Australian Journal of Crop Science AJCS
AJCS 8(2):232-242 (2014) ISSN:1835-2707

Determination of the relationship between water use efficiency, carbon isotope discrimination and proline in sunflower genotypes under drought stress

Öner Canavar*[1,2], Klaus-Peter Götz[3], Frank Ellmer[2], Frank-Michael Chmielewski[3], Mustafa Ali Kaynak[1]

[1]Adnan Menderes University, Faculty of Agriculture, Crop Science Department, Aydın–09100 Turkey
[2]Current address: Humboldt University, Agriculture and Horticulture Faculty, Crop Science Department, Berlin–14195 Germany
[3]Humboldt University, Agriculture and Horticulture Faculty, Agroclimatology Department, Berlin–14195 Germany

***Corresponding author: ocanavar@adu.edu.tr**

Abstract

The aim of this study was to determine the effect of drought stress on four sunflower genotypes (Tarsan, Sanbro, TR-3080 and DKF-2525) and the relationship between some physiological and phenological characters at 30% (drought stress) and 60% (well-watered) irrigation from soil water capacity. Stress treatment was started from the emergence until R1 stage (bud visible) under the controlled conditions of greenhouse. The results showed that plants responses in some parameters such as leaf, stem and total dry weight, total leaf area, young fully developed leaf area, plant height, WUE (water use efficiency), RWC (relative water content), SLW (specific leaf weight) and $\Delta^{13}C$ isotope (carbon isotope discrimination) are gradually declined under drought stress condition. On the other hand, some responses such as to proline content and chlorophyll of leaves are increased at the same drought condition. The highly significant relationships were found between both WUE and $\Delta^{13}C$ isotope and proline, especially under drought condition. Generally, sunflower genotypes with lower SLW (leaf thickness) showed higher water content than those with higher SLW mainly due to highly negative relationship with RWC, under drought stress condition. However, the Sanbro cultivar can be used as a parental genotype in sunflower breeding programs due to its high RWC, WUE, total dry weight, high proline accumulation and low $\Delta^{13}C$ isotope under drought stress conditions. Our study suggested that selection for WUE using indirect $\Delta^{13}C$ isotope can presumably be of great value in sunflower breeding programs aimed at developing drought tolerant genotypes. It can also be confirmed that measuring young fully developed leaf area can be enough to determine the leaf area because there is a close correlation between total leaf area and the young fully developed leaves on a plant in both normal and drought conditions.

Key words: Sunflower, drought stress, proline, $\Delta^{13}C$ isotope, RWC, WUE.
Abbreviation: P.H_Plant Height, ChyII_Chlorophyll, TLA_Total Leaf Area, YFDLA_Young Fully Development Leaf Area, RWC_ Relative Water Content, LDW_Leaf Dry Weight, SDW_Stem Dry Weight, TDW_Total Dry Weight, LN_Leaf Number, SLW_ Specific Leaf Weight, $\Delta^{13}C$ isotope_Carbon Isotope Discrimination, WUE_Water Use Efficiency, FC_Field Capacity, WW_Well Watered, DS_Drought Stress.

Introduction

Drought stress is one of the most severe natural limitations of productivity in agricultural ecosystems not only in arid and semi-arid regions of the world but also around the world. It has a seriously vital role in plant growth and development in all plant stages. Many studies have explained that drought stress is caused by limited rainfall during the growing season that affects plants biochemical, molecular, and physiological attributes and influences various cellular and whole plant processes, which significantly reduces crop yield and quality (Andrich et al., 1996; Krizmanic et al., 2003). Therefore, drought resistance and its components are almost constantly being redefined to express the outstanding inventive capacity for terminology. Genetic improvements through enhanced drought tolerance, which are believed to be very rewarding, have not received the significant attention of crop breeders (Yordanov et al., 2000). This is mainly due to the physiological complexities involved in the development of adequate screening techniques in order to document precisely the available variability (Nagarathna et al., 2012). Leaf water status may accurately define the demand and supply of water. Relative Water Content (RWC) is an important determinant of metabolic activity and the survival of leaves (Sinclair and Ludlow, 1986) and is an attribute for discriminating drought tolerant and sensitive genotypes (Rauf, 2008). Ritchie et al. (1990) reported that high relative water content is a resistant mechanism to drought stress; thus, high relative water content is the result of more osmotic regulation. The use of chlorophyll fluorescence measurements has been shown to be useful in quantifying the impact of drought and heat stress on plants (Oukarroum et al., 2007; Ristic et al., 2007). Chlorophyll concentration is known to be one of the major factors affecting photosynthetic capacity (Zobayed et al., 2005). There are many conflicting opinions in the literature about the change in chlorophyll content of leaves under drought stress. Some studies carried out to investigate chlorophyll content under drought stress have reported

