IJAFLS
e-ISSN: 2602-4381
International Journal of Agriculture, Forestry and Life Sciences
Original Article Open access Int J Agric For Life Sci (2021) 5(2): 217-225

Antioxidant enzymes activities of walnut nursery trees to drought stress progression

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Abstract

This research was carried out to determine the biochemical responses of Chandler and Fernor walnut cultivars saplings grafted on the *Juglans regia* seedling rootstock under water stress in 2020. In accordance with this purpose; three different irrigation levels were applied to the one-year-old seedlings in polyethylene tubes in the greenhouse for three months: 1) full irrigation as control (100% of potted field capacity (PFC)) and 2) two different levels of restricted water application (50 and 25% of PFC). The amount of decreasing water was provided every 5 days. The antioxidant enzyme activities including ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) in the leaves were determined every 15 days. In terms of the properties examined, statistically significant differences were found between the applications in the all analysis periods. In general, as the severity and duration of the water stress increased, the antioxidant enzyme activities gradually increased and while the highest values were determined in the 25% PFC application, the lowest values were determined in the 100% PFC control application.

As a result, the walnut saplings developed antioxidant defense mechanisms against water stress, demonstrating a possible tolerance. This suggests that the tolerance may be due to the activation of antioxidant systems and the reduction of oxidative damage. It has been determined that the antioxidant enzyme activities have different tendencies for both cultivars in response to the oxidative damage. While POD and CAT activities were at the higher levels in the Chandler cultivar; SOD and APX activities were at the higher levels in the Fernor cultivar.

Keywords: *Juglans regia*, Fernor, Chandler, Water stress, Enzyme

Introduction

Abiotic stress factors such as drought, salinity and extreme temperatures are among the main causes of crop yield losses in the world (Oliveira et al., 2013; Shahzad et al., 2016; Soni et al., 2017; Ilyas et al., 2020). As compared to the salt stress, the drought problem is more common and creates bigger economical problems (Vahdati and Lofti, 2013; Soni et al., 2017). Although drought is seen in all climatic zones in the world, the arid and semi-arid areas are more affected by the drought due to the lack of moisture and high variability of precipitation. The drought, which occurs in certain periods, especially in the semi-arid regions, can turn into an important disaster. In addition, it has been reported that the fresh water resources, 70% of which are currently used in the agriculture, will decrease seriously due to the population growth and global warming, and the world's water demand will increase by 55% until 2050 (Smedley, 2017). This situation raises the concerns regarding the drought. For this reason, it is very important to determine the fruit species that are tolerant to severe drought events and recover afterwards and their ability to cope with environmental stresses for the sustainable fruit growing.

Cite this artile as:
Yildirim, F., Esen, M., Binici, S., Celik, C., Yildirim, A.N., Karakurt, Y. (2021). Antioxidant enzymes activities of walnut nursery trees to drought stress progression. Int. J. Agric. For. Life Sci., 5(2): 217-225.
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Received: 09.12.2021 **Accepted:** 26.12.2021 **Published:** 26.12.2021
Year: 2021 **Volume:** 5 **Issue:** 2 (December)
Available online at: http://dergipark.gov.tr/ijafls

Juglans regia (walnut) species, which is in the *Juglans* genus of the Juglandaceae family, has been an important and strategic product of the daily diet of human beings since ancient times, especially because its fruits can be stored for a long time (Sen, 2011; Vahdati, 2014). Walnut, which is considered as a drought sensitive species, is mostly grown in the arid and semi-arid areas in the world (Vahdati, 2014). Therefore, the increasing danger of drought and severe water scarcity in these areas are the biggest limitations in the walnut cultivation. Although walnut roots go deep, since most of the roots are collected in the topsoil, the water taken from deep roots in the arid conditions is insufficient to prevent stress (Vahdati and Lofti, 2013). As a matter of fact, it has been reported that the walnut plant growth slows down under water stress, and its yield and quality decrease (Cohen et al., 1997; Simsek et al., 2017). Thus, it is economicaly important to know the capacity of walnut species and varieties to withstand drought stress. Liu et al. (2019) have reported that *Juglans mandshurica* Maxim and *Juglans regia* L. cv. Jizhaomian species were more tolerant to the drought stress as compared to the *Juglans nigra* L. species.

