

Ali Doğru^{1*}, Nesrin Ecem Bayram²

20.04.2016 Geliş/Received, 25.07.2016 Kabul/Accepted

doi: 10.16984/saufenbilder.25673

ABSTRACT

Bu çalışmada üç mısır (Zea mays L.) kültivarının (FR13, FRB73, TTM815) kuraklık stresine toleranslarının araştırılması amacıyla bazı morfolojik ve fizyolojik parametreler analiz edilmiştir. Yirmi bir günlük bitkilere, Hoagland besin çözeltisi uygulaması sonlandırılarak 2 (hafif kuraklık stresi), 5 (orta dereceli kuraklık stresi) ve 8 (şiddetli kuraklık stresi) gün olmak üzere üç farklı kuraklık rejimi uygulanmıştır. FR13 ve TTM815 kültivarlarında orta dereceli ve şiddetli kuraklık stresi kök büyümesini önemli derecede inhibe ederken, gövde büyümesi mısır kültivarlarında tüm kuraklık uygulamalarından etkilenmemiştir. Elde ettiğimiz sonuçlar mısır kültivarlarında hafif ve orta dereceli kuraklık stresi koşullarında toplam bitki boyundaki azalmanın, kök büyümesindeki inhibisyondan kaynaklandığını ancak FRB73'ün daha az etkilendiğini göstermiştir. Tüm kültivarlarda kuraklık uygulamaları taze ve kuru biyomasın artmasına neden olmuş fakat FRB73'de daha belirgin bir artış meydana gelmiştir. Bu sonuçlar kuraklık kosulları altında nispeten daha stabil su miktarına sahip olduğu için FRB73'deki su iliskilerinin daha uygun sekilde regüle edildiğini göstermektedir. FR13 ve TTM815'de "klorofil a" miktarı uygulamalar sonunda azalırken, FRB73'de kuraklık stresinden etkilenmemiştir. Diğer yandan FR13 ve FRB73'de "klorofil b" içeriği kuraklık uygulamalarından daha az etkilenmistir. Kuraklık uygulamaları sonucunda tüm kültiyarlardaki malondialdehit birikimi önemli derecede artmıs ancak FRB73'de daha az birikim belirlenmistir. Toplam fenolik madde iceriği tüm kültivarlarda özellikle orta dereceli ve şiddetli kuraklık sonucunda artmıştır. FR13 ve TTM815'de toplam çözünür şeker miktarı orta dereceli ve şiddetli kuraklık uygulamaları sonucu azalırken, FRB73'de değişmemiştir. Elde ettiğimiz sonuçlara göre FRB73'ün, FR13 ve TTM815 ile karşılaştırıldığında, kuraklık stresi altında kök ve gövde büyümesini sürdürme yeteneği, fenolik madde, taze ve kuru biyomas artışı, su, fotosentetik pigment (klorofil a ve b) ve şeker miktarının daha az etkilenmesi ve malondialdehit birikiminin daha az olması nedeniyle kuraklığa daha toleranslı bir mısır kültivarı olduğu söylenebilir.

Keywords: drought stress, maize, phenolics, soluble sugar, Zea mays L.

Bazı mısır (Zea mays L.) kültivarlarında kuraklık stresi toleransı üzerine bir çalışma

ÖZ

In this study, some morphological and physiological parameters of three maize (*Zea mays* L.) cultivars, cv. FR13, FRB73 and TTM815, were analysed to investigate their tolerance to drought stress. Twenty-one-day old plants were subjected to three different regime of drought stress by withholding Hoagland's nutrient solution for 2 (mild drought stress), 5 (moderate drought stress), and 8 (severe drought stress) days. Root growth in cultivars FR13 and TTM815 was significantly inhibited by moderate and severe drought stress while shoot growth in all maize cultivars was not affected under all drought treatments. Our results showed that inhibited root growth under mild and severe drought conditions was responsible for decreased total plant lenght in all cultivars although FRB73 was less affected. Fresh and dry biomass increased in all cultivars under all drought treatments, however it was more significant in FRB73.

^{*} Sorumlu Yazar / Corresponding Author

¹ Sakarya Üniversitesi, Fen Edebiyat Fakültesi, Biyoloji Bölümü, Sakarya – adogru@sakarya.edu.tr

² Bayburt Üniversitesi, Mühendislik Fakültesi, Gıda Mühendisliği Bölümü, Bayburt - nesrin.ecem@hotmail.com

These results may indicate that water relations in FRB73 can be regulated more properly under drought stress, as indicated by relatively constant water content. "Chlorophyll a" content in FR13 and TTM815 was decreased at the end of the treatments while it was not affected in FRB73 by drought stress. "Chlorophyll b" content in FR13 and FRB73, on the other hand, was less affected by drought treatments. Malondialdehyde accumulation in all cultivars increased considerably as a result of all drought treatments, however to a less extent in FRB73. Total phenolic contents in all cultivars were increased especially by moderate and severe drought stress. Total soluble sugar contents in FR13 and TTM815 were decreased significantly by mild and severe drought stress while it remained constant in FRB73 under all drought treatments. According to our results, it may be concluded that FRB73 is more drought tolerant maize cultivar because of the ability of maintaining root and shoot growth, accumulation of phenolics, fresh and dry biomass, relatively less affected water, photosynthetic pigment (chlorophyll a and b) and sugar content and lower level of malondialdehyde under drought stress when compared to FR13 and TTM815.

Anahtar Kelimeler: kuraklık stresi, mısır, fenolik madde, çözünür şeker, Zea mays L.