reductions in cotton (Massacci et al., 2008), sunflower (Kiani et al., 2008) and *Vaccinium myrtillus* (Tahkokorpi et al., 2007). In contrast, increases in chlorophyll content under drought stress were reported by Mensah et al. (2006) in sesame, Beeflink et al. (1985) in onion and Rahman et al. (2004) in maize, who explained the reason of this increase due to the shift of chlorophyll 'a' absorbance to a shorter wavelength, and slower growth. One reason for these inconsistent findings may be the differences in study conditions such as stress intensity and duration. High chlorophyll content types are associated with stress tolerant plants under drought stress (Kraus et al., 1995). Munns et al. (2000) explained that both cell expansion and cell division can be influenced by relative drought stress, even before photosynthesis or respiration is affected. Leaf area expansion is often decreases under drought stress, such that the expansion and development of the transpiration surface is drastically reduced. Leaf expansion is among the most sensitive growth processes to drought (Alves and Setter, 2004). When plants are exposed to drought stress, soluble sugar content in partly expanded leaves of plants is reduced under the abiotic stress (Shahbaz et al., 2011). In addition to sugars, some plants also accumulate other low or high molecular mass compounds, such as proline (Bajji et al., 2001). Proline, which is one of the amino acids highly synthesized under drought stress as a messenger, is considered to be one of the first metabolic responses to stress (Hare and Cress, 1997, Zengin and Kirbağ, 2007). Cechin et al. (2006) reported that the accumulation of proline under drought was dependent on the leaf age and a higher content of proline was especially found in young leaves. The Water Use Efficiency (WUE) characteristic as an important screening technique can help in discovering significant variation among the genotypes and is defined as the amount of biomass or yield accumulated per unit of water used (Condon et al., 2004; Paussioura, 1986). Carbon is continually fixed by the leaf; thus, $\Delta^{13}C$ isotope provides an integrated average across the life of the tissue. This suggests that $\Delta^{13}C$ isotope can differentiate genotypes compared to instantaneous gas exchange measurements (Ebdon and Kopp, 2004). Carbon isotope discrimination provides information about intrinsic transpiration efficiency on the basis of data from the time-integrated internal carbon dioxide concentration (Farquhar et al., 1989a). Several studies have shown strong negative correlations between carbon isotope discrimination $\Delta^{13}C$ isotope and WUE (Johnson and Basset, 1991, Morgan et al., 1993, Ebdon et al., 1998). Condon and Richards (1992) also reported that $\Delta^{13}C$ isotope, measured early in the season, has shown to provide the highest repeatability and heritability. According to Condon et al (2004), if a low $\Delta^{13}C$ isotope is the result of high photosynthetic capacity, the expectation might be a higher rate of photosynthesis per unit leaf area and; thus, a faster crop growth rate. Many researchers have suggested that isotopic methods could represent another alternative parameter to select genotypes in many plant species under drought stress. In the literature, studies on sunflower $\Delta^{13}C$ isotope and its biochemical basis under stress condition is limited. Indeed, sampling for Δ is easy for researcher. Plant sample is harvested, oven-dried, ground and analysed by mass spectrometry. We think that $\Delta^{13}C$ isotope can play a crucial role in the screening of the adaptation capabilities and responses of sunflower genotypes under drought stress in breeding programs because of measurements of photosynthetic parameters are costly (such as transpiration efficiency, internal CO_2 concentration, stomatal conductance, net assimilation rate). We investigated the relationship between the physiological traits of four sunflower genotypes with different thousand seed weights, dry matters, leaf numbers and seed yields which had already been determined without irrigation in a field experiment in Berlin (Canavar et al, 2010). Following all of these statements, the main aims of our study were (1) to determine selected and used cultivars which may be tolerant against drought stress, (2) to investigate the relationship between plant biomass, RWC, WUE, proline and $\Delta^{13}C$ isotope discrimination and other traits in sunflower cultivars, (3) to validate $\Delta^{13}C$ isotope discrimination as a useful criterion to obtain more tolerant genotypes under drought stress.

