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SEASONAL ANTIOXIDANT CAPACITY OF *ASPHODELUS AESTIVUS* BROT. ROOT TUBERS IN RESPONSE TO ARIDITY FROM DEGRADED MEDITERRANEAN ENVIRONMENTS

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ABSTRACT

Asphodelus aestivus Brot. (Walter) is the dominant geophyte species on degraded areas of eastern Mediterranean Basin. Because it has a stress-tolerant strategy and it is a competitive ruderal. We studied the activities of antioxidant enzymes (ascorbate peroxidase, APX; superoxide dismutase, SOD; catalase, CAT) in the root tubers of *A. aestivus* from Bursa, Turkey over one year. There was a seasonal variation in antioxidant enzyme activities. The CAT and APX showed similar variation model in which the activity was highest in July (9.3 ± 2.7 units/mg protein and 141.9 ± 52.7 units/mg protein) and lowest in August (1.2 ± 0.6 units/mg protein and 14.5 ± 2.3 units/mg protein). The SOD activity increasing in summer months was reached up the highest level in late summer (297.6 ± 48.1 units/mg protein). The significant negative correlations were also found between enzyme activities and water contents of soils ($p<0.05$). The increased antioxidant enzyme activities in summer and late summer periods may be a protective and acclimation mechanism against drought stress. These results indicate that drought can induce enzymatic antioxidant defence system in *A. aestivus* and this system may help to this species to spread on degraded Mediterranean environments.

Keywords: Drought, Antioxidant enzymes, *Asphodelus aestivus*, Root tubers, Mediterranea

1. INTRODUCTION

Many conservations studies pay attention to Mediterranean ecosystems because of their high biodiversity [1, 2]. These ecosystems are under the effects of Mediterranean climate conditions. Hot and dry summers are characteristic to Mediterranean climate and they cause to seasonally repeating aridity conditions [3]. Plant growth in Mediterranean-type ecosystems is usually limited by water deficiency which is accompanied by high solar radiation and high temperatures during the summer [4]. Water deficit periods are also common in Mediterranean winter and there has been an increase in the frequency and severity of aridity in the Mediterranean basin[5]. Under these extreme environmental conditions, the nutrient and water acquisition capability of plants, which determine the ecosystem sustainability, are restricted. In considering the climate change, many studies report that the changes in Mediterranean climate comprise elevated mean temperatures, lower precipitation as well as heat waves and droughts [6, 7, 8]. According to climate change projections which are reporting the higher temperatures and changes in seasonal precipitation patterns, Mediterranean ecosystems potentially will meet the intensified seasonal aridity conditions [9, 10]. Therefore, it is important to know the responses of Mediterranean plant to these conditions.

Plants have some mechanisms for coping with extreme conditions resulting from the low water availability related to high light levels and temperatures during the summer period. Such mechanisms include osmotic adjustment, regulation of stomatal opening, modification of cell wall characteristics and extensive root system [11]. As well as other environmental stress types, aridity may cause to

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decreased stomatal conductivity [12]. This leads to decreased leaf internal CO_2 and the formation of reactive oxygen species (ROS) by enhanced leakage of electrons to molecular oxygen [13]. Although ROS are involved in growth regulation, development, responses to environmental stimuli and cell death, they can be harmful for cellular components. Therefore, ROS scavenging mechanism plays a crucial role in adaptation capability of plants to stress conditions and their survival on these conditions. Plants have a cooperative system composed of both enzymatic and non-enzymatic antioxidants to overcome the excesses ROS [14]. Some compounds of non-enzymatic system antioxidants like tocopherols, carotenoids, flavones, anthocyanins can protect plant cells against oxidative injury. Additionally, plants possess enzymatic mechanisms to detoxify the ROS, including activation of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) [15].