When plants are exposed to the water stress, they show many reactions on a morphological, physiological, biochemical and molecular bases (Buyuk et al., 2012; Soni et al., 2017; Liu et al., 2019). The most important biochemical response that they show is the accumulation of reactive oxygen species (ROS) such as H_2O_2. Excessive accumulations of these highly reactive ROSes cause oxidative stress that results in the programmed cell death by rapidly damaging macromolecules (e.g. DNA, protein, lipid and carbohydrate) (Khaleghi et al., 2019). On the other hand, plants have developed many defense and adaptation mechanisms (synthesis of substances such as stress proteins, osmolytes, antioxidants, etc.) that can increase their ability to survive and grow under short or long-term drought stress (Ashraf and Foolad, 2007; Akinci and Lösel; 2012; Anjum et al., 2011; Buyuk et al., 2012; Marcin´ska et al., 2013; Vahdati and Lofti, 2013). Antioxidant enzymes the synthesis of which is probably the main process in the plant tolerance to the environmental stresses play an important role in the scavenging of these reactive oxygen species (Khaleghi et al., 2019). For example, it has been reported that the plants have develped enzymatic (superoxide distumase (SOD), peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT) and non-enzymatic (glutathione, β-carotene, ascorbic acid, α-tocopherol) antioxidant defense mechanisms to reduce the oxidative damage (Anjum et al., 2011; Buyuk et al., 2012; Farajzadeh et al., 2017; Popovic et al., 2017; Selmi et al., 2017). In a study conducted on two different types of walnuts, it was determined that drought stress promoted SOD and POD activity and increased the proline content (DaPei et al., 2018). In another study, it was reported that there were significant increases in the POD and APX activities in both leaves and roots of tolerant walnut genotypes starting from the 7th day of drought application (Lofti et al., 2010). As a result, many biochemical reactions are activated in the cell in terms of the tolerance levels of the genotypes under oxidative stress.

In this study, it was aimed to determine the tolerance mechanisms of one year old Chandler and Fernor walnut cultivars grafted on the *J. regia* seedlings against the increasingly limited water stress created by different irrigation levels, by the activity of some antioxidant enzymes.

Material and Method

Plant material and treatment

The research was carried out in the Agricultural Research and Application Center located in the campus of Isparta University of Applied Sciences (ISUBÜ) in 2020, in a plastic greenhouse of approximately 175 m^2 with manual ventilation from the side. The plant material of the study consisted of one-year-old tuberous seedlings of the Chandler and Fernor cultivars grafted on the *Juglans regia* seedlings. One year after grafting the walnut seedlings were planted in 8-liter plastic tubes with holes from the bottom filled with a 1:2:1 mortar containing sand, peat and perlite, and kept outdoors until they were put into the trial. Then the tubed saplings were transplanted into the greenhouse before the treatments were made. The growing medium used in the experiment was neutral, low in lime, salt-free and high content of organic matter, and has a clay loam structure (Anonymous 2020a). Irrigation water (C2S1) was in the class suitable for irrigation (Anonymous 2020b).

Three different irrigation levels were applied to the potted one-year-old saplings in a greenhouse for three months as follows: 1) full irrigation as control (100% of the pot field capacity (PFC)) and 2) two deficit irrigation treatments (50% of the PFC and 25% of the PFC). Irrigation treatments were started in the last week of June (24 June 2020) and ended in the third week of September (17.09.2020). Irrigation was performed once every 5 days to make up for the water missing from the pot capacity. The average temperature and humidity values measured in the greenhouse from July to September during the trial were given in Table 1.

Table 1. Average temperature and humidity values measured in the greenhouse from July to September in 2020, when the experiment was conducted.