Result

Growth parameters

It was determined that drought stress had a statistically significant impact on the physiological and phenological traits during the experiment (Table 2) (ANOVA Table). In contrast, drought stress did not have a statistically significant influence on plant height measured at the first and second measuring times (early stages) (Table 2 and Fig. 1). Drought stress × cultivar interaction was also statistically significant in terms of plant height measured at the 3rd, 4th and 5th measuring times (Table 1 and Fig. 1). The Sanbro cultivar was statistically in the first group at the 3rd, 4th and 5th measuring times under the drought stress condition (Fig. 1). All sunflower cultivars showed a reaction (elicited) by shortening the length of their plant heights against the drought stress (Fig. 1). Results showed that the maximum reduction in plant height was caused in the TR-3080 cultivar under drought stress at all measuring times because the plant heights of TR-3080 were generally the highest in all stages under the well-watered condition (Fig. 1). Furthermore, morphological components such as leaf, stem and total dry weight of each sunflower cultivars were found to be mostly influenced by drought stress (Table 1). Although Tarsan cultivar had the highest dry weight value under the well-watered condition, the highest total dry weight obtained was 9.57 g from the Sanbro cultivar under the drought stress condition, due to having high leaf and stem dry weight accumulation. The lowest reduction in terms of leaf, stem and total dry weight was obtained from the Sanbro cultivar as compared with the other sunflower cultivars under the drought stress (Table 1). Analysis of the combined variances (Table 2 and Fig. 2a) showed that drought stress did have a significant effect on the leaf numbers of all sunflower cultivars. There were also statistically significant differences in terms of the leaf numbers among the sunflower genotypes in both well-watered and drought stress conditions. Drought stress caused a decrease in leaf numbers of all sunflower genotypes of 16.8% (Fig. 2a). The highest reduction in leaf number was observed in TR-3080 (16.8%) and DKF-2525 (15.1%) sunflower cultivars under drought stress as compared to the Sanbro sunflower cultivar. The leaf number of the Sanbro cultivar stayed stable because it showed the lowest reduction of 3% in leaf number under drought stress.

Leaf thickness (SLW) and its relationship with RWC

Drought treatment substantially inhibited the SLW (specific leaf weight) (leaf thickness) of all sunflower genotypes by decreasing water availability, leading to a reduction in SLW (30.7%) in Sanbro, as compared to the well-watered treatment (Fig. 2b). SLW had a significant negative relationship with relative water content (-0.48**) and positively with water use efficiency (0.32*) (Table 3).

Table 1. Leaf, stem and total dry weight of four sunflower hybrid cultivars under the well watered and drought stress.

Cultivars	Leaf Dry Weight (g)		Stem Dry Weight (g)		Total Dry Weight (g)	
	WW (60%)	DS (30%)	WW (60%)	DS (30%)	WW (60%)	DS (30%)
Tarsan	4.65a	2.85b	10.22a	5.05a	14.88a	7.89ab
Sanbro	3.39c	3.40a	8.51c	5.41a	11.07c	8.88a
TR-3080	3.58c	1.87c	9.03b	3.70c	12.61b	5.57c
DKF-2525	4.11b	2.61b	8.46c	4.14b	12.50b	6.74bc
Mean	3.93a	2.68b	9.05a	4.57b	12.77a	7.27b