1. INTRODUCTION

During their lifetime, plants often encounter unfavorable environmental conditions that have adverse effects on normal plant growth and crop yield. These limiting environmental conditions can be classified as biotic and abiotic stress factors according to their origins. Abiotic stress factors have been thought to be responsible for yield losses worldwide more than 50 % for major crops [1, 2]. Among the various abiotic stresses, drought is the major factor that limits crop productivity [3].

The effects of drought stress on plants have been classified as visible and invisible syndromes. Visible syndromes are leaf wilting, decrease of plant height, number and area of leaves and delay in occurence of buds and flowers [1, 4]. On the other hand, injuries of cytoplasmic membranes, disturbances in water status in different plant organs and decrease in chlorophyll content have been regarded as invisible effects of drought [5-9]. However, plants may give more complex metabolic responses to drought stress. One of the earliest responses of plants against drought is stomatal closure induced by hydraulic and chemical signals generated by cell turgor status and abscisic acid [10-12]. The expected result of stomatal closure in plants is limitation of CO₂ diffusion into leaves, leading to reduced NADP+ regeneration by Calvin cycle. Under these conditions, electrons are donated to molecular oxygen instead of NADP⁺ and thus the rate of generation of reactive oxygen species (ROS) is accelerated [13]. Among cellular organelles, especially chloroplasts are considered to be the main sites of ROS generation. Finally, accumulation of these cytotoxic ROS can disturb normal metabolic reactions in plant cells as a consequence of protein degradation, lipid peroxidation and pigment bleaching [14]. However, plants can alleviate the adverse effects of ROS through concerted action of both enzymatic and non-enzymatic antioxidant mechanism [15]. A number of studies have shown that detoxification of ROS is a common stress response for

plants [16]. Moreover, a remarkable relationship between enhanced antioxidant capacity and increased resistance to environmental stress factors has been reported in many plant species [17, 18].

Under drought stress conditions, the role of phenolic compounds with respect to detoxification of ROS is highly remarkable. Phenolic compounds are responsible for mainly preventing ROS formation in plant cells. When absorbing radiation, phenolic compounds transform highly destructive radiation into the blue radiation which is less-destructive to cellular structures in leaves, including photosynthetic apparatus [19]. In addition, phenolic compounds are capable of scavenging ROS because of their unique chemical structure and they have also been regarded as effective plant antioxidants [20].

Another important stress response observed in different plant species is the accumulation of low molecular weight metabolites including proline, polyamines, glycine betaine and soluble sugars. These metabolites are known to be soluble compounds and nontoxic at high concentrations [21]. The most common hypothesis explaining the role of these metabolites in plant stress tolerance is that they serve as osmolytes, helping plant cells for osmotic adjustment when exposed to drought stress [22]. It has been reported that drought stress affects photosynthetic efficiency and carbohydrate metabolism in some plant species [23, 24]. Moreover, the close relationship between soluble sugar content and ROS production in plant tissues and also the role of soluble sugars on oxidative-stress-regulated gene expression have been well established [25].

The objective of this work is to investigate the effect of drought stress on biomass, plant growth, lipid peroxidation, photosynthetic pigment, total soluble carbohydrate and phenolic substances in three maize cultivars.

2. MATERIAL AND METHODS

2.1. Plant Material, Growth Conditions and Experimental Design

Seeds of maize cultivars (Zea mays L. cvs. FR13, FRB73 and TTM815) were obtained from Sakarya Agricultural Research Institute, Sakarya, Turkey. All seed samples were surface sterilized in 5% sodium hypochlorite solution for 10 minutes before sowing. After washing in distilled water, five seeds of each cultivar were sown in plastic pots (14 cm in diameters) containing perlite. The experiment was performed in greenhouse conditions. The perlite moisture was maintained at field capacity for 21 days (d), after which half of the pots from each cultivar were exposed to drought stress by withholding nutrient solution supply. The control plants were well watered for additional eight days with Hoagland nutrient solution. At 2nd, 5th and 8th days of drought exposure plants were harvested and morphological measurements were performed immediately. Leaf samples taken for biochemical analysis were kept frozen at -20 °C until use. All analysis was done in the 4th fully expanded mature leaves of both in well-watered and drought-exposed plants. During experiment, changes in some climatic parameters such as daily mean temperature (°C) and relative humidity (%) were given in Figure 1A and B, respectively.



Figure 1. Changes in climatic parameters in greenhouse conditions (A) temperature and (B) relative humidity during 29 days

2.2. Morphological Measurements and Biomass Production

Root length, shoot length and total plant length were recorded as cm at each harvesting time. After harvesting, maize seedlings were weighed for fresh weight (FW) determination. Dry weight (DW) of plants was measured after drying in hot-air oven at 70 °C for 2 d. Water content (%) of plants was calculated according to Gibon et al. [26].

2.3. Photosynthetic Pigment Analysis

Photosynthetic pigments were extracted from leaf segments in 5 ml of 100 % acetone. After extraction, the homogenate was centrifuged at 10,000 rpm for 10 min. The absorbance of the supernatant was measured at 470, 644.8 and 661.6 nm using a Shimadzu UV mini 1240 UV VIS spectrophotometer. The concentrations of "chlorophyll a" (chl a) and "chlorophyll b" (chl b) were calculated according to Lichtenthaler [27].

2.4. Determination of Malondialdehyde (MDA) Content

Approximately 0.1g leaf material was powdered in liquid nitrogen and extracted in 5 % trichloroacetic acid according to Ohkawa et al. [28]. The extract was centrifuged at 12,000 rpm for 20 min. 0.4 ml of the supernatant was reacted with 0.4 ml of trichloroacetic acid-thiobarbituric acid mixture at 100°C for 1 h. Reaction was stopped in an ice bath and reaction mixtures were centrifuged at 10,000 rpm for 10 min. MDA content in leaves was calculated by using absorbances at 532 and 600 nm.