Month	Average Maximum Temperature (°C)	Average Minimum Temperature (°C)	Average temperature (°C)	Average Maximum Humidity (%)	Average Minimum Humidity (%)	Average Humidity (%)
July	40.2	19.5	29.9	59.3	16.5	37.9
August	40.5	18.1	29.3	73.1	16.7	44.9
September	38.1	14.6	26.4	70.1	17.0	43.6

Biochemical analyses

Leaf Sampling: Leaf sampling was started 15 days after the first irrigation application and continued every 15 days until the end of the experiment. After washing the leaves with distilled water, they were frozen in liquid nitrogen and stored at -80 °C until the analysis.

Ascorbate peroxidase (APX) enzyme activity: APX enzyme activity analysis was performed according to the method described by Nakano and Asada (1981). For this purpose, 12 ml of 50 mM potassium phosphate buffer (Ph:7.3) containing 1 mM (EDTA), 2 mM DTT and 1 mM ascorbic acid was added to 4 g of leaf sample and the homogenate was centrifuged at 10,000 g and 4°C for 15 minutes. 0.9 ml of 0.05 M sodium phosphate buffer (Ph: 7.0) containing 0.5 mM ascorbate, 0.1 mM EDTA Na_2 and 1.2 mM H_2O_2 was added to the 0.1 ml enzyme extract and the absorbance values were readed at 470 nm. The results were expressed as mol/min/g protein.

Catalase (CAT) enzyme activity: CAT enzyme activity was determined as using the method described by Beers et al. (1952). For this purpose, 10 g sample and 25 ml 50 mM cold sodium phosphate buffer containing 0.5 g polyvinyl polypyrolidine (PVPP) was prepared (Ph: 7.0) and the lysed samples were centrifuged at 27,000 g and 4°C for 50 minutes. Then, 2 ml of sodium phosphate buffer (50 mM and pH: 7.0), 0.5 ml of H_2O_2 (40 mM) and 0.5 ml of enzyme extract were mixed and the absorbance values of the samples were read at 240 nm wavelength. The results were expressed as U/mg protein.

Peroxidase (POD) enzym eactivity: POD enzyme activity was determined using the method described by Jiangetal. (2010). According to this; 10 g of fresh leaf sample and 25 ml of cold 100 mM sodium phosphate buffer containing 0.5 g of polyvinyl polypyrolidine (PVPP) (pH: 6.4) were were homogenized, and then centrifuged at 27,000 g at 4°C for 50 minutes and then the supernatant was collected. After that, 100 mM sodium phosphate buffer (Ph: 6.4) containing 8 mM quaiacol was added to the 0.5 ml enzyme extract and incubated at 30°C for 5 minutes. The absorbance values were read at 460 nm wavelength and the results were expressed as Δ_{A460}/min/mgprotein.

Superoxide dismutase (SOD) enzyme activity: SOD enzyme analysis was performed as described by Jiang et al. (2010). For this purpose, 10 g of fresh leaf sample was added to 25 ml of cold 100 mM sodium phosphate buffer containing 0.5 g of polyvinyl polypyrolidine (PVPP) (pH: 6.4). After the samples were crushed with the help of a homogenizer, they were centrifuged at 27,000 g at 4°C for 50 minutes and the supernatant was collected. The analyses were made according to the method described by Constantine and Stanley (1977). 2.9 ml of 50 mM sodium phosphate buffer (Ph:7,8) containing 13 mM methionine, 75 µM NBT, 10 µM EDTA and 2 µM riboflavin and 0.1 ml of sample were mixed and the absorbance values were read in the spectrophotometer at 560 nm wavelength. The results were defined as the amount of enzyme reducing the amount of SOD by 50% as a result of NBT and expressed as U/mg protein.

Statistical Analysis: The experiment was established according to the completely randomized plots experimental design with 3 replications and 5 plants per replication. The data was subjected to the variance analyses ($p<0.05$) and the differences between the means were evaluated with the Tukey test.