WW: Well Watered, DS: Drought Stress

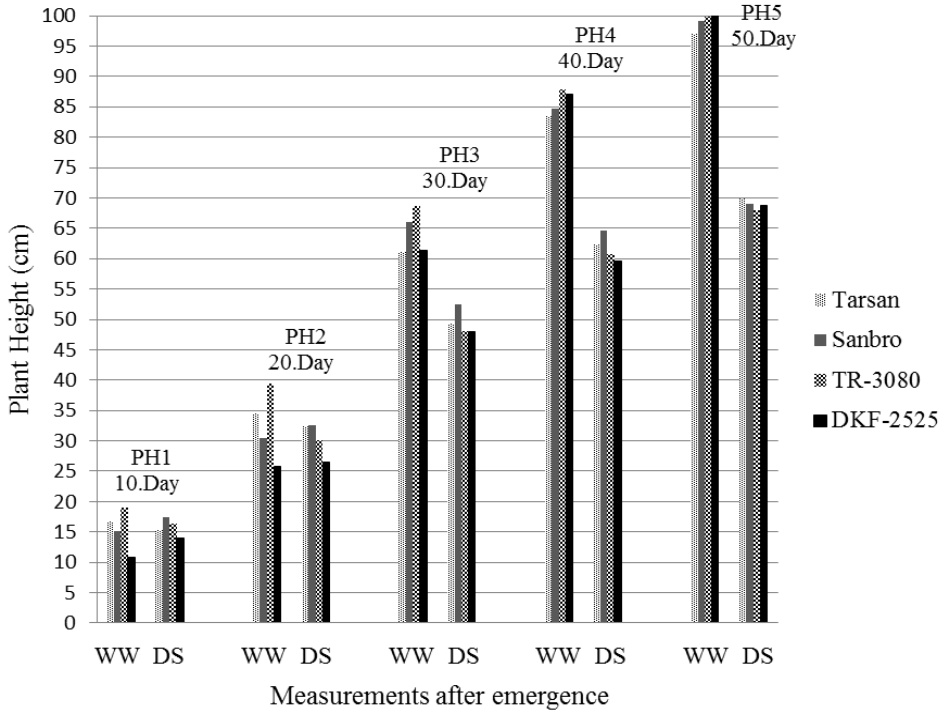

Fig 1. Plant height of sunflower from emergence to harvest time (bud visible) under the well watered (WW) and drought stress (DS). (PH1, 2, 3, 4, 5: Plant Height).

It was also found that the RWC and SLW of all sunflower cultivars were decreased in parallel with drought stress. There was a conversely high relationship between RWC and SLW (R^2= 0.91) under drought stress condition (Fig. 4). All sunflower cultivars had statistically different characteristics in terms of SLW under drought stress (Table 2). Fig. 4 distinctly shows that although the Sanbro cultivar is in the lowest group (based on SLW under drought stress), it was the highest group regarding the RWC under drought stress. Generally, this high regression relationship (R^2=0.91) among all sunflower genotypes under drought stress clearly shows that if any sunflower genotype has a low SLW under drought stress, it has a high RWC under drought stress (Fig. 4).

Responses to leaf area and water use efficiency (WUE)

Drought stress considerably affected the total leaf area and young fully developed leaf area in all sunflower cultivars (Fig. 2c and 2d and ANOVA of Table 2). The total leaf areas and young fully developed leaf areas of all sunflower cultivars under drought stress were lower compared to well-watered conditions (Fig. 2c and Fig. 2d).

All sunflower genotypes also showed different genetic reactions not only under well-watered conditions but also under drought stress. The biggest leaf area was obtained from Tarsan and DKF-2525 in terms of both total leaf area and young fully developed leaf area under well-watered conditions. In contrast, the biggest leaf area under drought stress was obtained from the Sanbro cultivar (Fig. 2c and Fig. 2d). The percentage of reduction in terms of leaf area was lowest in the Sanbro cultivar under the effect of drought stress (Fig. 2c and Fig. 2d). A significant effect of drought stress was revealed in the WUE (water use efficiency) of all sunflower cultivars (Fig. 3a and ANOVA of Table 2). However, all sunflower cultivars showed statistically significant differences in terms of the value of WUE in both conditions. Generally, the WUE of all sunflower cultivars were markedly reduced by drought stress (Fig. 3a). The WUE values of the Sanbro cultivar obtained under drought stress were higher, compared to other cultivars (Fig. 3a), because of having high significant positive correlations with leaf area and total dry weight (Table 3). However, the Tarsan sunflower cultivar was shown to be higher with regards to WUE than those of the TR-3080 and DKF-2525 cultivars in both conditions. But, the reduction percentage in the WUE value of the Tarsan was very high under drought stress (Fig. 3a).

Relative water content and chlorophyll parameters

Our study showed that the RWC (relative water content) was negatively influenced in response to drought stress condition

. **Table 2.** The result of variance analyses for all components measured of sunflower under the well watered and drought stress conditions.

Variance Source	d.f	Calculated of Mean Square								
		PH1	PH2	PH3	PH4	PH5	LN	TLA	YFDLA	SLW
D.S	1	ns	ns	2176.510**	5722.382**	9075.156**	22.500**	4076630.952**	27417.267**	536.923**
C	3	50.410*	142.819**	52.592**	ns	ns	6.867**	263684.211**	1139.491**	24.537**
D.S x C	3	ns	ns	38.490*	37.454**	10.823**	4.700**	ns	866.237**	37.336**
$LSD_{0.05\ D.S}$		2.435	3.529	2.087	1.820	0.676	0.720	91.986	8.030	1.156
$LSD_{0.05\ C}$		3.443	4.991	2.951	2.573	0.957	1.018	130.088	11.356	1.635
$LSD_{0.05\ D.S\ x\ C}$		4.869	7.058	4.174	3.639	1.353	1.440	183.973	16.059	2.312

