Türk Tarım ve Doğa Bilimleri Dergisi 6(1): 71–77, 2019

https://doi.org/10.30910/turkjans.515352

TÜRK
TARIM ve DOĞA BİLİMLERİ
DERGİSİ

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TURKISH
JOURNAL of AGRICULTURAL
and NATURAL SCIENCES

Research Article

The Effect of Drought Stress on Antioxidative Enzyme and Nutrient Exchange in Some Tomato Genotypes[&]

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Received: 19.09.2018 Received in Revised: 19.12.2018 Accepted: 26.12.2018

Abstract

Along with global warming, serious reductions occurred in water resources. The drought stress resulting from global warming has greatly affected production. As well as reducing yield and quality in production, drought also reduces farmer's income. One of the measures to be taken in order to minimize the damage caused by the effect of drought stress is to determine the tolerance of the genotypes to drought. Accordingly, three hybrids varieties, three standards varieties and three landraces of tomatoes were used in the present study. Tomato seeds were sown in a 2 liters-pot that contains 1:1 mixture of peat + perlite. After the true leaves emerged, the seedlings were irrigated with the Hoagland nutrient solution. While irrigation was carried on the control plants until the end of the application, irrigation was completely terminated during the seedling period in the plants that are exposed to drought. 12 days after the irrigation cut in tomato genotypes, the changes in the level of catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), potassium (K), calcium (Ca) and magnesium were examined. It was observed that there were significant differences in these parameters examined for the determination of tomato genotypes that are tolerant and sensitive to drought stress.

Keywords: Antioxidant, nutrient, drought stress, *Solanum lycopersicum* L.

Kuraklık Stresinin Bazı Domates GenotiplerindeAntioksidatif Enzim ve Besin Elementi Değişimleri Üzerine Etkileri

Özet

Küresel ısınmayla birlikte su kaynaklarında ciddi azalmalar meydana gelmiştir. Küresel ısınmanın sonucu ortaya çıkan kuraklık stresi üretimi büyük oranda etkilemiştir. Kuraklık stresi verim ve kaliteyi azaltmasının yanı sıra üreticilere de maddi sıkıntılar yaşatmaktadır. Kuraklık stresinin etkisine bağlı olarak oluşan hasarların en aza indirgenmesi amacıyla alınacak önlemlerden bir tanesi de kuraklığa tolerant çeşitlerin belirlenmesidir. Bu nedenle mevcut çalışmada kuraklığa tolerans gösteren domates genotiplerinin belirlenmesi amaçlanmıştır. Çalışmada üç adet hibrit, üç adet standart ve üç adet mahalli domates çeşidi kullanılmıştır. Domates tohumları 1:1 oranında torf + perlit karışımı içeren 2 litrelik saksılara ekilmiştir. Gerçek yapraklar oluştuktan sonra fideler, Hoagland besin çözeltisiyle sulama yapılmıştır. Sulama, kontrol bitkilerinde çalışma bitirilinceye kadar devam ederken, kuraklık uygulanan bitkilerde ise fide döneminde sulama tamamen kesilmiştir. Sulama kesildikten 12 gün sonra domates genotiplerindekatalaz (CAT), süperoksitdismutaz (SOD), askorbatperoksidaz (APX), potasyum (K), kalsiyum (Ca) ve magnezyum (Mg) içeriklerindeki değişimler incelenmiştir. Kuraklık stresine tolerant ve duyarlı domates genotiplerinin belirlenmesinde incelenen bu parametrelerde bariz farklar oluştuğu gözlemlenmiştir.

Anahtar kelimeler: Antioksidant, besin elementi, kuraklık stresi, *Solanum lycopersicum* L.

