA (0, 0.1, 0.3, 0.5 mM), different FA X B (0.1 mM FA X 0.1 mM B, 0.1 mM FA X 3 mM B) for a 4 weeks. As a result, shoot length, root length, weight of plant by the 0.1 mM B and 0, 0.1 mM FA were higher than other applications. The application of 3, 5 mM B and 0.3, 0.5 mM FA caused yellowing, root length reductions. It was not observed the root growth the application of 0.1 mM FA X 1 mM B and 0.1 mM FA X 3 mM B, but the plant length application of 0.1 mM FA X 1 mM B was higher than 0.1 mM FA X 3 mM B. It was determined an increase in proline for amount the application of different FA, B and FA X B. Also, the amount of protein of different concentrations of 0.1, 0.3 and 0.5 mM FA was determined lower than other application and control plants. However, the amount of the enzyme peroxidase (POD) was found lower in the application of 0.3 and 0.5 mM FA than other application and control plant.

Keywords: Fusarium spp., cv Agria, boron, fusaric acid, peroxidase, proline.

## **INTRODUCTION**

Potato is the third most important food crop in the world after rice and wheat in terms of human consumption (Anonymous 2014). The plants are exposed to biotic and abiotic stress conditions such as pathogen attack, drought, salt, heat, and cold (Vinocur and Altman 2005, Mittler 2006). The growth, metabolism, and yield of the plants under stress conditions are negatively affected. There are quite a few diseases in the areas where potatoes are grown, and Fusarium spp. is a fungal pathogen that is quite prevalent in those areas. Fusarium spp. which causes the disease on potato plants in store houses and farms gives rise to dry rot and wilt (Anonymous 2012). The dry rot on potato is particularly caused by Fusarium solani, Fusarium sambucinum, Fusarium culmorum, Fusarium avenaceum, Fusarium oxysporum, etc. Fusarium species produce a range of mycotoxins compounds such as fusaric acid (FA), fumonisins, enniatin, moniliformin, deoxynivalenol (DON), zearalenone, and trichothecenes (Bacon et al. 1996, Idris et al. 2003, Vogelgsang et al. 2008, Jestoi et al. 2008). Mycotoxins are toxic low molecular weight compounds produced by fungi which infect food and feed. The toxic effect of mycotoxins on animal and human health is referred to as mycotoxicosis. Mycotoxins result in high economic loss to handlers, producers, processors, and marketers of crops. Mycotoxins have significant economic impacts in numerous crops, especially wheat, maize, potato, and other nut crops, cottonseed, and coffee (Vogelsang et al. 2008). It has been estimated that 25% of the world's crops are affected by mycotoxins each year, with annual losses of around 1 billion metric tons of foods and food products (Anonymous 2016a). Fusaric acid is a well-known phytotoxin that is produced by several Fusarium species that may have some detrimental effects on human and animal health (Delen

2007, López-Berges et al. 2013). *Fusarium* spp. is a quiet prevalent fungal disease in the areas in our country where potato is grown (Eken et al. 2000, Anonymous 2016b).

The compound and quantity of the elements in the soil are known as the factors that affect the development and yield in plants as well as the biotic stress in order to grow potato. The quantity of these elements negatively affects the yield and growth in plants. Boron (B) which is one of the micro nutrition elements has a significant role in yield and growth of plants. Plants are negatively affected by boron deficiency or toxicity. The level of B toxic is a problem that limits the plant farming in many parts of the world (Reid and Fitzpatrick 2009). Additionally, potato's yield is negatively affected by B toxicity in some regions in which potato is grown in our country (Eskişehir-Kırka, Eskişehir-Sultanözü, Balıkesir-Bigadiç and Kütahya-Emet) (Doğan et al. 2005).

It is known that plants accumulate proline in order to avoid dehydration stress caused by several factors such as disease, drought, and salinity. Proline is an amino acid, which the plant produced under stress conditions and played a role in defense mechanism (Yashu et al. 1997) Proline accumulation is a symptom of adaptation towards the environmental stresses such as temperature, nutritional deficiency, exposure to heavy metals, and high level of acidity (Barnett and Naylor 1966, Stewart and Lee 1974, Stewart et al. 1977, Stewart 1978, Aspinall and Paleg 1981, Öncel 1988, Ergün 2005). It has been determined that there are some changes in enzyme activities that protect the plants that are under biotic and abiotic stress from the threat of reactive oxygen species (ROS) (O'Brien et al. 2012). Peroxidase enzyme (POD) has a substantial role in vital processes such as hormonal activities in plants, defense mechanism, and adjustment of the quantity of the indole acetous acid during the growth of the vegetables and fruits and lignin biosynthesis. Some changes occur in the quantity of peroxidase enzyme under disease and abiotic stress conditions (Türkan et al. 2005, Sotiropoulos et al. 2006a, Molassiotis et al. 2006, Güçlü 2010).