		LDW	SDW	TDW	ChyII1	ChyII2	WUE	RWC	Proline	$\Delta^{13}C$
D.S	1	16.189**	205.268**	300.299**	409.920**	402.908**	0.794**	0.013**	248042.025**	9.073**
C	3	1.849**	4.452**	25.806**	20.399**	35.822**	0.154**	0.003**	14641.642**	2.398**
D.S x C	3	1.842**	2.852**	16.914**	ns	ns	0.056**	ns	14758.155**	1.401**
$LSD_{0.05\ D.S}$		0.290	0.301	0.890	1.028	1.298	0.067	0.014	26.084	0.341
$LSD_{0.05\ C}$		0.410	0.425	1.250	1.312	1.816	0.095	0.020	36.889	0.482
$LSD_{0.05D.SxC}$		0.580	0.602	1.780	1.855	2.597	0.135	0.029	51.168	0.681

D.S: Drought Stress, C: Cultivars, d.f.: Degree of Freedom, ns: non-significant; $^{*}P< 0.05$; $^{**}P< 0.01$.

P.H.: Plant Height, ChyII: Chlorophyll, TLA: Total Leaf Area, YFDLA: Young Fully Development Leaf Area, RWC: Relative Water Content, LDW: Leaf Dry Weight, SDW: Stem Dry Weight, TDW: Total Dry Weight, LN: Leaf Number, SLW: Specific Leaf Weight, $\Delta^{13}C$ isotope: Carbon Isotope Discrimination, WUE: Water Use Efficiency.

Table 3. Correlation coefficients among the all parameter under the well watered and drought stress.

	Condition	LA	YFDLA	TDW	Chyll	SLW	RWC	WUE	Proline	$\Delta^{13}C$
Condition	-	-0.84**	-0.85**	-0.89**	0.83**	-0.81**	-0.57**	-0.67**	0.81**	-0.59**
LA	-	-	0.86**	0.91**	-0.67**	0.74**	0.43**	0.78**	-0.58**	0.43**
YFDLA	-	-	-	0.84**	-0.69**	0.68**	0.45**	0.63**	-0.62**	0.56**
TDW	-	-	-	-	-0.76**	0.63**	0.41**	0.88**	-0.62**	0.40**
Chyll	-	-	-	-	-	0.63**	0.64**	0.47**	0.57**	-0.38*
SLW	-	-	-	-	-	-	-0.48**	0.32*	-0.69**	0.49**
RWC	-	-	-	-	-	-	-	ns	-0.45**	ns
WUE	-	-	-	-	-	-	-	-	-0.37*	-0.67**
Proline	-	-	-	-	-	-	-	-	-	-0.70**
$\Delta^{13}C$	-	-	-	-	-	-	-	-	-	-

ChyII2: Chlorophyll, TLA: Total Leaf Area, YFDLA: Young Fully Development Leaf Area, RWC: Relative Water Content, TDW: Total Dry Weight, SLW: Specific Leaf Weight, $\Delta^{13}C$: Carbon Isotope discrimination, WUE: Water Use Efficiency.