2.5. Determination of Total Phenolic Compounds Content

Total phenolic compound content of leaves was determined according to Chandler and Dodds [29]. Accordingly, 0.2 g leaf material was powdered in liquid nitrogen and extracted in 80% methanol. This mixture was placed in a refrigerator at 4 °C for 48 h. Homogenates were centrifuged at 4,000 rpm for 10 min. Appropriate amount of supernatant was reacted with 50% Foline Ciocalteu Reagent (FCR) and 5% sodium carbonate and kept at room temperature at a dark place for 1 h. The mixture was vortexed and absorbance was read at 725 nm. Total phenolic compound content of the leaves was calculated by using a standard curve prepared with gallic acid.

2.6. Determination of Total Soluble Sugar (TSS) Content

TSS content in leaves was determined by the phenol sulphuric method according to Dubois et al. [30]. For this purpose, leaf material (50 mg) was oven dried until constant dry weight was reached. Dried leaf material was powdered in a mortar and pestle and total soluble sugars were extracted by 70% ethanol. After centrifugation of extract at 3,500 rpm for 20 min, a reaction mixture was prepared. This mixture consisted of 1,000 μ l supernatant, 300 μ l phenol and 2,000 μ l concentrated sulphuric acid. Absorbances of these mixtures were read at 470 nm and sugar content of the leaves was calculated by a standard curve using sucrose.

2.7. Statistical Analysis

The experimental design was a complete randomised block with three independent replicates. The replicated block consisted of five pots of five seedlings. The significance of difference between controls and applications (mean values) was determined by one way ANOVA at 95% confidence level by using SPSS 11.0 statistical program for Windows (SPSS Inc., Chicago, IL, USA). Means and s.e. values represent five replicates (n=5) for root lenght, shoot length and total plant length and tree replicates (n=3) for other analysis of each treatments.

3. RESULTS

3.1. Effect of Drought Stress On Plant Growth And Biomass Production

Drought stress at 5th and 8th day produced significant growth inhibition in roots of FR13 and TTM815 cultivars compared with their controls (P<0.05) while root length of FRB73 was not affected by drought application during 8 days (P>0.05) (Fig. 2A). Root growth in TTM815 was inhibited by 22% which was the highest ratio among all cultivars. Shoot length, on the other hand, was not affected by drought stress significantly (P>0.05) (Fig. 2B). Changes in total plant length were found to be similar as in the root length of drought-affected maize cultivars (Fig. 2C), but total plant length was significantly lower in FRB73 at 8th day (P<0.05) (Fig. 2C).

Drought stress affected total fresh weight in all maize cultivars studied. In FR13, total fresh weight was found to be significantly higher than its control only at 8^{th} day (26% of control) while all drought application caused significant increases in total fresh weight in FRB73 (P<0.05) (Fig. 3A) and maximum fresh weight was reached at 8^{th} day with a 79% higher than control. In

TTM815, however, total fresh weight showed a progressive and significant increase until 5^{th} day of drought application (75% of control) and then dropped sharply and reached nearly to the level of control at 8^{th} day.

Drought application caused increases in total dry weight of all maize cultivars. In FR13 and FRB73, increases in total dry weights were progressive and 62% and 69% higher dry mass than related controls were reached at 8th day, respectively (P<0.05) (Fig. 3B). Total dry weight of TTM815 was increased by 68% at 5th day of drought application significantly and then decreased by 49%. Nevertheless it was significantly higher than its own control (P<0.05). Total water contents of FR13 and TTM815 were dropped significantly with regard to their controls at 8th day of drought while FRB73 did not show significant water loss during drought application (P>0.05) (Fig. 3C).

3.2. Effect of Drought Stress On Photosynthetic Pigment Content

Our results showed that chlorophyll contents in leaves of all maize cultivars were drastically affected by drought stress (Fig. 4A-B). "Chlorophyll a" content showed more severe and significant decrease in FR13 leaves with ratios ranging between 48%-51% when compared to their controls (P<0.05) (Fig. 4A).

In FRB73 leaves, however, decline in "chlorophyll a" content was not found to be significant (P>0.05). TTM815 represented a progressive "chlorophyll a" loss with the increase of drought. "Chlorophyll a" content of TTM815 was reduced by 40% at 5th day of drought, while for 8th day of drought, this decline was about 51%. "Chlorophyll b" content in FR13 declined progressively during stress application but this decline was not significant (P>0.05) (Fig. 4B). "Chlorophyll b" content was found to be significantly lower than control in FRB73 leaves at 8th day (51% of control) and in TTM815 at 5th and 8th day (35% of control) of drought stress (P<0.05) (Fig. 4B). According to our results, it seemed that "chlorophyll b" content in all maize cultivars were less affected by drought stress than "chlorophyll a" content.



A study on drought stress tolerance in some maize (Zea mays L.) cultivars

A

□C □2D □5D ■8D

A □C □2D □5D ■8D 6 Total fresh weight (g plant⁻¹) 5 4 3 2 1 0 FR 13 FRB 73 TTM 815 B 1 □C **□**2D **□**5D **■**8D Total dry weight (g plant -1) 0,8 0,6 0,4 0,2 0 FR 13 TTM 815 **FRB 73** С □C **□**2D **□**5D **■**8D
 100
 80

 80
 60

 40
 20

 0
 20
 0 FR13 FRB73 TTM815 Cultivars

Figure 2. Effect of drought stress on some growth parameters in maize cultivars. (A) Root length, (B) shoot length and (C) total plant length. Significant differences from controls (P<0.05) are marked with an asterisk. Abbreviations and statistical evaluations are the same for the following figures

Figure 3. Effect of drought stress on some growth parameters in maize cultivars. (A) Total fresh weight, (B) total dry weight and (C) total water content



Figure 4. Effect of drought stress on photosynthetic pigment contents (mg g^{-1} FW) in maize cultivars. (A) Chlorophyll a content and (B) chlorophyll b content

3.3. Effect of Drought Stress On Malondialdehyde (MDA) Content

MDA content showed considerable and progressive increase in maize cultivars at all drought application (P<0.05) (Fig. 5). According to our results, MDA content in FR13, FRB73 and TTM815 leaves was 288%, 100% and 400% higher than their controls at the end of drought period, respectively.