Results and Discussion

The accumulation of ROS'es comes as a biochemical response in the plants exposed to the water stress. However, excessive accumulation of ROS'es inevitably causes oxidative stress by inducing the lipid peroxidation, protein reduction and DNA fragmentation in the cell. Plants have also developed antioxidant defense mechanisms to reduce this oxidative damage (Buyuk et al., 2012; Farajzadeh et al., 2017; Popović, 2017; Selmi et al., 2017). In many studies, it has been determined that the enzyme activities such as SOD, POD, APX, CAT and GR increased in the plants under water stress (Farajzadeh et al., 2017; Popović, 2017; Selmi et al., 2017).

The APX enzyme, which is highly expressed in the plant cells, plays an important role in the defense mechanism by reducing H_2O_2, one of the reactive oxygen species (Buyuk et al., 2012; Sharma 2012). In this study, a significant difference was determined between the treatments in terms of APX activity in all analysis periods, and the APX activity increased linearly as the severity of water stress increased (Table 2, Figure 1). Moreover, as the period progressed in the study, APX activity increased at higher rates in the all treatments until the 60th day analysis, and this increase was realized at the higher levels in the limited water stress applications. However, APX activity remained slightly increased or decreased at similar levels with the 60th day analysis results in the last two analysis periods. In the measurements made at the end of the experiment (90th day), the APX activity increased 1.5 times in the 50% PFC application and 2.1 times in the 25% PFC application as compared to the 100% PFC application. According to the results of the 15th day analysis, at the end of the period, it was 206.0% in 100% PFC, 171.1% in 50% PFC and 182.4% in 25% PFC. These results were similar to the literature. As a matter of fact, it was determined that there was a significant increase in the APX activities in both leaves and roots of tolerant walnut genotypes starting from the 7th day of drought treatment (Lofti et al., 2010). Moreover, similar results were also obtained in the studies conducted on different plants (Gur, 2018; Faaek, 2018; Babalik, 2012).

In the study, the difference between the cultivars was significant in terms of APX enzyme activity (Table 2). Fernor cultivar showed higher activity. These results may indicate that the drought tolerance of cultivars may be closely related to the antioxidant enzyme capacity. Indeed, Lofti et al. (2010) indicated that the tolerant walnut genotypes showed higher APX activity, but Lofti et al. (2019) reported that the APX enzyme increased as the drought severity increased in the seedlings of the Chandler cultivar. Moreover, Vahdati (2014) stated that the differences between the walnut genotypes that show resistance or sensitivity to abiotic stresses could be attributed to the differences in the mechanisms underlying oxidative stress damage and subsequent tolerance to abiotic stress.

Table 2. Effects of water stress treatments on the APX enzyme activity (mol/min/g)

Cultivar	Water Stress Treatments (% PFC)	Analysis Dates 15. Day	30. Day	45. Day	60. Day	75. Day	90. Day
Chandler	100 (control)	0.70	0.91 d	1.41 d	1.39	1.66	2.35 d
	50	1.34	1.82 bc	2.75 c	2.84	2.91	3.33 c
	25	1.59	2.55 ab	3.60 b	4.49	5.35	5.32 a
Fernor	100 (control)	0.96	0.85 d	1.39 d	2.62	1.84	2.73 cd
	50	1.50	1.52 cd	2.44 c	3.50	3.41	4.38 b
	25	2.16	3.22 a	4.71 a	5.72	5.73	5.31 a
Treatment Average	100 (control)	0.83 c	0.88 c	1.40 c	2.00 c	1.75 c	2.54 c
	50	1.42 b	1.67 b	2.59 b	3.17 b	3.16 b	3.85 b
	25	1.88 a	2.89 a	4.16 a	5.11 a	5.54 a	5.31 a
Cultivar Average	Chandler	1.21 b	1.76	2.59 b	2.91 b	3.31 b	3.66 b
	Fernor	1.54 a	1.87	2.84 a	3.95 a	3.66 a	4.14 a

PFC: Pot Field Capacity; *: There is no statistically significant difference between the means shown with the same letter in the columns ($p<0.05$).