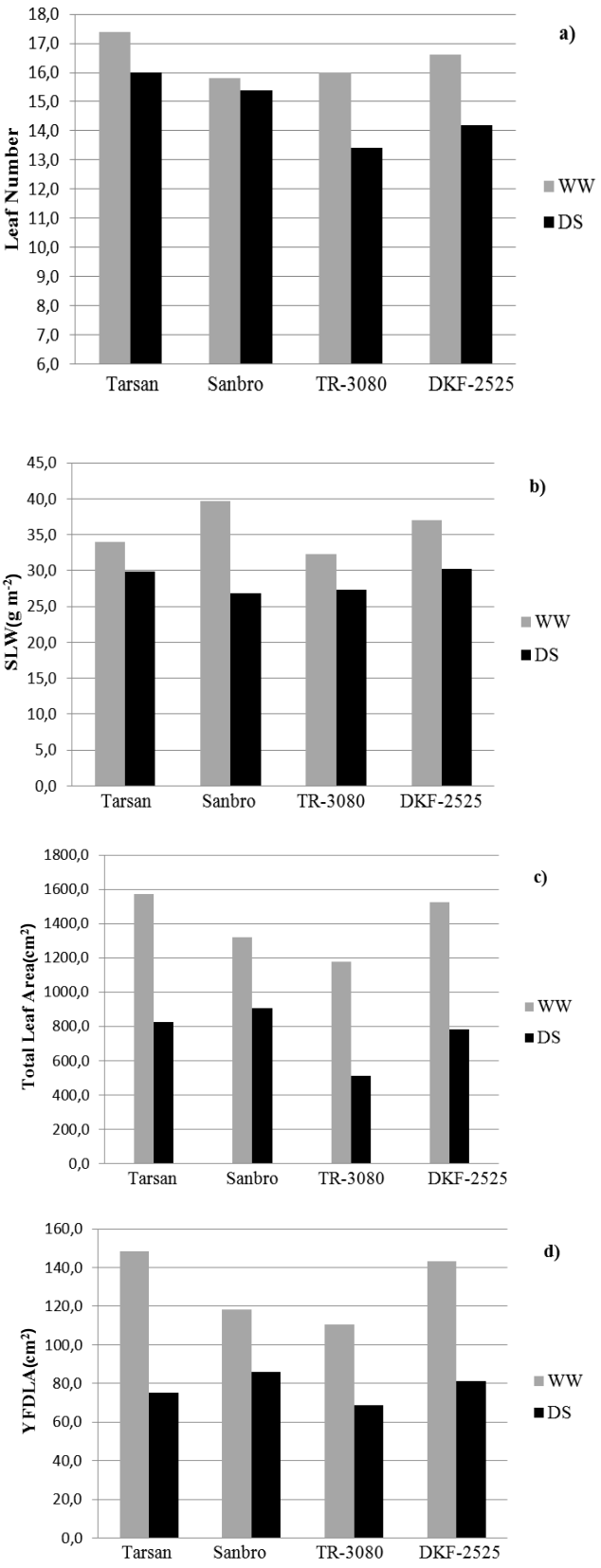

Fig 2. Leaf number (number) (a), specific leaf weight (g/m^2) (b), total leaf area (cm^2) (c) and young fully developed leaf area (cm^2) (d) of sunflower cultivars under the well watered (WW) and drought stress (DS).

in all genotypes (Fig. 3b). It was shown in Table 2 and Fig. 3b that there are significant differences among the genotypes in terms of the effect of drought stress on the RWC of sunflowers. The RWC values of all sunflower cultivars were reduced by 3% under drought stress as an average of all genotypes. The highest reduction in RWC was obtained from the DKF-2525 cultivar under drought stress. However, the differences in this reduction were statistically significant (Fig. 3b). Also, all sunflower cultivars showed statistical differences under both conditions (Fig. 3b and ANOVA Table 2). Although the percentage reduction in RWC value of Sanbro cultivar was very high under drought stress, the highest RWC value was obtained from the Sanbro under drought stress (Fig. 3b).

The result obtained from two measuring times showed that there were significant effects of drought stress on the chlorophyll of young fully developed leaves in all genotypes (Fig. 3c and Table 2). Statistically, the chlorophyll of all genotypes increased under the drought stress. On average, the increasing tendencies of 42.35 obtained for the first measuring time and 44.52 obtained for the second measuring time in well-watered conditions, respectively; whereas, they were statistically different by 6.60 and 6.40 at $p \leq 0.001$ regarding the chlorophyll under drought stress (Fig. 3c). Also, there were statistically significant differences among all sunflower cultivars in terms of chlorophyll measured by SPAD at both measuring times. The highest chlorophyll was obtained from the TR-3080 cultivar for both conditions and both measuring times (Fig. 3c). However, when all sunflower cultivars were exposed to drought stress, the highest increase percentage among all the cultivars was observed in Tarsan and DKF-2525 as 19.5% and 20% under drought stress at the second measuring time (Fig. 3c). Generally, we determined that all sunflower cultivars can show different reactions and responses to drought stress during the growth under the drought stress (Fig. 3c).

Carbon isotope discrimination and relationship with WUE and proline accumulation

The $\Delta^{13}C$ isotope in Fig. 3e also showed a significant variability in its value ranging from 34.25 to 35.70 ‰ among the genotypes under the drought stress condition. As shown in Fig. 3e, the data obtained from both conditions indicated that the $\Delta^{13}C$ isotope (‰) of all sunflower cultivars significantly ($p \leq 0.01$) decreased by drought stress. The Tarsan, Sanbro and DKF-2525 genotypes seem to be more efficient in terms of $\Delta^{13}C$ (‰) than the TR-3080 cultivar when they are exposed to drought stress.