3.4. Effect of Drought Stress On Total Phenolic Compounds Content

Total phenolic compounds content was 21% less than control in FR13 at 5th day of drought stress whereas it increased at 8th day and reached a 18% higher and significant level than control (P<0.05) (Fig. 6). In FRB73 leaves, total phenolic compounds were accumulated until 5th day of drought and then stayed constant. It was significantly and 41% higher than respective control (P<0.05). We detected significant and continuous increases in phenolic compounds content in TTM815 leaves during drought stress (P<0.05) and it was 86% higher than its control at 8th day.







Figure 6. Effect of drought stress on total phenolic compounds (mg g⁻¹ FW) in maize cultivars.

3.5. Effect of Drought Stress On Total Soluble Sugars

Total soluble sugar content in FR13 leaves dropped significantly lower level (56% of control) at 5th day of drought (P<0.05) and then increased (Fig. 7). Nevertheless, it was 21% less than control level. In FRB73 under drought stress, changes in total soluble sugar were not found to be remarkable when compared to control (P>0.05). Total soluble sugar content was significantly higher than control in TTM815 leaves at 2nd day of drought (P<0.05). However, it showed a continuous and significant drop and reached to minimum level with a reduction ratio of 45% at the and of drought period (P<0.05).



Figure 7. Effect of drought stress on total soluble sugar content (mg g⁻¹ DW) in maize cultivars

4. DISCUSSION

It has been well documented that drought stress is one of the most important environmental constraints that affects crop growth and crop production worldwide [31, 32]. According to statistical data, up to 45% of the world agricultural lands are subjected to drought stress [33]. Crop tolerance to abiotic stress factors, such as drought, is very complex at the whole plant and cellular level [34, 35]. Drought stress is mainly characterized by inhibition of both cell elongation and expansion of plant cells at the initial phase of growth and establishment [36, 37]. Accordingly, our results showed that exposure of maize cultivars to drought stress dramatically reduced their root growth except FRB73 (Figure 2A). In FR13 and TTM815, this inhibition was proportional to stress duration and reached to significantly higher level only at 5th and 8th days of drought exposure. In parallel with our results, several authors have reported that drought stress reduced root growth in several plant species. Kusaka et al. [38], for example, recorded that drought stress caused significant reduction on root growth of Pennisetum glaucum plants due to inhibition effect of drought on cytogenesis and cell growth in roots. Similar results have been obtained with different plant species [39-42]. Shoot length, on the other hand, was not affected significantly during drought exposure although some degree of growth inhibition was observed in all maize cultivars (Figure 2B). Similar to root growth, total plant length was decreased significantly by drought exposure in all maize cultivars and we observed earlier and more remarkable inhibitory effects in FR13 and TTM 815 (Fig. 2C). Our results may show the existence of different degree of tolerance between maize cultivars in response to drought stress. Variability in growth under drought stress conditions has been indicated by several researchers. For example, Blum [43] found differences in growth reduction caused by drought stress in different barley cultivars. Yin et al. [44] reported significant interspecific differences in total plant height between two Populus species and concluded that this variation in drought responses may be used as a selection and improvement

for the reduction of total plant length because of no remarkable changes were observed in shoot length during drought exposure of maize cultivars.
It has been reported that the restriction of water supply from the soil in relation to genotype reduced plant biomass [48]. Moreover, a common adverse effect of drought strong on oran plants has hean known to be the

biomass [48]. Moreover, a common adverse effect of drought stress on crop plants has been known to be the reduction in fresh and dry biomass production [32]. In our study, however, total fresh and dry weights of maize cultivars were generally increased during drought exposure in a time dependent manner (Fig. 3A, B). Our results was in accordance with the result of Türkan et al. [49] which showed that *Phaseolus acutifolius* was more tolerant to drought than P. vulgaris because of higher dry matter accumulation under PEG (poly ethylene glycol) induced water stress. Greater plant fresh and dry weights under drought stress conditions are desirable characteristics [50]. In addition, plant productivity under drought stress is strongly related to dry matter production and distribution [42]. Drought stress is primarily characterized by reduction of water content and turgor loss in plant cells [51]. Our results showed minor water losses in all maize cultivars as a result of drought exposure (Fig. 2C), one of the most important characteristics of drought-resistance cultivars [52]. Higher water retaining ability during drought conditions is an important strategy for acquiring resistance [53]. It has been stated that plants can provide cellular osmotic regulation under drought stress by accumulating some organic compounds and protect themselves from adverse effects of water loss [54].

criteria. Similar results have been recorded with cultivars

of sorghum [45], canola and Indian mustard [46] and amaranth [47]. Another important point of our results

was that reduction in root growth was mainly responsible

The photosynthetic activity of higher plants is known to decrease as the relative water content decrease as a result of drought [55]. It has been confirmed that reduced photosynthetic pigment content is mainly responsible for limitation of photosynthesis in sunflower under drought conditions [56]. Our results showed that photosynthetic pigment contents (chl a and chl b) were less affected by drought stress in FRB73, while effect of drought was more pronounced in the other cultivars (Fig. 4A-B). It should be noted that pigment content in different cultivars of the same species may exhibit differential sensitivity to drought. The former literature indicates that drought-resistant cultivars exhibited no or minor changes in pigment content compared to drought-sensitive ones [56]. Therefore, relatively higher pigment content in the leaves of FRB73 may show that this cultivar is more tolerant to drought stress in comparison to FR13 and TTM815. Similar to our results, it has been demonstrated decreased chlorophyll content upon exposure to

oxidative stress and a comparatively higher chlorophyll content in tolerant wheat and maize cultivars under stress conditions than in susceptible ones [57, 58].