In addition to extreme environmental conditions leading to drought stress in plants, Mediterranean ecosystems are under the anthropogenic pressure by various activities and these ecosystems have been changed or degraded [16]. The plant cover is represented by 'maquis' and geophytes in degraded areas and the typical geophyte species in last degradation stage of these ecosystems in the eastern Mediterranean Basin is *Asphodelus aestivus* Brot. (Walter). According to Ayyad and Hilmy [17] and Le Houérou [18] ''asphodel geophyte-deserts'' or ''asphodel-semi deserts" terms can be used to express these ecosystems. Since *A. aestivus* can handle the difficulties of Mediterranean climate [19] and it is resistant to disturbance [20], it can be dominant on degraded areas such as slopes of agricultural lands, roadsides and on calcareous soils in pastures Mediterranean environments. According to Pantis et al. [19] the wide distribution of *A. aestivus* on many countries of Mediterranean Basin may be explained by it's a competitive ruderal and stress-tolerant strategy. In addition, we previously showed that the high nitrate assimilation [21] and heavy metal accumulation capacity [22] could help to *A. aestivus* for dominating on degraded Mediterranean environments. According to Kırmızı et al. [23] seasonal variations in proteases (endopeptidase, leucineaminopeptidase and carboxypeptidase) of tubers appear to be harmonized with the Mediterranean climate and can ensure the domination of this species in degraded areas. We hypothesize that the seasonal responses of Mediterranean geophytes to aridity can alleviated by activities of some antioxidative enzymes of root tubers. Thus, we evaluated the activities of three antioxidant enzymes, SOD, CAT and APX, in the root tubers for 1 year.

2. MATERIAL AND METHOD

Study area. The study site is located (40°16′21″ N and 28º39′11″ E) on Bursa City from north-west part of Turkey. It is characterized by Mediterranean climate with wet and mild seasons from autumn to spring and with dry and warm seasons from spring to autumn. The annual mean temperature is 14.6 °C and the coldest month is January (1.7 °C). The mean maximum temperature reaches up to 30.9 °C in August. The precipitation generally occurs in the form of rain during winter and spring with an annual precipitation of 697 mm [26]. The soil moisture and temperature regimes of study site are xeric and thermic [27]. Agriculture and animal husbandry are the main land use practices around the study area.

Asphodelus aestivus Brot. (*A. microcarpus* Viv.) spreading all over the Mediterranean basin is a perennial tuberous root geophytes [20]. It has two phonological phase within its lifecycle. One of them is photosynthetic active. This phase begins with leaf emergence at early autumn and ends up with senescence of the above-ground plant parts at late spring. The other is inactive phase which represented by belowground parts [19, 24]. Flat leaves (40-90 cm in length and 2-4 cm in width) developed by over-wintering root tubers from a shoot apex in February-March. Flowering stalks (70-170 cm tall) bears 60-200 flowers occurs during April-May. Many tuberous roots attached mother plant. After senescence at June, many tuberous roots stand out to the mother plant [25].

Sampling and analyses. The study was conducted in a natural habitat of degraded maquis vegetation around the Taşpınar village of Bursa City between 09.05.2007 and 06.05.2008. Three plant samples were harvested from a 10x10 m area on each sampling date. After the tuberous roots were removed from the plants, they were washed with tap water and then with distilled water, respectively. Three subsamples of tuberous roots were taken from each plant for analysis (n=9). The tuberous roots which are not used were stored at -70°C in a freezer.

Fresh plant material (1 g) was homogenized with 3mL buffer solution (50mM Na-phosphate buffer; pH 7.8), 1mM EDTA and 2% PVP in an ice bath for the enzyme extraction and assays. After homogenization, plant materials were centrifuged at 14 000g for 40 min at 4°C. The supernatants were used for the determination of the SOD and CAT activities, as well as the soluble protein content. For the determination of the APX activity, 2mM ascorbate was added to the above buffer solution. While, SOD activity was determined according to the method described by Beauchamp and Fridovich [28], the determination of CAT and APX activities was made according to Lester et al. [29] and then protein content was determined [30]. Tissue water contents were determined and these data were used for calculation of enzyme activities and protein determination.

The activities of enzymes are expressed as units per mg protein. One-way ANOVA was used to evaluate differences among the investigated enzyme activities. Tukey’s honest significant difference test was used to determine the differences among the periods. The simple correlations between antioxidant enzyme activities and soil water contents (%) provided by our previous study [21] were also tested. All the statistical tests were performed at a significance level of 0.05 using SPSS 16.0 for Windows [31].