Mineral elements are applied to increase crop yields and improve overall plant health and quality. Mineral nutrients have different effects on plant disease, and in many situations they are the front line of defense against disease. It is considered that plant disease is long overdue and have encouraged through mineral nutrient. The aim of this study was investigate understanding of the effect of B on Fusarium dry rot on potato. In this study, it was researched to different concentrations of boron (B), *Fusarium* spp. toxin fusaric acid (FA) reactions and interaction of B X FA applied shoot-tip culture of potato plants (*Solanum tuberosum* cv. Agria) against the increase in the amount of proline and peroxidase enzyme (POD) *in vitro*.

## MATERIAL AND METHOD

#### Plants, explants, surface sterilization, culture condition

This study was carried out in the Laboratory of Plant Pathology and Plant Cell and Tissue Culture, Department of Plant Protection, Agricultural Faculty, University of Suleyman Demirel, in Isparta. In this experiment certified seeds of potato cv. Agria (Sürde Tarım), FA (Sigma-Aldrich) and B (BOH<sub>3-</sub> Sigma Aldrich) were used. Shoot tips of Agria were surface sterilized first by washing under running tap water. Under a laminar flow shoot surface sterilized by immersing in 70% ethanol for 2 minutes, washed three times with sterilized distilled water to remove the trace of alcohol then immersed in 3% (v/v) sodium hypochlorite solution supplemented with 1-2 drops of tween 20 for 15 minutes and 5% sodium hypochlorite solution for 10 minutes, finally rinsed three times for 5 minutes each time with sterilized distilled water. Explants were cultured in magenta containing MS basal media (Murashige and Skoog 1962) supplemented with 3% saccharose + 7 g Agar (Sigma Aldrich) and the pH was adjusted to  $5.7 \pm 0.1$  with 1 M NaOH before autoclaving at 121°C, 1.2 atmosphere pressure, and 20 minutes. All cultures that were cultured into MS during all the phases of the tissue culture process were left for growth in culture rooms that were arranged for 25±1°C, 16 hours darkness and 8 hours daylight and 3000 lux light intensity.

## FA treatment

The shoot tips cv. Agria (approximately 20 mm) was used in tissue culture experiment. After FA was dissolved in purified water, it was rendered sterile by cold sterilization being filtered with a 0.22 pore  $\mu$ M span. MS media was sterilized at 121°C and 1.2 atm for 20 minutes. Sterilized FA was added with different concentration (0, 0.1, 0.3, 0.5 mM) into MS. FA was not added into the MS media in which plant material was used for control (Arici 2006). The shoot tips were cultured into the MS media containing FA. After 4 weeks, the following physiological attributes were measured.

#### **B** treatment

Different concentrations of B (1, 3, 5, 10 mM) was added into MS media so as to create B stress and sterilized at 121°C and 1.2 atm pressure for 20 minutes. The shoot tips (approximately 20 mm) of the cv. Agria type were used in tissue culture experiment. B was not added to the MS for control plant. Plantlets were cultured into the MS media containing different concentration B (0, 1, 3, 5, 10 mM). After 4 weeks, the following physiological attributes were measured.

#### FA and B treatment

B and FA concentration and combination were used that were appropriately arranged after FA and B treatments in this study. A set of morphological, physiological and biochemical changes were observed as a result of FA-B

interaction (FA X B) by adding 3 mM B and 1-3 mM FA into MS media. After 4 weeks, the following physiological attributes were evaluated.

#### **Protein determination**

Enzyme extraction was conducted according to Molassiotis et al. (2006). Ground tissues were homogenized in extraction buffer (50 mM Na-fosfat buffer; pH: 5.6, %2 PVP, 1 M NaCl), then 45 ml extraction buffer was added to 1.5 g samples and homogenized at 10.000 rpm for 30 min at 4°C. Supernatant was used for electrophoresis. Protein was estimated by the method of Bradford (1976), using bovine serum albumin as a standard.

#### **Proline determination**

Leaves taken from plants (0.5 g) were homogenized by using 10 ml 3% sulfosalicylic acid and they were filtered by Whatman No 2. Proline was determined as spectrophotometric at 540 nm (Bates et al. 1973).