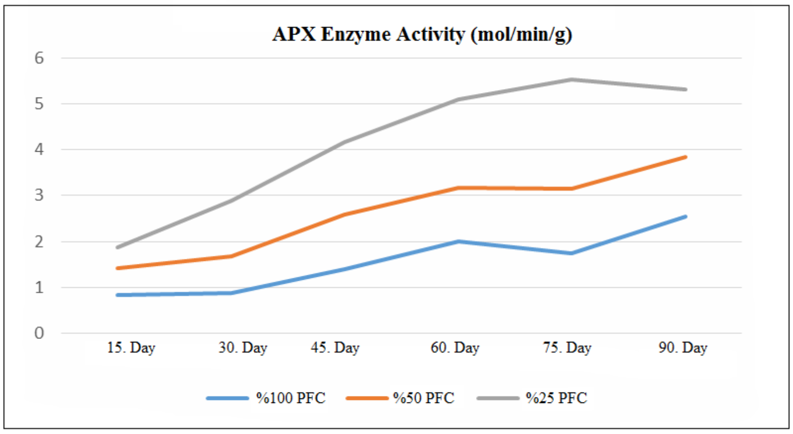

Figure 1. Variation of APX activity according to the periods in different water stress applications

CAT is one of the important antioxidant enzymes that play a role in the decomposition of harmful H_2O_2 into H_2O and O_2, which occurs under stress conditions (Buyuk et al., 2012). In this study, a significant difference was found between the treatments in all analysis periods in terms of CAT activity. As the severity of water stress increased, CAT enzyme activity increased and the highest CAT values were determined in 25% PFC treatment, while the lowest values were determined in 100% PFC control treatment (Table 3 and Figure 2). Moreover, in 100% PFC and 50% PFC treatments, CAT activity increased at high rates until the 45th day analysis, while it followed a slight increase or decrease in similar levels with the 45th day analysis results in the following periods. On the other hand, there was a significant increase in the CAT activity in the 15th day analysis of 25% PFC application and this increase continued as the period progressed. In the measurements made at the end of the experiment (90th day), CAT activity increased 1.7 folds in 50% PFC application and 2.4 folds in 25% PFC application as compared to the 100% PFC treatment. According to the results of the 15th day analysis, it was observed as 40.9% for 100% PFC, 109.0% for 50% PFC and 103.2% for 25% PFC at the end of the period. These results clearly show that CAT enzyme activity plays a role under the drought stress in walnuts. This was particularly evident under the severe stress conditions. Similar results have also been reported in the studies performed on the other plants (Bolat et al., 2014; Hassan et al. 2018; Tiryaki, 2018).

Table 3. Effects of water stress treatments on the CAT enzyme activity (U/mg)

Cultivar	Water Stress Treatments (% PFC)	Analysis Dates					
		15. Day	30. Day	45. Day	60. Day	75. Day	90. Day
Chandler	100 (Control)	22.03	27.47 cd	20.18 d	30.86 cd	28.79 c	25.22
	50	20.91	45.96 ab	36.09 b	42.00 b	46.92 b	44.29
	25	29.66	53.70 a	57.62 a	52.52 a	60.69 a	58.00
Fernor	100 (Control)	12.21	23.25 d	21.94 cd	24.68 d	21.07 d	23.03
	50	18.31	34.25 c	26.41 c	39.52 bc	26.27 cd	37.67
	25	27.81	36.05 bc	37.50 b	58.14 a	55.15 a	58.75
Treatment Average	100 (Control)	17.12 b	25.36 b	21.06 c	27.77 c	24.93 c	24.12 c
	50	19.61 b	40.10 a	31.25 b	40.76 b	36.59 b	40.98 b
	25	28.73 a	44.88 a	47.56 a	55.33 a	57.92 a	58.37 a
Cultivar Average	Chandler	24.20 a	42.38 a	37.96 a	41.79	45.67 a	42.50
	Fernor	19.44 b	31.18 b	28.62 b	40.78	34.16 b	39.81

PFC: Pot Field Capacity; *: There is no statistically significant difference between the means shown with the same letter in the columns ($p<0.05$).