Introduction

Climate changes caused by global warming have adversely affected tomato production in recent years. As a result of global warming; drought, humidity, light and temperature stresses were started to be seen frequently in plants. Only 10% of the world's available agricultural land is not subject to any environmental stress conditions. It is emphasized that about 26% of the remaining 90% of agricultural land is threatened by drought stress (Blum et al., 1986; Ozer et al., 1997; Asraf and Foolad, 2007 ;Bagcı 2010; Arslan et al., 2018). Many plants accumulate solutes in their cells in response to drought stress. The increase in the intracellular solute level is very important for the hold of cell's water. Among the solutes which are effective on the osmotic regulation, are many ions, especially K^+, sugars and amino acids (Kacar et al., 2006; Ozen and Onay, 2007).In a study of the interrelationships between the morphological, physiological and biochemical responses of tomato, eggplant and melon genotypes to drought, K^+, MDA content, and antioxidative enzyme activities are important criteria for determine the tolerance to drought stress in the mentioned vegetables species, along with other physiological and morphological characters (Kıran et al., 2015). It was emphasized that water stress applied to the tomato plant caused the decrease of yield and fruit quality while the antioxidant content was increased in the sensitive genotypes (Sanchez et al., 2010; Alp and Kabay, 2017a; Alp and Kabay, 2017b). Drought stress also has an important effect on enzyme activity and enzyme amount in plants. In addition, the abscisic acid amount increases 40 times in leaves, while these increase are less in the other part of plant including root. Abscisic acid prevents the transpiration of water by closing the stoma (Kacar et al., 2006). Genotypes with higher antioxidative enzymes (SOD, CAT, APX and GR) and higher tolerance to stress conditions in tomato, eggplant, pepper, cucumber, melon, watermelon, bean and okra species have been determined. It was indicated that antioxidant enzyme activities may be regarded as an important parameter for detecting sensitive and tolerant plants in separating genotypes that are very close to tolerance levels (Yasar et al., 2013).It has been reported that CAT and APX antioxidant enzyme activities increased in drought stress conditions in drought-tolerant CU 196 and susceptible CU 3 melon genotypes (Kusvuran et al., 2008).

It has been shown that MDA, CAT, SOD and APX activities changes different levels in bean genotypes applied drought stress; K and Ca contents are lower in drought sensitive varieties, whereas drought tolerant genotypes are close to control plants (Kabay and Sensoy, 2016; Kabay and Sensoy, 2017; Kabay et al., 2017).

In the stoma cells, the water potential is reduced as a result of the K accumulation; then, with the entry of water into the these cells and potassium deficiency in these cell, sugar and starch accumulation causes water need. However, in the case of addition K, it has been ameliorated that the intracellular electrolyte balance, which has deteriorated. Because the amount of K that compete with Na increases in the same membrane-bound regions and the intracellular Na/K balance, which has deteriorated, is readjusted to regulate metabolic activities. Two of the basic elements required for growth and development are Ca and K ions. Abiotic stress negatively affects Ca intake in addition to K. Sodium (Na) is replaced by Ca in the cell membrane, which results an increase in Na/Ca ion ratio in the apoplast. In this case, the physiological and functional structure of the membrane deteriorates and the Ca balance of the cell deteriorates (Kaya and Tuna 2010).

It is stated that plants in drought conditions show different sensitivity and defense system in terms of K, Ca, Na ion contents. Na ion creates necrotic spot starting from the mature leaves to the shoot and young leaves in plants (Aktas, 2002; Dasgan et al., 2006; Aybeke, 2016). Genotypes with higher K and Ca ions content in the green parts and roots are more resistant to stress conditions in melon and it is also indicated that genotypes in drought stress cause an increase in oxidative and antioxidative enzyme activities (Kusvuran 2010).

The present study aimed to determine the changes of antioxidative enzyme and some nutrient contents under drought stress in some tomato genotypes grown in our country. As a result of this study, it is expected that tomato genotypes tolerant to drought stress will benefit from future breeding study and will be able to select a suitable genotype for the producer.

Materials and Methods

The effects of drought stress on catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), potassium (K), calcium (Ca) and magnesium (Mg) contents at the $12t^h$ day of tomato genotypes exposed to drought stress were investigated. In the study, 3 landraces (Lice, Hilvan and Ahlat), 3 commercial F_1 hybrid cultivars (BT986, BT1134, and Tokat) and 3 standard

varieties (H2274, Rio Grande and Falcon) produced in our country were used. Tomato seeds were sown in 2 liters pots containing 1: 1 mixture of peat + perlite and 2 seeds in each pot. The study was carried out in a controlled climate room with 23 ± 2 ºC temperature and 8000 lux light intensity. The planted seeds were irrigated from the beginning of study with tap water. Three weeks after the first true leaves were formed; the seedlings were irrigated with a Hoagland standard nutrient solution until the end of the experiment. The "drainage solution / applied solution" ratio took into account for irrigation. While irrigation was carried on the control seedlings until the end of the application, irrigation was completely terminated and there was no irrigation for 12 days after the seedlings exposed to drought (Kusvuran, 2010; Kabay, 2014; Alp, 2017).