3. RESULTS

In our previous study, we determined water contents (%) in the soils of *A. aestivus* and we found annual fluctuations (21). This data were presented in Figure 1. Soil water contents were decreased sharply lower in summary season. Variations in the activities of antioxidant enzymes (SOD, CAT and APX) during the 1-year study period are shown in Figures 2-4. Additionally, correlations between soil water contents and antioxidant enzyme activities were presented in Figure 5. Similar to soil water contents, the antioxidant enzyme activities changed significantly during the growth period ($P < 0.05$). For instance, while the highest SOD activity level was observed in the root tubers taken at September 2007 (297.6 ± 48.1 units/mg protein), we found the lowest SOD activity value in January 2008 (46.57 ± 8.38 units/mg protein). The SOD activity levels determined in February, March, April and May 2008 were low (70.3 ± 12.7 units/mg protein, 83.3 ± 25.0 units/mg protein, 65.0 ± 13.1 units/mg protein, 91.9 ± 32.9 units/mg protein; respectively) and similar to the SOD activity level determined in January (Figure 2). We observed significant negative correlation between SOD activity in root tubers and soil water contents (r^2=0.375; $p<0.05$) (Figure 5).

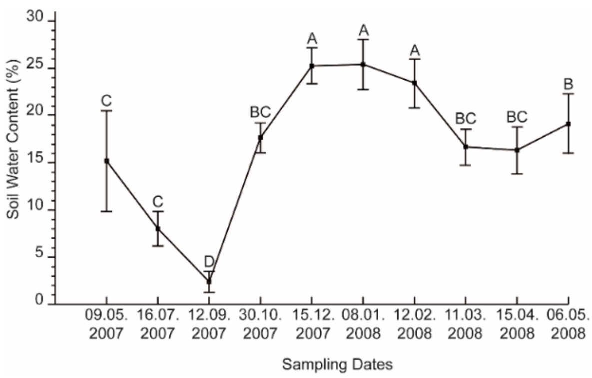

Figure 1. Seasonal fluctuations of soil water content (%), [The values with the same letters are not significantly different at the α= 0.05 level. Values are the means ± standard errors].

The APX activity patterns during a 1-year period are shown in Figure 2. APX activity was highest (141.9 ± 52.7 units/mg protein) in July 2007. However, it began to decrease in September (102.5 ± 14.6

units/mg protein). The APX activity was lower in the winter months, January to February 2008 (14.5 ± 2.3 units/mg protein and 21.4 ± 6.4 units/mg protein). Interestingly, it began to increase in April (76.9 ± 7.4 units/mg protein) but then decreased in May 2007 (35.5 ± 5.9 units/mg protein) (Figure 3). A significant negative correlation was found between APX activity in root tubers and soil water content (r^2=0.454; $p<0.05$) (Figure 5).

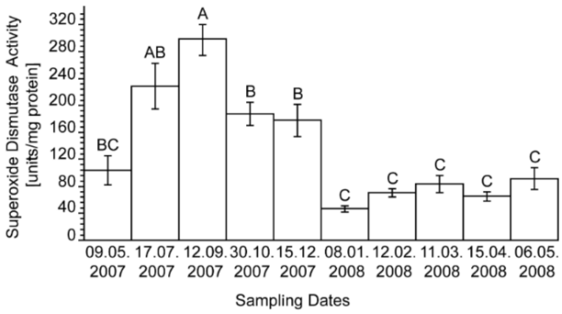

Figure 2. Seasonal fluctuations of SOD activity in root tubers of *Asphodelus aestivus*. [The values with the same letters are not significantly different at the α= 0.05 level. Values are the means ± standard errors].

We also found that CAT activity in root tubers of *A. aestivus* changed during the studied year ($p<0.05$); it varied between 1.2 ± 0.6 units/mg protein and 9.3 ± 2.7 units/mg protein (Figure 4). The seasonal fluctuation model for CAT activity was similar to the APX fluctuation model. The highest CAT activity value was observed in July 2007, while the lowest value was measured in January 2008. Additionally, we found a significant negative correlation between CAT activity in root tubers and the soil water

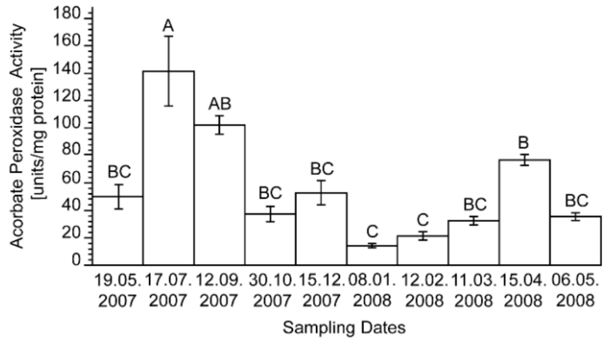

Figure 3. Seasonal fluctuations of APX activity in root tubers of *Asphodelus aestivus*. [The values with the same letters are not significantly different at the α= 0.05 level. Values are the means ± standard errors].