### Polyacrylamid gel electrophoresis (PAGE)

Polyacrylamid gel electrophoresis (PAGE) was performed with a mini Protean II electrophoresis unit (Bio-Rad). Protein (50  $\mu$ g) from original extracts was loaded per well on polyacrylamide gel. Gels were stained for peroxidase using the method of Laemmli (1970) as described by Molassiotis et al. (2006). 7.5% separation gel and 4% stacking gel were used with the exception that SDS was omitted from all buffers. 20  $\mu$ L sample was loaded and electrophoresis was run at 150 V for about 2 h and at 4°C. POD bands were detected by immersing the gels in a solution of 3-amino-9-ethyl carbazol, 30% Hydrogen peroxide, 0.05 M Sodium acetate (pH 5.0) (Wendel and Weeden 1989).

#### **Statistical analysis**

Statistical analysis of the data was performed using the program SPSS-16 Chicago, USA) by means of one-way ANOVA. The mean values were compared with the Tukey Test ( $P \le 0.01$ ). Each treatment included at least 30 replications (tubes).

#### **RESULTS AND DISCUSSION**

#### **Determination of FA and BA concentration**

In this study, the effects of different concentrations of FA (0, 0.1, 0.3, 0.5 mM), B (0, 1, 3, 5, 10 mM) and FA X B concentration on plant growth (*S. tuberosum* cv. Agria) were investigated. It was observed that plant's wet weight decreased in when FA applications. While plantless wet weight was obtained 0.09 g in control, 0.02 g was obtained in 0.5 mM FA. It was also identified that FA applications decreased the plantless wet weight at a rate of 77%. The plants in control had the best wet weight (0.09 g). When it comes to the effects of the FA on the potato's length, significant differences were statistically observed ( $P \le 0.01$ ). It was

identified that plant's length decreases at the rate of 45% in FA application. The best length was determined to control and 0.05 mM FA application (4.20 and 3.30 cm). There was no root growth when the FA was applied. FA application prevented the root growth at the rate of 100%. The plants had the best root length in control application (Table 1). In addition, it was observed statistically differences between applications on the leaf growth (P $\leq$ 0.01). FA applications decreased the leaf growth at the rate of 80%. The best leaf growth was 0.60 cm in control application (Table 1). Moreover, FA toxicity drastically prevented the plant growth with the increased FA applications. It was also determined that FA toxicity prevented the plant growth in other studies, as well (Chawla and Wenzel 1987, Bouizgarne et al. 2004, Bouizgarne et al. 2005, Bouizgarne et al. 2006, Arıcı 2006, Wu et al. 2008, Türkkan and Dolar 2010).

It was determined that the plant growth decreased in high level of B concentration. However, 1 mM B application had much more effect on the growth of potato plant than control application or other B applications (Table 2). With the increased B applications, there was an important decrease in plant's wet weight. It was determined 0.04 g plant's wet weight in 10 mM B application, while plants wet weight was 0.22 g in control. B applications decreased the plant's wet weight at the rate of 81%. Plants length decreased in increased B applications while plants in control application were 7.50 cm, plants in 10 mM application was 1.60 cm. Furthermore, B application decreased the plants' length at the rate of 78%. It was detected that there was significant decline in plant's root length while the root length was 3.60 cm in control, there was no root formation in 5 and 10 mM B applications. Increased B applications prevented the root formation at the rate of 100%. According to the results of the experiment, 1 mM B application had no negative effect on the plants but increased B applications gave rise to B toxicity. It was reported that B was a necessary element for the plants' growth; however, if it was too much in the soil, it caused to stress, shoot tip or edge burns, dead areas on indefinite areas or vessels (Demirtas 2005). It was obtained that there was a decline in the chlorophyll quantity when salt or B was applied cherry rootstock. Nonetheless, positive consequences occurred in the wet weight of the shoot tips and leaves when salt and B were applied in a limited and small quantity (Sotiropoulos et al. 2006 a, b). It was determined that too much B applications prevented in root and leaf numbers in vines. Besides, there was some decline in APX activity with regard to control application whereas there was some increase in SOD and CAT activities (Güneş et al. 2006).

Interaction experiments were conducted along with the appropriate FA and B doses. Statistically differences between FA X B applications on potato plant's wet weight were detected ( $P \le 0.01$ ). While plant's wet weight was observed 0.14 g in control, plant's wet weight was determined 0.7 g in 0.1 mM FA X 1 mM B application. 0.1 mM FA X 1 mM B and 0.1 mM FA and there had no important differences in plant's wet weight in statistically ( $P \le 0.01$ ) in 3 mM B applications.

FA X B decreased the plant's wet weight at the rate of 50% to compare with control (Table 3). The best length result was 3.90 cm in 0.1 mM FA X 1 mM B and control. There was no root formation in 0.1 mM FA X 1 mM B.