It has been well known that drought stress induced the production of reactive oxygen species (ROS), which led to lipid peroxidation of membrane lipids reflecting the stress induced damage in tissues [59]. Malondialdehyde (MDA) content, an indicator of the extent of lipid peroxidation resulting from oxidative stress, significantly increased in leaves of all maize cultivars in response to drought stress in the present work (Fig. 5). However, FRB73 showed lower MDA accumulation than FR13 and TTM815 under drought stress. In consistence with our results, Sairam and Srivastava [60] reported lower MDA content in drought-tolerant wheat cultivar. The lower MDA content in drought-stressed FRB73 may indicate a better protection from oxidative stress and greater drought tolerance as demonstrated by Bor et al. [18] and Türkan et al. [49] in Beta maritima and drought-tolerant Phaseolus acutifolius, respectively. In FR13 and TTM815, structure and function of the cellular membranes may not be maintained probably due to higher level of MDA accumulation under drought stress.

Phenolic compounds and their metabolism are important processes that have been related to the responses of drought stress in plants [61]. Phenolic compounds are believed to prevent the formation of ROS under drought stress [62]. The amount of phenolic compounds increased in FRB73 and TTM815 as the exposure time to drought increased (Fig. 6). These data agree with those of Agastian et al. [63] and Muthukumarasamy et al. [64], who reported increases in phenolic content in different tissue of mulberry and Raphanus sativus under salt stress, respectively. Parida et al. [65] also reported that increases in phenol content in plant tissues ameliorate the ionic effect of NaCl. Therefore, enhanced level of phenolic compounds in FRB73 and TTM815 under drought stress may be beneficial to achieve tolerance to water-deficit stress as demonstrated by several authors in many kind of plants [66-68]. Hura et al. [69] and Sanchez-Rodriguez et al. [61], however, have reported more pronounced level of phenols in winter triticale and tomato genotypes more sensitive to drought stress and concluded that the accumulation of phenolics is not a tolerance mechanism, but can be used as a stress indicator. We believed further investigations are needed to clarify physiological role of phenolic compounds in plants under drought stress.

It has been well known that plants can accumulate compatible solutes to avoid water loss during drought stress. Several studies have indicated that soluble sugars play an important role in osmotic adjustment in plants under stress. Sanchez et al. [70], for example, has reported that the measured sugar concentration represented between 34 and 46% of the osmotic adjustment depending on cultivar. Decreased level of soluble sugar in FR13 and TTM815 on the 5th day of drought stress may be attributed to the inhibition of photosynthetic CO₂ assimilation as shown by Martinez et al. [71]. Increases in soluble sugar content in FR13 at 8th day and in TTM815 at 2nd day may also be associated with stimulation of starch degradation. It has been shown an increase in soluble sugar and concomitant decrease in starch content in safflower (*Carthamus maeroticus* L.) plants under drought stress [72]. However, it is possible to find contradictory results in the literature about the effect of drought stress on sugar accumulation. Some studies have reported the sugar content rose [73] while others have found sugar content decreased [74] or remained constant [75, 76] under drought stress.

In summary, root and shoot growth were not affected by drought stress in FRB73 and inhibition of root growth was responsible for the decreased total plant length in FR13 and TTM815. Exposure of all maize cultivars to drought stress resulted in higher fresh and dry weights while water content in FRB73 was not affected. The more stable photosynthetic pigment content and the constant soluble sugar level in FRB73 under drought stress may be explained by less oxidative damage in thylakoid membranes, which was in accordance with lower MDA and higher phenolic compounds accumulation in the same cultivar. As a result, we could identify FRB73 as the most drought-tolerant cultivars while FR13 was the most sensitive one. TTM815, on the other hand, represented moderately tolerant to drought. Nevertheless, further studies are required to establish more reliable validity of this phenomenon in drought tolerance.

ACKNOWLEDGEMENT

The authors wish to thank Sakarya University Commission for Scientific Research Projects (Project no: 2008-50-01-028) for supporting this study financially and Sakarya Agricultural Research Institute for providing maize seeds.

REFERENCES

- [1] J. S. Boyer, "Plant productivity and environment", Science, vol. 218, pp. 443-448, 1982.
- [2] E. A. Bray, J. Bailey-Serres and E. Weretilnyk, "Responses to abiotic stresses", in *Biochemistry and Molecular Biology of Plants*, W. Gruissem, B. Buchanan, R. Jones, Ed. American Society of Plant Physiologists, 2000.
- [3] B. Valliyodan and H. T. Nguyen, "Understanding regulatory networks and engineering for enhanced drought tolerance in plants", Curr. Op. Plant Biol., vol. 9, pp. 189-195, 2006.