Antioxidative enzyme analyzes

The frozen 1 g leaf sample (third leaf from the bottom of the plants) was homogenized with a mixture of 5 ml of cold 0.1 M Na-phosphate, 0.5 mM Na-EDTA and 1 mM ascorbic acid (pH: 7.5), then the samples were homogenized at 4 ºC for 30 minutes at 18000 rpm. The ascorbate peroxidase (APX) activity was determined immediately in the homogenate with this prepared. Catalase (CAT) 1 g of frozen leaf sample was homogenized with 5 ml of cold 0.1 M Na-phosphate, 0.5 mM Na-EDTA mixture (pH: 7.5), and the homogenate was centrifuged at 18000 rpm for 30 minutes at 4 ºC for the determination of CAT and superoxide dismutase (SOD) activity. CAT activity was detected in a portion of the homogenate and the rest of the extract was stored at -20 °C for SOD determination (Jebara et al. 2005; Bagcı 2010; Kabay, 2014; Alp, 2017).

Catalase (CAT) activity

Catalase activity was determined by monitoring the disappearance of H_2O_2 at a wavelength of 240 nm. 0.05 M phosphate buffer (KH_2PO_4), 1.5 mM H_2O_2 mixture (pH: 7.0) was used as the reaction solution. 2.5 ml of reaction solution and 0.2 ml of plant extract were mixed. In spectrophotometer, 0 and 60 second readings were taken at 240 nm wavelength. The reaction was started by the addition of 0.1 ml enzyme extract. The evaluation was made taking into account the change in absorbance within 1 minute (Jebara et al. 2005; Bagcı 2010; Kabay, 2014; Alp, 2017).

Superoxide dismutase (SOD) activity

It was determined the inhibition of nitroblue tetrazolium (NBT) by at a wavelength of 560 nm. As the reaction solution, a mixture of 50 mM Na-phosphate buffer (Na_2HPO_4 x $H_2O_2^-$), 0.1 mM Na-EDTA, 33 µM NBT, 5 µM riboflavin, 13 mM methionine (pH: 7.0) were used. 2.5 ml of reaction solution was mixed with 0.1 or 0.2 ml of plant extract. The reaction was achieved at 25 ºC with 75 µmol m-2 s-1 (40 W) for 10 minutes under light. The control solution was left in the dark condition for the same period without enzyme. Control and reaction solution read at 560 nm. As SOD activity unit, 50% of NBT was determined as reductive (Jebara et al. 2005; Bagcı 2010; Kabay, 2014; Alp, 2017).

Ascorbate peroxidase (APX) activity

Ascorbate peroxidase activity was measured at 290 nm depending on the ascorbic acid reducing $H_2O_2^-$. As the reaction solution, 50 mM phosphate buffer (KH_2PO_4), 0.5 mM ascorbic acid, 0.1 mM EDTA, 1.5 mM H_2O_2 mixture (pH: 7.0) were used. 3 ml of reaction solution and 0.1 ml of plant extract were mixed. The 0.and 60 second readings were taken at 290 nm wavelength in the spectrophotometer. The reaction was started by the addition of 0.1 ml enzyme extract. The evaluation was made taking into account the change in absorbance within 1 minute (Jebara et al. 2005; Bagcı 2010; Kabay, 2014; Alp, 2017).

Mineral element analysis

At the end of the drought experiment, the shoot samples from stressed and control plants were dried in an oven at 65 ºC until reaching to a constant weight. Then, the dried samples (200 mg) were grounded, pre-lit by ethyl alcohol, and lit till ash formation at 550 °C. The ash samples were dissolved with a 3.3 % HCl solution, filtered with a blue-band filter paper, and Mg, K, and Ca was determined in an atomic absorption device (Thermo trade brand serial no: ice3000 series aa spectrometer) (Kusvuran 2010; Bagcı 2010; Kabay and Sensoy 2016).

The statistical analysis

Analyses of variances based on general linear models (Yesilova and Denizhan 2016) were carried out by SAS 9.4.1 statistical program (SAS, 1999). Duncan multiple Comparison tests was used to measure the statistical differences between genotype (Duncan, 1955).

Results and Discussion

Drought stress, one of the abiotic stress conditions that adversely affect vegetable production, may cause specific necrotic symptoms on plants. Depending on the intensity of these symptoms, it is often possible to determine that

the plants are tolerant or sensitive. The results were quite different between the control group and the tomato genotypes that are exposed to drought in this study. At the end of drought stress, the antioxidative enzymes (CAT, APX and SOD) values measured from the leaves of tomato genotypes are given in Table 1. When this table was examined, it was determined that all genotypes had an increase in CAT, APX and SOD activities, and the resulting changes varied among genotypes.