Fusar ic Acid (FA)	Wet Weight (g)	Length (cm)	Root Length (cm)	Root Number (number )	Leaf Latitude (cm)	Leaf Length (cm)	Whole Leaf (number )
Contr	$0.09 \pm 0.02$	4.20±1.5	$1.60\pm0.4$	4.50±1.0	0.50±0.1	$0.60{\pm}0.0$	0.90±0.1
ol	*a	3a	1a	5a	4 a	8a	1a
0.1	$0.06 \pm 0.03$	3.30±0.8	$0.00{\pm}0.0$	$0.00{\pm}0.0$	0.20±0.1	$0.40\pm0.1$	0.50±0.2
mM	b	1a	0b	0b	5b	6b	1b
0.3	$0.05 \pm 0.02$	2.40±0.5	$0.00{\pm}0.0$	$0.00{\pm}0.0$	0.20±0.1	0.30±0.1	$0.40\pm0.2$
mM	b	7b	0b	Ob	7b	9b	4b
0.5	$0.02{\pm}0.01$	2.30±0.7	$0.00{\pm}0.0$	$0.00{\pm}0.0$	0.10±0.0	0.10±0.0	0.20±0.1
mM	c	5b	0b	0b	4c	7c	2c

Table 1. The effects of the different FA concentrations on potato plant's growth

\*The differences between figures that are shown in the same column with different letters are important in terms of statistic.

Boro n (B)	Wet Weight (g)	Length (cm)	Root Length (cm)	Root Number (number )	Leaf Latitude (cm)	Leaf Length (cm)	Whole Leaf (number )
Contr	$0.22 \pm 0.09$	$7.50 \pm 3.4$	3.60±2.1	6.00±3.3	$0.60\pm0.2$	$0.80\pm0.2$	$1.00{\pm}0.3$
ol	*a	1a	b	3b	1b	2b	2b
1 mM	0.27±0.11	7.90±2.6	5.30±2.8	7.90±3.7	0.80±0.2	$1.00\pm0.2$	1.30±0.4
	а	9a	7a	1a	2a	8a	0a
3 mM	0.13±0.05	3.90±1.1	2.20±1.6	3.40±2.6	0.50±0.2	$0.60\pm0.2$	0.80±0.2
	b	8b	b	0b	4b	3b	9b
5 mM	$0.07 \pm 0.02$	2.10±0.2	$0.00{\pm}0.0$	$0.00{\pm}0.0$	0.30±0.2	0.30±0.2	0.50±0.3
	с	5c	0c	0c	5c	7c	5c
10	$0.04{\pm}0.01$	$1.60\pm0.9$	$0.00{\pm}0.0$	$0.00{\pm}0.0$	0.10±0.1	0.10±0.1	0.10±0.1
mM	с	5c	0c	0c	0d	4d	7d

Table 2. The effects of the different B concentrations on potato plant's growth

\*The differences between figures that are shown in the same column with different letters are important in terms of statistic ( $P \le 0.01$ ).

Table 3. The effects of FA X B applications with different concentrations on growth criteria of potato plants *in vitro* 

FA X B	Wet Weight (g)	Length (cm)	Root Length (cm)	Root Number (number )	Leaf Latitude (cm)	Leaf Length (cm)	Whole Leaf (number )
Contr	$0.14{\pm}0.06$	3.90±1.3	$1.60{\pm}1.1$	3.70±2.8	$0.40\pm0.3$	$0.50\pm0.3$	$0.70\pm0.4$
ol	*	4a	8a	6a	0a	2a	0a
1 mM B X 0.1m M FA	0.10±0.05 ab	3.90±0.9 0a	0.00±0.0 0b	0.00±0.0 0b	0.40±0.1 8a	0.50±0.2 5a	0.70±0.3 1a
3 mM B X 0.1m M FA	0.07±0.02 b	2.90±0.4 5b	0.00±0.0 0b	0.00±0.0 0b	0.30±0.2 3a	0.40±0.2 7a	0.50±0.3 5a

\*The differences between figures that are shown in the same column with different letters are important in terms of statistic ( $P \le 0.01$ ).

FA X B prevented the root formation at the rate of 100%. The best average root number was 3.70 in control. When the effects of the FA X B application on the leaf growth were investigated, and there was no statistically difference between applications, but it was observed that the plant's length was much better in control and 0.1 mM FA X 1 mM B than 0.1 mM FA X 3 mM B. The reason of this situation could be because appropriate concentration of B protects the plant against the stress conditions like plant disease. Keane and Sackston (1970) reported that B deficiency was observed inoculated with Fusarium oxysporum f. sp. lini on linen. Guerra and Anderson (1985) reported that application of  $FeCl_3$  and  $FeCl_3 + B$  on bean (Phaseolus vulgaris) decreased in lesions of Fusarium solani f. sp. phaseoli on plants. After the  $FeCl_3 + B$  application, it is stated that B cause to decline in lesions on plant's hypocotyls. In addition, it was reported that potassium tetraborate was effective from room temperature to 0°C grapes to the control of Botrytis cinerea. B had broken down the cell wall of fungal pathogens and caused the loss of cytoplasmic material of fungal hyphae. B prevented B. cinerea spore germination and elongation of the germ tube and spreading mycelium (Qin et al. 2010). Shi et al. (2012) reported that B caused to mitochondrial deterioration in spores of Colletotrichum gloeosporioides and had antifungal effect against C. gloeosporioides.