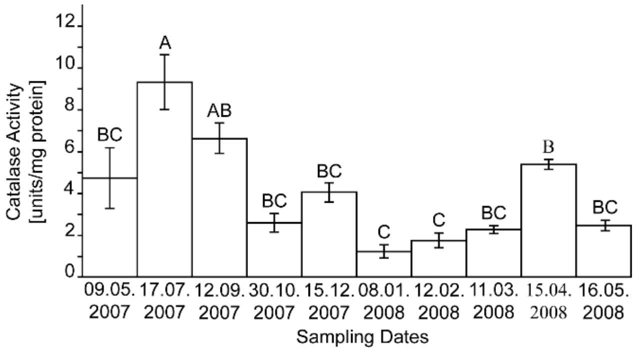

Figure 4. Seasonal fluctuations of CAT activity in root tubers of *Asphodelus aestivus*. [The values with the same letters are not significantly different at the α= 0.05 level. Values are the means ± standard errors].

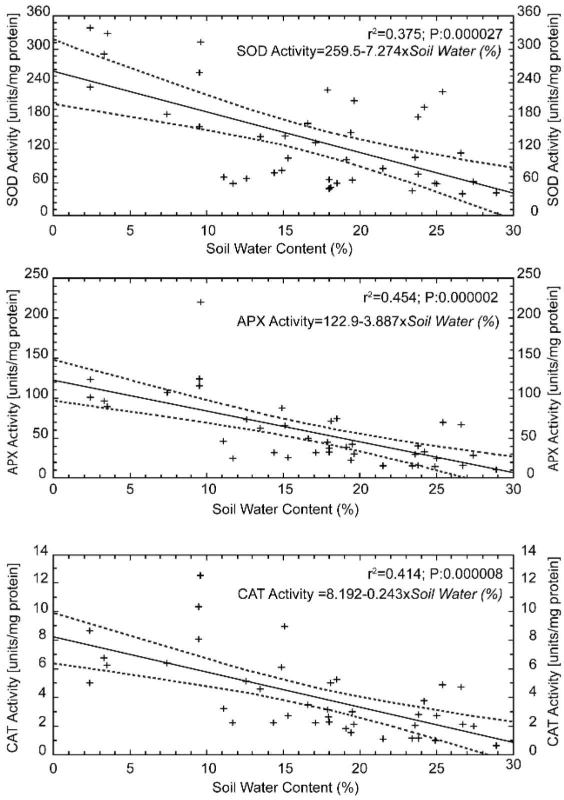

Figure 5. Simple correlation coefficients (r2), significant levels (possibility, P), and linear regression equations (Y = a + bx) between water content of the soil samples and antioxidative enzyme activities in root tubers of *Asphodelus aestivus*. ($P < 0.05$ significant correlation)

4. DISCUSSION

Sakar et al. [21] measured previously variations in soil moisture content of *A. aestivus* which is accordance with the pattern of Mediterranean climate (Figure 1). The hot and dry summer period is typical for Mediterranean climate and leads to seasonally recurring aridity [3, 10]. Despite these conditions, *A. aestivus* has ability to maintain it's survival and to become dominant on degraded areas of Mediterranean regions. This situation can be related to the capacity of this species to cope with difficulties of Mediterranean climate [19] and to resist the most common disturbances in its habitat [20]. Moreover, it has been reported that *A. aestivus* is a competitive ruderal and shows stress-tolerant strategies, which may indicate the distribution of this species on the Mediterranean environments in the whole world [19].

The present study was aimed to better understand the response of *A. aestivus* to drought stress related to low soil water content. We found that the examined antioxidant enzyme activities showed seasonal fluctuations synchronized with the soil water contents. The high APX and CAT activities in the root tubers were determined in the summer, when soil water contents decreased although the activities of these enzymes were low during the winter. These results and significant negative correlations between enzyme activities and soil water contents ($p<0.05$) indicate that the enzymatic antioxidant defence mechanism of this Mediterranean plant species was induced by drought. These findings are in agreement with the results of the few studies indicating the contribution of antioxidant enzymes in the defence systems of species such as *Capparis ovata* Desf. [12] and *Quercus ilex* L. [32].