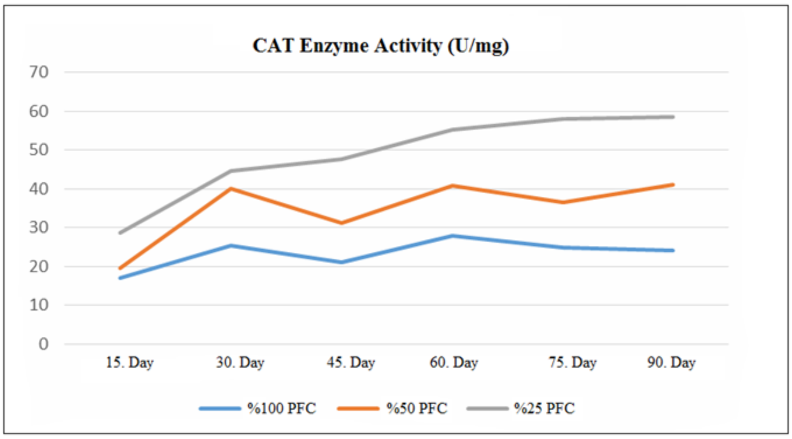

Figure 2. Variation of CAT activity according to the periods in different water stress treatments

Significant differences were found between the varieties in terms of CAT activity. Accordingly, Chandler cultivar had the higher CAT enzyme activity in all analysis periods. Similarly, Gur (2018) reported the differences in the CAT activities in the different pear rootstocks under drought stress.

SOD, a metalloprotein, is the first enzyme to play a role in the cellular defense mechanisms against reactive oxygen species (Buyuk et al., 2012; Khaleghi et al., 2019). SOD catalyzes (produces H_2O_2) and eliminates the superoxide (O_2-) radical (Khaleghi et al., 2019). H_2O_2 is converted to H_2O and O_2 by CAT (Zhu et al., 2020). In this study, a significant difference was found between the treatments in terms of the SOD activity in all analysis periods, and the SOD activity increased linearly as the severity of water stress increased (Table 4 and Figure 3). In the study, there was no increase in SOD activity in the first two months in the control application (100% PFC), but an increase was found in the analyzes of the 75th day with the progression of the period. On the other hand, as the period progressed in the 50% PFC and 25% PFC treatments, the SOD activity values gradually increased. In the measurements made at the end of the experiment (90th day), the SOD activity increased 2.3 times in the 50% PFC treatment and 3.2 times in the 25% PFC treatment as compared to the control. According to the results of the 15th day analysis, at the end of the period, it was 14.2% for 100% PFCs, 70.0% for 50% PFCs and 71.7% for 25% PFCs. This increase in the SOD activity in walnut leaves with water stress may be associated with the mechanism of protection against oxidative stress-related damage. In the studies conducted on walnuts, it was also determined that the SOD enzyme activity increased under drought stress (JiMing et al., 2012; DaPei et al., 2018). Similar results have also been reported in the studies performed on the other plants (Tiryaki, 2018; Khaleghi et al., 2019).

In terms of SOD activity, a significant difference was found between the cultivars in the all period analyses except the 15th day and 45th day analyses. Accordingly, Fernor cultivar had the higher SOD enzyme activity in all analysis periods (Table 4). Zhu et al. (2020) also reported that there were differences between the Cassava cultivars that they examined in terms of SOD content, and one cultivar showed the higher SOD activity under severe drought. Similarly, Gur (2018) reported that there were significant differences in the SOD activity among the pear rootstocks that they examined.