- [4] J. B. Passioura, A. G. Condon and R. A. Richards, "Water deficits, the development of leaf area and crop productivity", in *Water Deficits. Plant Responses from Cell to Community*, J. A. C. Smith, E. Griffiths, BIOS Scientific Publications, Oxford, 1993.
- [5] A. Blum and A. Ebercon, "Cell membrane stability as a measure of drought and heat tolerance in wheat", Crop Sci., vol 21, pp. 43-47, 1981.
- [6] N. Trapani and E. Gentinetta, "Screening of maize genotypes using drought tolerance tests", Maydica, vol. 29, pp. 89-100, 1984.
- [7] P. Martiniello and C. Lorenzoni, "Response of maize genotypes to drought tolerance tests", Maydica, vol. 30, pp. 361-370, 1985.
- [8] J. P. Palta, "Stress interactions at the cellular and membrane level", HortSci., vol. 25, pp. 1337-1381, 1990.
- [9] S. Grzesiak, "Genotypic variation between maize (Zea mays L.) single cross hybrids in response to drought stress", Acta Physiol. Plant., vol. 23, pp. 443-456, 2001.
- [10] A. L. S. Lima, F. M. DaMatta, H. A. Pinheiro, M. R. Totola and M. E. Loureiro, "Photochemical responses and oxidative stress in two clones of *Coffea canephora* under water deficit condition", Environ. Exp. Bot., vol. 47, pp. 239-247, 2002.
- [11] J. E. Muller and M. S. Whitshitt, "Plant cellular responses to water deficit", Plant Growt. Regul., vol. 20, pp. 41-46, 1996.
- [12] L. Taiz and S. C. E. Zeiger, "Plant Physiology", University of California, Los Angeles, Sinauer Associates, Inc., Publisher, pp. 726-735, 1998.
- [13] C. H. Foyer and G. Noctor, "Redox haemostasis and antioxidant signalling: a metabolic interface between stress perception and physiological responses", Plant Cell, vol. 17, pp. 1866-1875, 2005.
- [14] M. K. Nikolaeva, S. N. Maevskaya, A. G. Shugaev and N. G. Bukhov, "Effects of drought on chlorophyll content and antioxidant enzyme activities in leaves of three wheat cultivars varying in productivity", Russ. J. Plant Physiol., vol. 57, no 1, pp. 87-95, 2010.
- [15] B. Halliwell, "Oxidative damage, lipid peroxidation and antioxidant protection in chloroplasts", Chem. Phys. Lipids, vol. 44, pp. 327-340, 1987.
- [16] R. G. Alscher, J. L. Donahue and C. L. Cramer, "Reactive oxygen species and antioxidants: Relationships in green cells", Physiol. Plant. vol. 100, pp. 224-233, 1997.
- [17] C. Bowler, M. V. Montagu and D. Inze, "Superoxide dismutase and stress tolerance", Annu. Rev. Plant. Physiol. Plant Mol. Biol., vol. 43, pp. 83-116, 1992.
- [18] M. Bor, F. Özdemir and İ. Türkan, "The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L and wild beet *Beta maritima* L.", Plant Sci., vol. 164, pp. 77-84, 2003.

- [19] W. Bilger, T. Johnsen and U. Schreiber, "UVexcited chlorophyll fluorescence as a tool for the assessment of UV-protection by the epidermis of plants", J. Exp. Bot., vol. 52, pp. 2007-2014, 2001.
- [20] O. Blokhina, E. Virolainen and K. V. Fagerstedt, "Antioxidants, oxidative damage and oxygen deprivation stress: a review", Ann. Bot., vol. 91, pp. 179-194, 2002.
- [21] T. H. H. Chen and N. Murata "Enhancement of tolerance of abiotic stress by metabolic engineering of betaines and other compatible solutes", Curr. Opin. Plant Biol., vol. 5, pp. 250-257, 2002.
- [22] G. A. Gilbert, C. Wilson and M. A. Madore, "Root zone salinity alters raffinose oligosaccharide metabolism and transport in Coleus", Plant Physiol., vol. 115, pp. 1267-1276, 1997.
- [23] S. Pelleschi, J. P. Rocher and J. L. Prioul, "Effect of water restriction on carbohydrate metabolism and photosynthesis in mature maize leaves", Plant Cell Environ., vol 20, no 4, pp. 493-503, 1997.
- [24] J. Y. Kim, A. Mahe, J. Brangeon and J. L. Prioul, "A maize vacuolar invertase, IVR2, is induced by water stress. Organ/tissue specificity and diurnal modulation of expression", Plant Physiol., vol. 124, pp. 71–84, 2000.
- [25] I. Couee, C. Sulmon, G. Gouesbet and A. El Amrani, "Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants", J. Exp. Bot., vol. 57, no 3, pp. 449-459, 2006.
- [26] Y. Gibon, M. A. Bessieres and F. Larher, "Is glycine betaine a non-compatible solute in higher plants that do not accumulate it?", Plant, Cell Environ., vol. 20, pp. 329-340, 1997.
- [27] H. K. Lichtenthaler, "Chlorophylls and carotenoids: Pigments of photosynthetic bio membranes", Methods Enzymol., vol. 148, pp. 350-382, 1987.
- [28] H. Ohkawa, N. Ohishi and Y. Yagi, "Assay of lipid peroxides in animal tissue by thiobarbituric acid reaction", Anal. Biochem., vol. 95, pp. 351-358, 1979.
- [29] S. F. Chandler and J. H. Dodds, "The effect of phosphate nitrogen and sucrose on the production of phenolics and socosidine in callus cultures of *Solanum laciniatum*", Plant Cell Rep., vol. 2, pp. 105-108, 1983.
- [30] M. Dubois, K. A. Gilles, K. J. Hamilton, P. A. Rebers and F. Smith, "Colorimetric method for determination of sugars and related substances", Anal. Chem., vol. 28, pp. 350-356, 1956.
- [31] M. Ashraf and M. R. Foolad, "Roles of glycine betaine and proline in improving plant abiotic stress resistance", Environ. Exp. Bot., vol. 59, pp. 206-212, 2007.
- [32] M. Farooq, A. Wahid, N. Kobayashi, D. Fujita and S. M. A. Basra, "Plant drought stress: effects,

mechanisms and management", Agron. Sustain. Dev, vol. 29, pp. 185-212, 2009.