Similarly, Daniels et al. [33] found an increase in non- enzymatic antioxidant capacity based on total polyphenol content in bulbs and roots of South African bulbous geophyte *Gethyllis multifolia* L. Bolus under drought stress. Additionally, Peksel et al. [34] showed that *Asphodelus aestivus* has powerful antioxidant activity that is based on non-enzymatic antioxidants. In our study, the high APX, CAT and SOD activities in the root tubers of *A. aestivus* can be related to this plant's life cycle, which consists of an active phase (autumn-late spring) and an inactive (summer) phase [19, 24]. These enzymes can help to maintain the survival during the drought period and to enable the leaf emergence in the autumn.

We concluded that there were seasonal fluctuations in the activities of antioxidant enzymes of *A. aestivus* which are harmonized with Mediterranean climate. The increased antioxidant enzyme activities in summer and late summer periods may be the part of a mechanism of this plant for protection and acclimation to arid conditions. Our results may help our understanding of the effects of the antioxidant defense mechanism on the domination of *A. aestivus* in the degraded areas in Mediterranean environments.

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REFERENCES

[1] Myers N, Mittermeier RA, Mittermeier CG, Da Fonseca GAB, Kent J. Biodiversity hotspots for conservation priorities. Nature 2000; 403: 853-858.

[2] Matesanz S, Valladares F. Ecological and evolutionary responses of mediterranean plants to global change. Environ Exp Bot 2014; 103: 53-67.

[3] Lionello P, Malanotte-Rizzoli P, Boscolo R, Alpert P, Artale V, Li L, Luterbarcher J, May W, Trigo R, Tsimplis M, Ubrichu, Xoplaki E. In: Lionello P, Malanotte-Rizzoli P, Boscolo R. editors. The Mediterranean climate: an overview of the main characteristics and issues. Mediterranean climate variability. Elsevier, Amsterdam, 2006. pp.1-26.

[4] Mooney HA. The impact of environmental stress on plant performance in mediterranean climate ecosystems: Differing levels on analysis. In: Tenhunen J. D, Catarino F. M, Lange OL, Oechel WC Editors. Plant Responses to Stress: Functional Analysis in Mediterranean Ecosystems. NATO ASI Series G, Vol 15. Springer-Verlag, Berlin, 1987. pp. 661-668.

[5] Houghton JT, DingY, Griggs D J, Noguer M, Van Der Linden PJ, Dai X, Maskell K., Johnson CA. Climate Change 2001: the Scientific Basis. Cambridge University Press; Cambridge, 2001.

[6] Gao X, Giorgi F. Increased aridity in the mediterranean region under greenhouse gas forcing estimated from high resolution simulations with a regional climate model. Global Planet Change, 2008; 62: 195-209.

[7] Lloret F, Escodero A, Iridondo MJ, Martinez-Villata J., Valladares F. Extreme Climatic Events and Vegetation: the Role of Stabilizing Processes. Global Change Biol 2012; 18: 797-805.

[8] Bussoti F, Ferrini F, Pollastrini M, Alessio F. The challenge of Mediterranean sclerophyllous vegetation under climate change: from acclimation to adaptation. Environ Exp Bot 2014, 103: 80-98.

[9] Meehl GA, Stocker TF, Collins WD, Friedlingstein P, Gaye AT, Gregory JM, Kitoh A, Knutti R, Murphy JM, Noda A, Raper SCB, Watterson IG, Weaver AJ, Zhao ZC. Global climate

projections. In: Solomon S, Qin D, Manning M, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, Miller HL Editors. Global climate projections. Cambridge University Press: Cambridge and New York, 2007. pp. 747-845.

[10] Niinemets U, Keenan T. Photosynthetic responses to stress in Mediterranean evergreens: mechanisms and models. Environ Exp Bot, 2014; 103: 24-41.

[11] Rhizopulou S, Psaras GK. Development and structure of drought tolerant leaves of the Mediterranean shrub *Capparis spinosa* L. Ann Bot 2003; 92: 377-383.

[12] Özkur O, Özdemir F, Bor M, Türkan İ. Physiochemical and antioxidant responses of the perennial xerophyte *Capparis ovata* Desf. to drought. Environ Exp Bot 2009; 66: 487-492.

[13] Miller G, Suzuki N, Çiftçi Yılmaz S, Mıttler R. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant Cell Environ 2010; 33: 453-467.