Table 4. Effects of the water stress treatments on the SOD enzyme activity (U/mg)

Cultivar	Water stress Treatments (% PFC)	Analysing Dates					
		15. Day	30. Day	45. Day	60. Day	75. Day	90. Day
Chandler	100 (Contol)	1.28	1.13	1.02	1.12	1.33 f	1.74 d
	50	1.85	2.30	2.54	2.55	2.83 d	3.39 c
	25	2.75	3.42	4.42	4.42	4.87 b	4.30 b
Fernor	100	1.40	1.13	1.32	1.10	1.93 e	3.32 d
	50	2.30	2.54	2.90	2.79	3.89 c	3.64 c
	25	2.90	3.72	4.51	4.59	5.59 a	5.41 a
Treatment Average	100 (Control)	1.34 c	1.13 c	1.17 c	1.11 c	1.63 c	1.53 c
	50	2.07 b	2.42 b	2.72 b	2.67 b	3.36 b	3.52 b
	25	2.83 a	3.57 a	4.47 a	4.50 a	5.23 a	4.86 a
Cultivar Average	Chandler	1.96	2.28	2.66 b	2.70 b	3.01 b	3.14 b
	Fernor	2.20	2.46	2.91 a	2.82 a	3.80 a	3.46 a

PFC: Pot Field Capacity; *: There is no statistically significant difference between the means shown with the same letter in the columns (p<0.05).

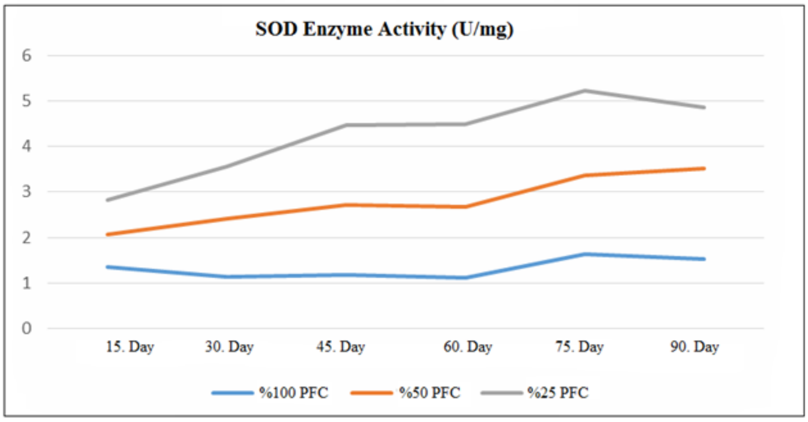

Figure 3. Variation of SOD activity according to the periods in different water stress treatments

Referring to Asada and Takahashi (1987), another important enzyme involved in the scavenging of H_2O_2 produced under stress is POD (Gokmen, 2011). Moreover, Li et al. (2017) reported that POD plays a role in the protective mechanism under water stress. In this study, POD activity linearly increased with the increasing water stress severity and stress duration (Table 5 and Figure 4). In the analyses performed at the end of the experiment (90th day), POD activity increased 2.1 times in the 50% PFC application and 2.6 times in the 25% PFC application as compared to the control. It was determined that the POD activity increased as the period progressed in all treatments. According to the results of the 15th day analysis, the increases at the end of the period were 53.4% for 100% PFCs, 109.3% for 50% PFCs, and 106.9% for 25% PFCs. In the previous studies on walnuts, POD enzyme activity was found to increase under drought stress (Lofti et al., 2010; JiMing et al., 2012; DaPei et al., 2018; Lofti et al., 2019). This increase in the POD activity in walnut leaves with water stress may be associated with the mechanism of protection against oxidative stress-related damage. However, different results have been reported in the literature regarding the POD activity. Renju et al. (2017) reported that the POD activity increased in a moderate water stress application, whereas in moderate and severe drought applications, it first increased and then decreased in the later stages of drought. Similarly, Hui et al. (2016) reported that POD activity increased in the drought-resistant potato variety but decreased in the non-durable potato variety. Tiryaki (2018) reported that the limited water applications in oil rose reduced POD activity. Zhu et al. (2020) also reported that restricted water applications significantly reduced POD activity in Cassava plant. These results indicate that water stress affects POD activity differently in different species. This suggests that POD activity can be explained by whether it is suppressed or not by the transcription factors (Zhu et al., 2020).