## Determination of proline and protein contents

The addition of different concentrations of FA and B in the MS medium resulted in a significant increase of proline content in the plants compared to control (Figure 1). With the increased FA applications, it was detected to increase in proline quantity at the rate of 86% with FA applications on plants. The most proline

quantity is 2.21 mmol proline /g in 0.5 FA applications to compare with control (0.30 mmol proline /g). B applications increased the proline quantity at the rate of 34% and the most proline rate was 0.46 or 0.45 mmol proline /g in 3 and 5 mM B applications. As the 10 mM B application causes toxic effect on the plant, it caused the death of plants. It was believed this reason that there was a decrease in proline quantity rather than other applications (3 and 5 mM B). With the increased FA X B applications, it was detected that there were significant increases in proline quantity on plants. It was determined 1.93 mmol proline /g in 0.1 mM FA X 3 mM, 0.30 mmol proline /g in control. The rate of proline quantity was 84% in FA X B applications. The proline most quantity was 1.93 mmol proline /g in 0.1 mM FA X 3 mM FA X 3 mM B application.



Figure 1. Proline content in potato plant in MS medium supplemented with different applications.

In this study, protein content range was from 1.22 to 1.29 mg/mL in control and B while FA X B was from 0.12 to 0.14 mg/mL in FA applications. FA decreased in protein contents on average 89% with regard to FA X B applications (Figure 2).



Figure 2. Protein content in potato plant in MS medium supplemented with different applications.

According to the results of experiment, it was detected that there was an increase in potato plant's proline content in FA and FA X B applications by 86% and 84% when compared to control plants. However, it was also detected that there was an increase in potato plant's proline content through B applications at a rate of 34% when compared to control plants. The proline content in B application was much lower than FA and FA X B.

Some researchers investigated the effects of salt stress to proline content. It was reported that proline that was synthesized with glutamine as a result of salt stress cumulates on rice plant (Lin et al. 2002). The proline and carbohydrate contents in the wheat types changed in the media that includes 0, 100, 200 and 3000 mM NaCl (Kafi et al. 2003). The antioxidant enzyme activities, hydrogen peroxide, malondialdehyde, and total phenolic substances were searched on etiola bean seedlings. Antioxidant enzyme activities, hydrogen peroxide, malondialdehyde, and proline contents had much more increased in etiola seedlings than control or de-etiola seedlings (Akgül 2010). The physiological and biochemical reactions of Shiraz grape (*Vitis vinifera* L.) grafted on 110R rootstock against NaCl and proline applications was investigated, it was detected that proline was highly effective in phase change, lipid peroxidation, and antioxidant enzyme systems (Özden et al. 2011). As a result, it is believed that the increase in proline content on plants could be efficient in defense mechanism. The increase in proline quantity could be a sign of tolerance against stress factor.

### Isoforms of peroxidase enzyme (POD)

Native-PAGE of plant extracts revealed 6 POD isoforms in all media (Figure 3). POD activity of leaves and stems of explants exposed to FA and B was increased. It was seen in this study that POD activity that was effective in plant's defense

mechanism considerably decreased in FA medium. It was assumed that B was effective in plant's defense mechanism during plant growth against the stress conditions. Antioxidant enzyme activities and total phenolic compounds were much higher than etiola plants in control. Therefore, B had a positive effect on plant growth in stress conditions (Demirtaş 2005, Akgül 2010)

In this study, 0.3 mM FA, and 0.5 mM FA band profiles were lighter than others. The reasons of this decline are thought as that even plant's defense mechanism could not affect due to FA. It was suggested that these combinations had lower POD activity. Some researcher investigated the stress condition in enzyme activity. It was reported that SOD decreased while POD increased in cucumber against salt stress. Nevertheless, there was no change in CAT and APX enzyme activities. Özden et al. (2011) reported that Shiraz Grape Type demonstrated physiological and biochemical reactions against NaCl and proline applications. Antioxidant enzyme activities such as EL, SBO, chlorophyll, proline, MDA and SOD, POD, CAT PPO were measured. Ionic current, chlorophyll degradation increased on leaf tissues against NaCl stress.