[14] Munne-Bosch S, Alegre L. Drought induced changes in the redox state of alpha-Tocopherol, Ascorbate, and the diterpene Carnosic Acid in chloroplasts of *Labiatae* species differing in Carnosic Acid contents. Plant Physiol 2003; 131: 1816-1825.

[15] Ashraf M. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. Biotechnol Adv 2009; 27: 84-93.

[16] Cody ML. Diversity, rarity and conservation in Mediterranean-climate regions. In: Soule ME Editor. Conservation biology: The science of scarcity and diversity. Sinauer Associate Inc., Sunderland, MA, USA, 1986. pp.122-152.

[17] Ayyad MA, Hilmy SH. The distribution of *Asphodelus microcarpus* and associated species on the western Mediterranean coast of Egypt. Ecology 1974; 55: 511-524.

[18] Le Houreou HN. Impact of man and his animals on Mediterranean vegetation. In: Di Castri F, Goodall D, Specht DWR Editors. Ecosystems of the world, Mediterranean-type shrublands. Elsevier, Amsterdam, 1981; pp. 479-521.

[19] Pantis JD, Sgardelis SP, Stamou GP. *Asphodelus aestivus*, an example of synchronization with the climate periodicity. Int J Biometeorol 1994; 38:28-32.

[20] Pantis JD, Margaris NS. Can systems dominated by Asphodels be considered as semi-deserts? Int J Biometeorol 1988; 32: 87-91.

[21] Sakar FS, Arslan H, Kırmızı S, Güleryüz G. Nitrate reductase activity (NRA) in *Asphodelus aestivus* Brot. (Liliaceae): distribution among organs, seasonal variation and differences among populations. Flora 2010; 205: 527-531.

[22] Güleryüz G, Arslan H, Leblebici Z, Kırmızı S, Aksoy A, SakarFS. Heavy metal content of *Asphodelus aestivus* Brot. from degraded areas in the Mediterranean environment (Bursa, Turkey). Fresen Environ Bull 2014; 23: 400-407.

[23] Kırmızı S, Güleryüz G, Arslan H. Protein mobilisation and proteolytic activities in root tubers of *Asphodelus aestivus*. Turk J Bot 2014; 38:302-308.

[24] Aschmann H. Distribution and peculiarity of Mediterranean ecosystem. In: Di Castri F, Mooney HA Editors Mediterranean type ecosystems. Springer-Verlag: Berlin, 1973. pp. 11-19.

[25] Sawidis T, Kalyva S, Delivopoulos S. The root-tuber anatomy of *Asphodelus aestivus*. Flora 2005; 200: 332-338.

[26] Güleryüz G. The studies on the nutrient turnover and productivity at some plant communities of the Uludağ alpine region. Ph.D. Disseration. University of Uludağ, Bursa, Turkey, 1992.

[27] Aydınalp C, Cresser MS. Iron and Zinc status in soils, water and plant Samples from a densely populated and industrialized region of Turkey. J Plant Nutr 2005; 28: 567-572.

[28] Beaucamp C, Fridovich I. Superoxide Dismutase; improved assays and an assay applicable to acrylamide gels. Anal Biochem 1971; 44: 276-287.

[29] Lester GE, Hodges DM, Meyer RD, Munro KD. Pre-extraction preparation (fresh, frozen, freeze-dried, or acetone powdered) and long-term storage of fruit and vegetable tissues: effects on antioxidant enzyme activity. J Agr Food Chem 2004; 52: 2167-2173.

[30] Bradford MM. A Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976: 72-248-254.

[31] SPSS 16.0 for Windows. SPSS Inc, Chicago, IL. 2007

[32] Nogués I, Llusiá J, Ogaya R, Munné-Bosch S, Sardans J, Peńuelas J, Loreto F. Physiological and antioxidant responses of *Quercus ilex* to drought in two different seasons. Plant Biosyst 2014; 148: 268-278.

[33] Daniels CW, Rautenbach F, Marnewick JL, Valentine AJ, Babajide OJ, Mabesula WT. Environmental Stress Effect on the Phytochemistry and Antioxidant Activity of a South African Bulbous Geophyte, *Gethyllis multifolia* L. Bolus. S Afr J Bot 2015; 96: 29-36.

[34] Peksel A, İmamoğlu S, Altaş Kıymaz N, Orhan N. Antioxidant and Radical Scavenging Activities of *Asphodelus aestivus* Brot. Extracts. Int J Food Prop 2013; 16: 1339-1350.