Table 1. Determination of catalase (CAT) (mmol / g F.W*), ascorbate peroxidase (APX) (mmol / g F.W*) and superoxide dismutase (SOD) (unit / g F.W*) enzyme activities of tomato genotypes in control and drought stress

Genotype	CAT (control)	CAT (drought)	APX (control)	APX (drought)	SOD (control)	SOD (drought)
Lice	0.00115 c	0.00170 c	0.286 e	0.318 h	281.59 a	357.29 ab
Hilvan	0.00135 bc	0.00340 b	0.107 f	0.950 e	190.52 bc	213.45 bc
Ahlat	0.00035 d	0.00115 cd	0.500 a	1.643 b	179.11 bc	242.37 bc
986	0.00006 d	0.00135 c	0.396 b	1.554 c	148.99 c	166.15 c
1134	0.00185 a	0.00340 b	0.286 e	1.214 d	218.82 b	340.28 ab
Tokat	0.00195 a	0.00370 b	0.393 b	0.804 f	172.08 bc	299.44 abc
H2274	0.00165 ab	0.00345 b	0.321 d	1.786 a	165.94 bc	204.17 bc
Rio grande	0.00190 a	0.00445 a	0.107 f	0.946 e	135.21 c	213.45 bc
Falcon	0.00115 c	0.00175 d	0.339 c	0.357 g	153.81 c	426.89 a

*= Fresh Weight

At the end of drought stress; the best result in CAT activity was found in the Tokat F1 genotype (control: 0.00195 mmol g^{-1} F.W, drought: 0.00370 mmol g^{-1} F.W), while the highest increase in CAT activity is 986 F1 genotype (control: 0.00006 mmol / g F.W, drought: 0.00135 mmol / g F.W). Sanchez-Rodriguez et al. (2010 reported that drought stress caused an increase in CAT enzyme activity in tomato cultivars. In a study conducted by Kusvuran (2010), it was reported that enzyme activities are higher in salt-tolerant genotypes and catalase (CAT) enzyme activity may be an effective parameter in determining the salt tolerance.

The highest APX ratio in the control group was found in the Ahlat genotype with a value of 0.500 mmol / g F.W whereas in the drought group the highest APX activity was 1.786 mmol / g F.W with the H2274 genotype and the lowest APX activity was Lice genotype with 0.318 mmol / g F.W. Nikoleva et al. (2010) reported that APX activity increased in the 3th and 5th days of drought stress in the wheat and it decreased in 7th day of stress in parallel with the increase in MDA amount. AzevedoNeto et al. (2006) reported an increase in APX activity under salt stress conditions in both salt tolerant and sensitive corn varieties.

In terms of superoxide dismutase (SOD) activity, the highest value in the control group was Lice genotype with 281.59 units / g F.W and the lowest SOD activity respectively was Falcon (153.81 units / g F.W), 986 F1 (148.99 units / g F.W) and Rio grande (135.21 units / g F.W) genotypes. The highest value of SOD activity in drought application was Falcon genotype (426.89 units / g F.W), while the lowest SOD activity was 986 F1 genotype (166.15 units / g F.W). Yong et al. (2006) reported that plants exposed to drought stress had an increase in SOD enzyme activity and the reduction of enzyme activity may occur as a result of continued stress conditions. Moussa and Abdel-Aziz (2008) emphasize that corn varieties exposed to drought stress are experiencing an increase in SOD activity and this increase is higher in drought-tolerant Giza 2 varieties. Yu and Rengel (1999) reported that drought and salt stresses resulted an increase in SOD enzyme activities and it is higher in drought stress than salt stress.

At the end of drought stress, Magnesium (Mg), calcium (Ca) and potassium (K) the nutrient contents measured from the leaves of tomato genotypes are given in Table 2. When this table was examined into, it was determined that all genotypes had a decrease in Mg, Ca and K content, and the resulting changes varied among genotypes. It was determined that the highest magnesium (Mg) content (control: 0.9947 %, drought: 0.7923 %) was found in the Tokat genotype both control and drought group and it was determined that the Hilvan genotype (drought: 0.5290 %) had the lowest content of magnesium (Mg) in the drought group.

Table 2. Determination of (Mg), calcium (Ca) and potassium (K) nutrient content of tomato genotypes in control and drought stress (% ppm)

Genotype	Mg (control)	Mg (drought)	Ca (control)	Ca (drought)	K (control)	K (drought)
Lice	0.9930 a	0.6917 ab	3.4433 a	2.9950 ab	7.837 a	6.579 a
Hilvan	0.6673 a	0.5290 b	2.9267 a	2.2230 b	7.552 a	5.812 a
Ahlat	0.7093 a	0.5597 ab	3.1750 a	2.9900 ab	7.162 a	5.744 a
986	0.6780 a	0.5540 ab	3.1310 a	1.9567 b	8.406 a	4.721 a
1134	0.7477 a	0.5970 ab	2.6060 a	2.4670 ab	6.274 a	6.252 a
Tokat	0.9947 a	0.7923 a	3.4917 a	3.3980 a	7.060 a	6.994 a
H2274	0.8470 a	0.5820 ab	2.8337 a	2.6940 ab	7.392 a	6.209 a
Rio grande	0.7363 a	0.6543 ab	3.5413 a	2.5223 ab	7.703 a	4.147 a
Falcon	0.8283 a	0.7343 ab	3.7970 a	2.6417 ab	6.927 a	6.424 a