Significant differences were found between cultivars in terms of POD activity. Accordingly, the Fernor cultivar provided higher POD activity in the 15th day and 45th day analyses, but the Chandler cultivar performed higher POD enzyme activity in the other periods (Table 5). Lofti et al. (2019) also reported that the POD enzyme activity increased with increasing drought severity in the seedlings of Chandler cultivar. Moreover, Zhu et al. (2020) reported that there were differences between the Cassava cultivars that they examined in terms of POD contents and the two cultivars showed lower POD activity.

Table 5. Effects of water stress treatments on the POD enzyme activity (Δ_{A460}/min/mg)

Cultivar	Water stress treatments (% PFC)	Analyses Dates					
		15. Day	30. Day	45. Day	60. Day	75. Day	90. Day
Chandler	100 (ontrol)	76.13	79.91 c	91.63 d	96.30 c	96.90 d	128.08 c
	50	125.06	135.70 b	207.81 b	169.78 b	231.87 b	308.00 a
	25	153.31	153.85 b	250.36 a	275.33 a	345.26 a	341.32 a
Fernor	100	90.88	60.23 c	93.08 d	99.91 c	142.41 c	128.15 c
	50	131.0	134.46 b	134.00 c	140.84 bc	166.71 c	227.94 b
	25	167.97	203.86 a	208.45 b	193.85 b	224.13 b	323.39 a
Treatment Average	100 (Control)	83.51 c	70.07 c	92.35 c	98.11 c	119.65 c	128.11 c
	50	128.03 b	135.08 b	170.90 b	155.31 b	199.29 b	267.97 b
	25	160.64 a	178.85 a	229.41 a	234.59 a	284.69 a	332.35 a
Cultivar Average	Chandler	118.17 b	123.15	183.26 a	180.47 a	224.67 a	259.13 a
	Fernor	129.95 a	132.85	145.18 b	144.87 b	177.75 b	226.49 b

PFC: Pot Field Capacity; *: There is no statistically significant difference between the means shown with the same letter in the columns ($p<0.05$).

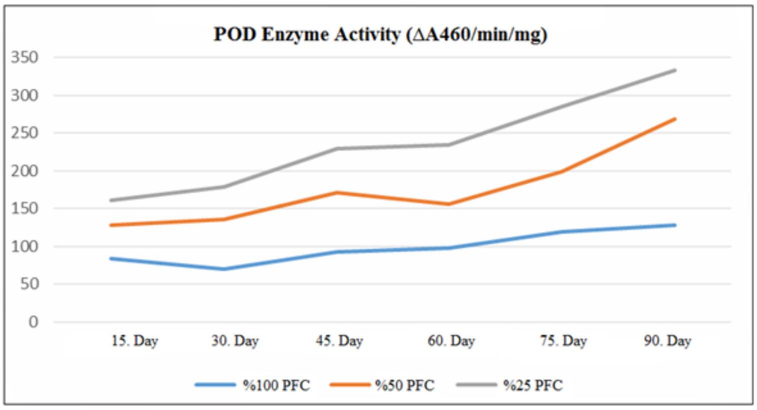

Figure 4. Variation of POD activity according to the periods in different water stress treatments

Conclusion

In the study, the responses of one-year-old tubedsaplings of commercially important walnut cultivars to the different levels of drought stress were investigated on the basis of some antioxidant enzymes. As a result, walnut saplings developed antioxidant defense mechanisms against water stress, demonstrating a possible tolerance. This tolerance suggests that it may be related to the activation of antioxidant systems and the reduction of oxidative damage. Antioxidant enzymes were found to have different affinities for both cultivars in response to the oxidative damage. While POD and CAT activities were at higher levels in the Chandler cultivar; SOD and APX activities were at higher levels in the Fernor cultivar. In addition, it was determined that especially the APX enzyme activity increased at a high level as the period progressed under the controlled conditions.

Acknowledgment

This project was supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK) with the number 119O806. We would like to thank TÜBİTAK. Also we would like to thank Prof. Dr. Yusuf UÇAR assiting in the calculation and application of irrigation water levels in the study.

Conflict of Interest

There is no conflict of interest between co-authors.

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