Figure 3. Native-PAGE and activity staining for POD activities in the potato cv. Agria in response to different culture media. 1: Control, 2: 1 mM B, 3: 3 mM B, 4: 5 mM B, 5: 10 mM B, 6: 0.1 mM FA, 7: 0.3 mM FA, 8: 0.5 mM FA, 9: 0.1 mM FA X 1 mM B and 10: 0.1 mM FA X 3 mM B.

Consequently, since the different doses of the FA prevented the root formation, plant growth was negatively affected by it. Different doses of B application are effective in plant's root formation; however, it could negatively affect the plants growth and development because the overuse of B creates toxic effect. Providing that B is in a required quantity for plants, it can have a role in protecting plant against the stress conditions. Isoperoxidase bands and salinity B studies were concentrated on some vegetables, but there are few studies about plant disease and B or salinity in vegetables. This study is new for these combinations in vegetables (potato). Our results can therefore be used for these specific responses.

## ACKNOWLEDGEMENT

This study was supported by the Süleyman Demirel University Research Fund, Project No. 3649-YL-1-13

#### REFERENCES

- Akgül B. 2010. Etiyole fasulye (*Phaseolus vulgaris* L.) fidelerinde antioksidan enzim aktivitelerinin ve total fenolik bileşiklerin incelenmesi. Yüksek Lisans Tezi, Gaziosmanpaşa Üniversitesi, Fen Bilimleri Enstitüsü, Tokat, 42s.
- Anonymous 2012. Patates Entegre Mücadele Teknik Talimatı. http://www.tarim.gov.tr/TAGEM/Belgeler/yayin/011\_patates.pdf (Erişim Tarihi: 07.12.2012).
- Anonymous 2014. FAO statistical databases, FAOSTAT. http://faostat3.fao.org/ (Accessed date: 8 June 2014).
- Anonymous 2016a. Food and Agriculture Organization of the United Nations.
- Anonymous 2016b. Patates hastalıkları ve zararlıları ile mücadele yöntemi. http://www.tarim.gov.tr/GKGM/Belgeler/Bitki%20Sa%C4%9Fl%C4%B1%C4%9F %C4%B1%20Hizmetleri/hastalik\_zararlilari\_ile\_m%C3%BCcadele\_dokumanlari/p atates.pdf
- Arıcı Ş. E. 2006. Somaklonal varyasyondan yararlanarak in vitro seleksiyonla buğday (*Triticum aestivum* L.)'da başak yanıklığına (*Fusarium* spp.) dayanıklı bitki elde edilmesi. Doktora Tezi, Çukurova Üniversitesi, Fen Bilimleri Enstitüsü, Adana, 167s.
- Aspinall D. and Paleg L.G. 1981. Proline accumulation: physiological aspects. In: Paleg L.G., Aspinall D. (eds). The Physiology and Biochemistry of Drought Resistance in Plants. pp 205–241. Academic Press, Sydney.
- Bacon C.W., Porter J.K. and Norred W.P. 1996. Production of Fusaric Acid by *Fusarium* Species. Applied and Environmental Microbiology, 62, 4039-4043.
- Barnett N. M. and Naylor A. W. 1966. Amino Acid and Protein Metabolism in Bermuda Grass During Water Stress. Plant Physiology, 41, 1222-1230.
- Bates L.S., Waldern R.P. and Teare I.D. 1973. Rapid Determination of Free Proline for Water Stress Studies. Plant and Soil, 39, 205-207.
- Bouizgarne B., Brault M., Pennarun A.M., Rona J.P., Ouhdouch Y., El Hadrami I. and Bouteau F. 2004. Electrophysiological Responses to Fusaric Acid of Root Hairs From Seedlings of Date Palm Susceptible and Resistant to *Fusarium oxysporum* f. sp. Albedinis. Journal of Phytopathology, 152, 321-324.
- Bouizgarne B., El-Maarouf-Bouteau H., Madiona K., Biligui B., Monestiez M., Pennarun A.M., Amiar Z., Rona J.P., Ouhdouch Y., El Hadrami I. and Bouteau F. 2005. A Putative Role for Fusaric Acid in Biocontrol of the Parasitic Angiosperm *Orobanche ramosa*. MPMI 19, 550-556.