In a study on Cape gooseberry (*Physalisperuviana* L.), it was determined that the lowest N (2.05%), P (0.14%), K (1,95%), Ca (0,83%) and Mg 67) was in 0 % water application; The highest amount of N (3,19 %), P (0,28 %), K (2,55 %), Ca (1,46 %) and Mg (1,02 %) was in 100 % water application (Celik, 2014). In a study of pepper given different levels of water, the highest N, P, K, Ca and Mg contents were found in control plants and the lowest values were found in 0 % water application (Pıtır, 2015).

When the genotypes were examined in terms of calcium (Ca) content in the control group, the highest value was Falcon genotype with 3.79 % and the lowest value was 1134 genotypes with 2.60 %.The highest Ca content in the drought group was found Tokat genotype with 3.3980 %, while the lowest content was 986 genotype with 1.95 %.According to Kucukkomurcu (2011), the average amount of calcium in the green parts of the okra genotypes was 1.91% in control plants and the average amount of calcium in the drought plants was 1.42%. Kaya and Dasgan (2013) determined the amounts of K and Ca of bean genotypes as 4.06 % and 2.11 % respectively in control group and 3.45 % and 0.90% respectively in the plants under drought stress.

When the content of potassium (K) among the genotypes was examined, it was found that both the highest content of potassium (K) in the control group was 986 genotype (8,406 %) and the lowest content of potassium (K) in the drought group was 986 genotype (4,721%). In addition, the highest content of potassium (K) in the drought group was Tokat genotype with 6.994 %.

According to Kaya (2011), average K amounts of bean genotypes were found 4,06 % in control plants and 3,45 % in drought plants. According to Kusvuran (2010), the amount of K and Ca in the genotypes was 3.62 % and 3.38 % at the end of drought stress in melon plant and this value decreased to 2.42 % and to 2.02 % in drought plants. In another study carried out by Kusvuran (2011) on okra genotypes, the average K and Ca contents in control plants were determined as 3,24 % and 3,03 % respectively, while these values were found as 1,88 % and 1,84 % in plants exposed to salt stress. In the study conducted by Dogan (2006) on the K of the leaves of bean plants, it was found that the amount of K in the control group was found 27,407 mg/g, in modarate-water stress was 23,01 mg/g and intense-water stress was 18,107 mg/g.

Conclusion

This study was conducted to determine the responses of tomato genotypes to drought stress as well as to reveal tolerant and sensitive genotypes and antioxidative defense mechanisms against drought stress and changes in some nutrient contents. It was determined that tomato genotypes showed different tolerance and sensitivity levels against drought stress.

It has been found that all genotypes are significantly affected in Mg, Ca and K values as a result of drought stress exposure and while decreases in these values are detected, CAT, SOD and APX values of antioxidative enzyme activities were increased.it has been found that the genotypes that are more tolerant to drought stress conditions had less CAT, SOD and APX content; the genotypes that are sensitive to drought stress conditions had significant increases in this amount. In addition, when examined in terms of nutrient content, genotypes that are more tolerant to drought stress showed less decrease in Mg, Ca and K contents, and sensitive genotypes showed more decrease in these parameters.

Considering all the parameters that were examined in our study, it was determined that the genotypes to be shown tolerant to drought stress were the Lice genotype which is a landrace, Falcon which is standard varieties and Tokat F1 which is commercial cultivars. So these genotypes were identified as least affected by drought stress conditions. Among genotypes that are most

damaged by drought stress that can be regarded as sensitive were determined Hilvan and Ahlat genotypes that are landraces, 986 F1 and 1134 that commercial cultivars and the H2274 which is standard varieties were found.

As a result of the study, we reached the conclusion that tomato genotypes identified as tolerant to drought stress can be protect and using these genotypes in breeding programs may be useful for obtain pure lines. In addition, we believe that drought tolerant plants will help to future breeding studies and reduce the negative impacts of yield and quality factors on tomato and will contribute significantly to future researchers.

[&]:The antioxidative enzyme data involved in this study were taken from the master's thesis.

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