The lowest reduction percentage of $\Delta^{13}C$ isotope was observed in TR-3080 (0.1% under drought stress) (Fig. 3e). However, there was relationship (-0.67**) between WUE and $\Delta^{13}C$ isotope in Table 3. The regression analysis in Fig. 5 shows that there is a strong negative relationship (R^2=0.75) between WUE and $\Delta^{13}C$ isotope under drought stress. The lowest $\Delta^{13}C$ isotope was determined in the Sanbro cultivar in both well-watered conditions and drought stress (Fig. 3e). In contrast, the highest proline content was determined in the Sanbro cultivar under drought stress. Generally, in contrast to $\Delta^{13}C$ (‰), all genotypes also showed that their proline contents were significantly increased by drought stress (Fig. 3d). The highest increase in proline content was approximately quintuplicate (from 68.79 to 331.73 µg g^{-1}) in the Sanbro cultivar under drought stress (Fig. 3d). In both conditions, the lowest proline content was determined in TR-

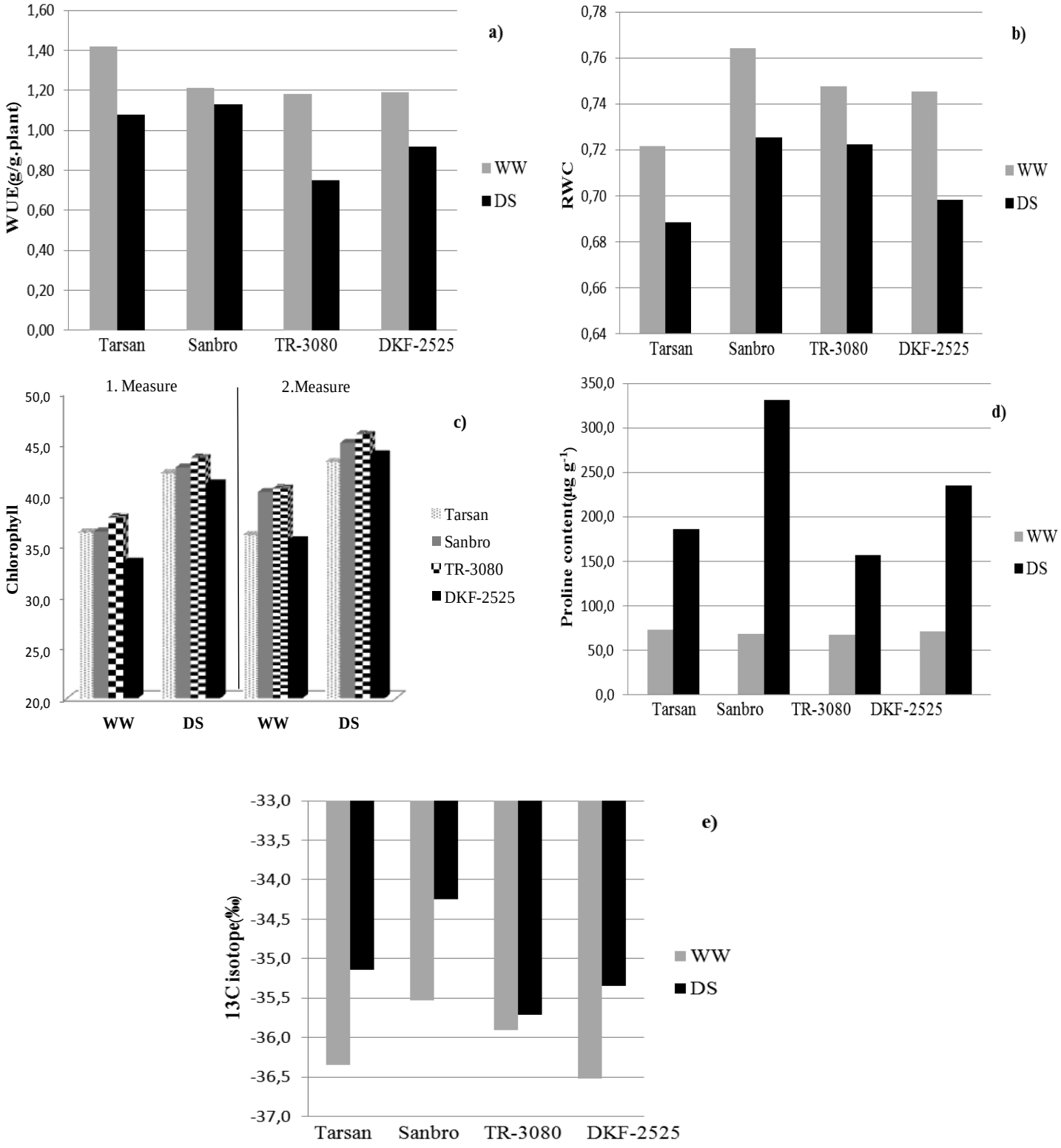

Fig 3. (a) Water use efficiency (g g.plant^{-1}), (b) relative water content (%), (c) chlorophyll, (d) proline content (µg g^{-1}) and (e) $\Delta^{13}C$ isotope (‰) of sunflower cultivar under the well-watered (WW) and drought stress (DS).

3080, while it also contained the highest $\Delta^{13}C$ (‰) under drought stress (Fig. 3d).

Correlation among the measured parameters

Looking at the correlation coefficients in Table 3, drought stress was negatively correlated with total leaf area, young fully development leaf area, relative water content, total dry weight, specific leaf weight, $\Delta^{13}C$ isotope and water use