- Bouizgarne B., El-Maarouf-Bouteau H., Frankart C., Reboutier D., Madiona K., Pennarun A.M., Monestiez M., Trouverie J., Amiar Z., Briand J., Brault M., Rona J.P., Ouhdouch Y., El Hadrami I. and Bouteau F. 2006. Early Physiological Responses of *Arabidopsis thaliana* Cells to Fusaric Acid: Toxic and Signaling Effects. New Phytologist, 169, 209-218.
- Bradford M.M. 1976. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Analytical Biochemistry, 72, 248–254.
- Chawla H.S. and Wenzel G. 1987. In-vitro Selection for Fusaric Acid Resistance Barley Plants. Plant Breeding, 99, 159-163.
- Delen S. 2007. Bazı *Fusarium* türlerinin teşhisini kolaylaştırmaya yönelik bilgisayar programı. Yüksek Lisans Tezi, Selçuk Üniversitesi, Fen Bilimleri Enstitüsü, Konya, 149s.
- Demirtaş A. 2005. Bitkide Bor ve Etkileri. Atatürk Üniversitesi, Ziraat Fakültesi Dergisi, 36 (2), 217-225.
- Doğan G., Sabah E. and Erkal T. 2005. Borun çevresel etkileri üzerine Türkiye'de yapılan bilimsel araştırmalar. Türkiye 19. Madencilik Kongresi, 09-12 Haziran 2005, IMCET 2005, İzmir, Türkiye, 425-431.
- Eken C., Demirci E. And Şahin F. 2000. Pathogenicity of the Fungi Determined on Tubers from Potato Storages in Erzurum, Turkey. The Journal of Turkish Phytopathology, 29 (2-3), 61-69.
- Ergün N. 2005. Buğday (*Triticum aestivum* L. cv. GÜN 91) fidelerinde bazı ağır metallerin ve ağır metal-hormon etkileşiminin fizyolojik ve biyokimyasal etkileri. Doktora Tezi, Ankara Üniversitesi, Fen Bilimleri Enstitüsü, Ankara, 67s.
- Guerra D. and Anderson A.J. 1985. The Effect of Iron and Boron Amendments on Infection of Bean by *Fusarium solani*. The American Phytopathologial Society, 75(9), 989-991.
- Güçlü S.F. 2010. Kirazlarda anaç/kalem ilişkilerinin biyokimyasal yöntemlerle incelenmesi. Doktora Tezi, Süleyman Demirel Üniversitesi, Fen Bilimleri Enstitüsü, Isparta, 113s.
- Güneş A., Söylemezoğlu G., Güneri E.G., Coban S. and Sahin O. 2006. Antioksidant and (Stomatal responses of grapevine *Vitis vinifera* L.) to Boron Toxicity. Scientia Horticulturae, 110, 279-284.
- Idris A.E., Abouzeid M. A., Boari A., Vurro M. and Evidente A. 2003. Identification of Phytotoxic Metabolites of a New *Fusarium* sp. Inhibiting Germination of *Striga hermonthica* Seeds. Phytopathologia Mediterranea, 42, 65-70.
- Jestoi M.N., Paavanen-Huhtala S., Parikka P. and Yli-Mattila T. 2008. In Vitro and In Vivo Mycotoxin Production of Fusarium Species Isolated from Finnish Grains. Archives of Phytopathology and Plant Protection, 41, 545–558.