- [33] A. J. Bot, F. O. Nachtergaele and A. Young, "Land resource potential and constraints at regional and country levels", Wold Soil Res. Rep., 90, in *Land and Water Development Division*, FAO, Rome, 2000.
- [34] M. R. Foolad, P. Subbiah, C. Kramer, G. Hargrave and G. Y. Lin, "Genetic relationships among cold, salt and drought tolerance during seed germination in an interspecific cross of tomato" Euphytica, vol. 130, pp. 199-206, 2003.
- [35] M. Ashraf and P. J. Harris, "Potential biochemical indicators of salinity tolerance in plants", Plant Sci., vol. 166, pp. 3-16, 2004.
- [36] M. Kusaka, M. Ohta and T. Fujimura, "Contribution of inorganic components to osmotic adjustment and leaf folding for drought tolerance in pearl millet", Physiol. Plant., vol. 125, pp. 474-489, 2005.
- [37] H. B. Shao, L. Y. Chu, M. A. Shao, C. Abdul Jaleel and M. Hong-Mei, "Higher plant antioxidants and redox signalling under environmental stresses", Comp. Rend. Biol., vol. 331, pp. 433-441, 2008.
- [38] M. Kusaka, A. N. Lalusin and T. Fujimura, "The maintenance of growth and turgor in pearl millet (*Pennisetum glaucum* L. Leeke) cultivars with different root structures and osmo-regulation under drought stress", Plant Sci., vol. 168, pp. 1-14, 2005.
- [39] R. E. Sharp, W. K. Silk and T. C. Hsiao, "Growth of the maize primary root at low water potentials", Plant Physiol., vol. 87, pp. 50-57, 1988.
- [40] T. I. Baskin, H. T. H. M. Meekes, B. M. Liang and R. E. Sharp," Regulation of growth anisotropy in well-watered and water-stressed maize roots. II. Role of cortical microtubules and cellulose micro fibrils", Plant Physiol., vol. 119, pp. 681-692, 1999.
- [41] H. Wang, J. Siopongco, L. J. Wade and A. Yamaguchi, "Fractal analysis on root systems of rice plants in response to drought stress", Env. Exp. Bot., vol. 65, pp. 338-344, 2009.
- [42] H. Kage, M. Kochler and H. Stützel, "Root growth and dry matter partitioning of cauliflower under drought stress conditions: measurement and simulation", Eur. J. Agron., vol. 20, pp. 379-395, 2004.
- [43] A. Blum, "Osmotic adjustment and growth of barley genotypes under drought stress", Crop Sci., vol. 29, pp. 230-233, 1989.
- [44] C. Yin, X. Wang, B. Duan, J. Luo and C. Li, "Early growth, dry matter allocation and water use efficiency of two sympatric *Populus* species as affected by water stress", Env. Exp. Bot., vol. 53, pp. 315-322, 2005.
- [45] G. S. Premachandra, H. Saneoka, K. Fujita and S. Ogata, "Leaf water relations, osmotic adjustment, cell membrane stability, epicuticular wax load and growth as affected by increasing water deficits in sorghum", J. Exp. Bot., vol. 43, pp. 1569-1576, 1992.

- [46] P. R. Wright, J. M. Morgan and R. S. Jessop, "Comparative adaptation of canola (*Brassica napus*) and Indian mustard (*B. juncea*) to soil water deficits: plant water relations and growth", Field Crops Res., vol. 49, pp. 51-64, 1996.
- [47] F. Liu and H. Stutzel, "Leaf water relations of vegetable amaranth (*Amaranthus* ssp.) in response to soil drying", Eur. J. Agron., vol. 16, pp. 137-150, 2002.
- [48] C. I. Ogbonnaya, B. Sarr, C. Brou, O. Diouf, N. N. Diop and H. Roy-Macauley, "Selection of cowpea genotypes in hydroponics, pots and field for drought tolerance", Crop Sci., vol. 43, pp. 1114-1120, 2003.
- [49] İ. Türkan, M. Bor, F. Özdemir and H. Koca, "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 Sci., vol. 168, pp. 223-231, 2005.
- [50] C. A. Jaleel, P. Mannivannan, A. Wahid, M. Farooq, H. J. Al-Juburi, R. Somasundaram and R. P. Vam, "Drought stress in plants: A review on morphological characteristics and pigments composition", Int. J. Agric. Biol., vol. 11, pp. 100-105, 2009.
- [51] M. F. Quartacci, C. Pinzino, C. L. M. Sgherri and F. Navarri-Izzo, "Lipid composition and protein dynamics in thylakoids of two wheat cultivars differently sensitive to drought", Plant Physiol., vol. 108, pp. 191-197, 1995.
- [52] D. S. Selote and R. Khanna-Chopra, "Drought acclimation confers oxidative stress tolerance by inducing co-ordinated antioxidant defence at cellular and subcellular level in leaves of wheat seedlings", Physiol. Plant., vol. 127, pp. 494-506, 2006.
- [53] S. Y. Hsu, Y. T. Hsu and C. H. Kao, "The effect of polyethylene glycol on proline accumulation in rice leaves", Biol. Plant., vol. 46, pp. 73–78, 2003.
- [54] D. W. Lawlor and G. Cornic, "Photosynthetic carbon assimilation and associated metabolism in relation to water deficit in higher plants", Plant Cell Environ., vol. 25, pp. 275-294, 2002.
- [55] A. R. Reddy, K. V. Chaitanya and M. Vivekanandan, "Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants", J. Plant Physiol., vol. 161, pp. 1189-1202, 2004.
- [56] I. G. Shmat'ko and O. E. Shvedova, "Vodnyi rezhim i zasukhoustoichivost' pshenitsy (Water regime and wheat drought tolerance)", Kiev: Naukova Dumka, 1977.
- [57] T. E. Kraus, B. D. McKersie and R. A. Fletcher, "Paclobutrazole induced tolerance of wheat leaves to paraquat may involve antioxidant enzyme activities", J. Plant Physiol., vol. 145, pp. 570-576, 1995.
- [58] G. M. Pastori and V. S. Trippi, "Oxidative stress induced high rate of glutathione reductase synthesis

in a drought resistant maize strain", Plant Cell Physiol., vol. 33, pp. 957-961, 1992.

- [59] H. H. Ratnayaka, W. T. Molin and T. M. Sterling, "Physiological and antioxidant responses of cotton and spurred anoda under interference and mild drought", J. Exp. Bot., vol. 54, pp. 2293-2305, 2003.
- [60] R. K. Sairam and G. C. Srivastava, "Water stress tolerance of wheat (*Triticum aestivum* L.): Variation in hydrogen peroxide accumulation and antioxidant activity in tolerant and susceptible genotypes", J. Agron. Crop Sci., vol. 186, pp. 63-70, 2001.
- [61] E. Sanchez-Rodriguez, M. Rubio-Wilhelmi, L. M. Cervilla, B. Blasco, J. J. Rios, M. A. Rosales, L. Romero and J. M. Ruiz, "Genotypic differences in some physiological parameters symptomatic for oxidative stress under moderate drought in tomato plants", Plant Sci., vol. 178, pp. 30-40, 2010.
- [62] A. M. Mayer and E. Harel, "Phenol oxidases and their significance in fruit and vegetables", Food Enzyme, vol. 32, pp. 373-398, 1991.
- [63] P. Agastian, S. J. Kingsley and M. Vivekanandan, "Effect of salinity on photosynthesis and biochemical characteristics in mulberry genotypes", Photosynthetica, vol. 38, pp. 287-290, 2000.
- [64] M. Muthukumarasamy, S. D. Gupta and R. Pannerselvam, "Enhancement of peroxidase, polyphenol oxidase and superoxide dismutase activities by triadimefon in NaCl stressed *Raphanus sativus*", Biol. Plant., vol. 43, pp. 317-320, 2000.
- [65] A. K. Parida, A. B. Das, Y. Sanada and Y. Mohanty, "Effects of salinity on biochemical components of the mangrove, *Aegiceras corniculatum*", Aquat. Bot., vol. 80, pp. 77-87, 2004.
- [66] R. A. Dixon, A. D. Choudhary and D. Dalkin, "Molecular biology of stress-induced phenylpropanoid and isoflavonoid biosynthesis in alfalfa", in *Phenolic metabolism in plants*, H. A. Stafford, R. K. Ibrahim, Ed. New York, Plenum Press, 1992, pp. 91-138.
- [67] R. M. Rivero, J. M. Ruiz, P. C. Garcia, L. R. Lopez-Lefebre, E. Sanchez and L. Romerao, "Resistance to cold and heat stress: accumulation of phenolic compounds in tomato and watermelon plants", Plant Sci., vol. 160, pp. 315-321, 2001.
- [68] A. K. Parida, V. S. Dagaonkar, M. S. Phalak, G. V. Umalkar and L. P. Aurangabadkar, "Alterations in photosynthetic pigments, protein and osmotic components in cotton genotypes subjected to shortterm drought stress followed by recovery", Plant Biotechnol. Rep., vol. 1, pp. 37-48, 2007.
- [69] T. Hura, S. Grzesiak, K. Hura, E. Thiemt, K. Tokarz and M. Wedzony, "Physiological and biochemical tools useful in drought-tolerance detection in genotypes of winter triticale: accumulation of ferulic acid correlates with drought tolerance", Ann. Bot., vol. 100, pp. 767-775, 2007.

- [70] F. J. Sanchez, E. F. de Andres, J. L. Tenorio and L. Ayerbe, "Growth of epicotyls, turgor maintenance and osmotic adjustment in pea plants (*Pisum sativum* L.) subjected to water stress", Field Crop Res., vol. 86, pp. 81-90, 2004.
- [71] J. P. Martinez, S. Lutts, A. Schanck, M. Bajji and J. M. Kint, "Is osmotic adjustment required for water stress resistance in the Mediterranean shrub *Atriplex halimus* L.?", Plant Sci., vol. 161, pp. 1041-1051, 2004.
- [72] L. E. Abdel-Nasser and A. E. Abdel-Aal, "Effect of elevated CO₂ and drought on proline metabolism and growth of safflower (*Carthamus maeroticus* L.) seedlings without improving water status", Pak. J. Biol. Sci., vol. 5, pp. 523-528, 2002.
- [73] I. Kerepesi and G. Galiba, "Osmotic and salt-stress induced alteration in soluble carbohydrate content in wheat seedlings", Crop Sci., vol. 40, pp. 482-487, 2000.
- [74] A. D. Hanson and W. D. Hitz, "Metabolic responses of plant water deficit", Annu. Rev. Plant. Physiol., vol. 33, pp. 163-203, 1982.
- [75] J. M. Morgan, "Osmotic components and properties associated with genotypic differences in osmoregulation in wheat", Aust. J. Plant Physiol., vol. 19, pp. 67-76, 1992.
- [76] M. El-Tayeb, "Differential responses of pigments, lipid per-oxidation, organic solutes, catalase, and peroxidase activity in the leaves of two *Vicia faba* L. cultivars to drought", Int. J. Agric. Biol., vol 8, no 1, pp. 116-122, 2006.