- Kafi M., Stewart W.S. and Borland A.M. 2003. Carbohydrate and Proline Contents in Leaves, Roots, and Apices of Salt-Tolerant and Salt-Sensitive Wheat Cultivars. Russian Journal of Plant Physiology, 50, 155-162.
- Keane E.M. and Sackston W.E. 1970. Effects of Boron and Calcium Nutrition of Flax on *Fusarium* Wilt. Canadian Journal of Plant Science, 415-422.
- Laemmli U.K. 1970. Cleavage of Structural Proteins During the Assembly of the Head of Bacteriophage T4. Nature, 227, 680-685.
- Lin C.C., Hsu Y.T. and Kao C.H. 2002. The Effect of NaCl on Proline Accumulation in Rice Leaves. Plant Growth Regulation, 36 (3), 275-278.
- Lopez-Berges M. S., Hera C., Sulyok M., Schafer K., Capilla J., Guarro J. and Di Pietro A. 2013. The Velvet Complex Governs Mycotoxin Production and Virulence of *Fusarium oxysporum* on Plant and Mammalian Hosts. Molecular Microbiology, 87, 49-65.
- Mittler R. 2006. Abiotic Stress, The Field Environment and Stress Combination. Trends in Plant Science, 11 (1), 15-19.
- Molassiotis A., Sotiropoulos T., Tanou G., Diamantidis G. and Therios I. 2006. Boron-Induced Oxidative Damage and Antioxidant and Nucleolytic Responses in Shoot Tips Culture of the Apple Rootstock EM 9 (*Malus domestica* Borkh). Environmental and Experimental Botany, 56: 54-62.
- Murashige T. and Skoog F. 1962. A Revised Medium for Rapid Growth and Bioassays with Tobacco Tissue Cultures. Physiologia Plantarum, 15, 473-497.
- O'Brien J.A., Daudi A., Butt V.S. and Bolwell G.P. 2012. Reactive Oxygen Species and Their Role in Plant Defence and Cell Wall Metabolism. Planta, 236, 765–779.
- Öncel I. 1988. The Proline Accumulation of Some Halophytes in the Vicinities of the Salt Lake. Commun. Fac. Sci. Univ. Ankara, 6: 219-225.
- Özden M., Dikilitaş M., Gürsöz S. and Ak B.A. 2011. 110 R Anacı Üzerine Aşılı Şiraz Üzüm (*Vitis vinifera* L.) Çeşidinin NaCl ve Prolin Uygulamalarına Karşı Fizyolojik ve Biyokimyasal Tepkileri. Harran Üniversitesi, Ziraat Fakültesi Dergisi, 15 (1), 1-9.
- Qin G., Zong Y., Chen Q., Hua D. and Tian S. 2010. Inhibitory Effect of Boron Against *Botrytis cinerea* on Table Grapes and its Possible Mechanisms of Action. International Journal of Food Microbiology, 138, 145–150.
- Reid R.J. and Fitzpatrick K.L. 2009. Redistribution of Boron in Leaves Reduces Boron Toxicity. Plant Signal Behavior, 4 (11), 1091–1093.
- Shi X., Li B., Qin G. and Tian S. 2012. Mechanism of Antifungal Action of Borate Against Collectorichum gloeosporioides Related to Mitochondrial Degradation in Spores. Postharvest Biology and Technology, 67, 138–143.
- Sotiropoulos T.E., Molassiotis A., Almaliotis D., Mouhtaridou G., Dimassi K., Therios I. and Diamantidis G. 2006a. Growth, Nutritional Status, Chlorophyll Content, and Antioxidant Responses of the Apple Rootstock MM 111 Shoots Cultured Under High Boron Concentrations in-vitro. Journal of Plant Nutrition, 29, 575–583.

- Sotiropoulos T.E., Therios I.N., Almaliotis D., Papadakis I. and Dimassi K.N. 2006b. Response of Cherry Rootstocks to Boron and Salinity. Journal of Plant Nutrition, 29, 1691-1698.
- Stewart G.R. and Lee J. A. 1974. The Role of Proline Accumulation in Halophytes. Planta, 120, 279-289.
- Stewart C.R., Boggess S.F., Aspinall D. and Paleg L.G. 1977. Inhibition of Proline Oxidation by Water Stress. Plant Physiology, 59, 930-932.
- Stewart C.R. 1978. Role of Carbohydrates in Proline Accumulation in Wilted Barley Leaves. Plant Physiology, 61, 775-778.
- Türkan İ., Bor M., Özdemir F. and Koca H. 2005. Differential Responses of Lipid Peroxidation and Antioxidants in the Leaves of Drought-Tolerant *P. acutifolius* Gray and Drought-Sensitive *P. vulgaris* L. Subjected to Polyethylene Glycol Mediated Water Stress. Plant Science, 168, 223-231.
- Türkkan M. and Dolar F.S. 2010. Fusarium oxysporium f.sp. ciceris'in fusarik asit üretimi ince tabaka kromatografisi ve spektofotometrik metodlar ile belirlenmesi. http://dergi.omu.edu.tr/index.php/ANAJAS/article/view/2538/1877 (ErişimTarihi: 08.12.2012).
- Vinocur B. and Altman A. 2005. Recent Advances in Engineering Plant Tolerance to Abiotic Stress, Achievements and Limitations. Current Opinion in Biotechnology, 16, 123–132.
- Vogelgsang S., Sulyok M., Hecker A., Jenny E., Krska R., Schuhmacher R. and Forrer H.R. 2008. Toxigenicity and Pathogenicity of *Fusarium poae* and *Fusarium avenaceum* on Wheat. European Journal of Plant Pathology, 122, 265–276.
- Wendel J.F. and Weeden N.F. 1989. Visualization and interpretation of plant isozymes. In: Soltis D.E and Soltis P.S (eds). Isozymes in Plant Biology, Dioscorides Press, Portland, Oregon, pp. 5-44.
- Wu H., Yin X., Liu D., Ling N., Bao W., Ying R., Zhu Y., Guo S. and Shen Q. 2008. Effect of Fungal Fusaric Acid on the Root and Leaf Physiology of Watermelon (*Citrullus lanatus*) Seedlings. Plant Soil, 308, 255-266.
- Yashu Y., Tomohiro K., Kazuo N. and Kazuko Y.S. 1997. Regulation of Levels of Proline as an Osmolyte in Plants Under Water Stress. Plant and Cell Physiology, 38, 1095-1102.