efficiency, while it was positively correlated with proline content and chlorophyll. We found that there is a significant positive correlations of 0.86** between total leaf area and young fully developed leaf area. In addition, a significant negative correlation coefficient of -0.70** was found between proline content and $\Delta^{13}C$ isotope. Generally, negative correlation was found between chlorophyll and total

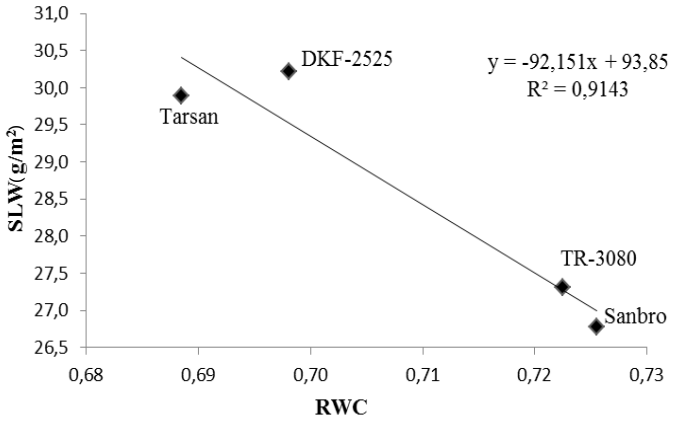

Fig 4. Relationship between specific leaf weight (g/m^2) and RWC (%) of sunflower cultivars under drought stress.

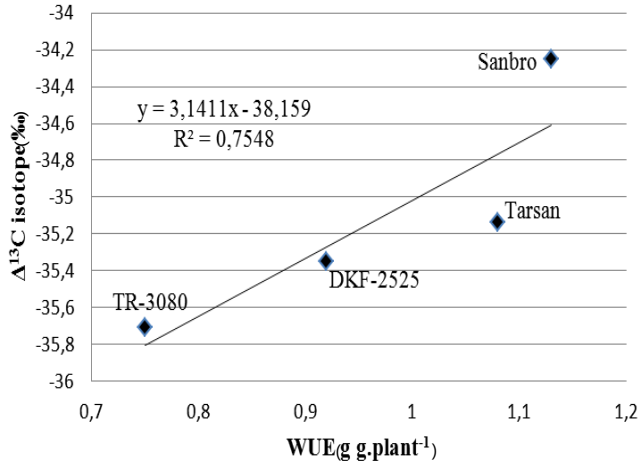

Fig 5. Relationship between WUE (g g.plant^{-1}) and Δ^{13}C isotope (‰) of four sunflower cultivars under drought stress.

leaf area and positive correlation between young fully developed leaf area and total dry weight. In contrast to chlorophyll, WUE was positively correlated with total leaf area, young fully developed leaf area, total dry weight and also with specific leaf weight (Table 3).

Discussion

The analysis of measured characteristics clearly showed that drought had a highly significant effect on the growth of all sunflower genotypes under drought stress. Our results indicated that the plant height and total dry weight of all sunflower genotypes were significantly decreased, probably by diminished cell expansion and cell division under drought stress (Munns et al., 2000). Paussioura (1986) explained that high production of biomass in plants is strongly associated with water use and water use efficiency. The results of our study, in which there was a positive significant correlation between WUE and total dry weight (0.78** and 0.88**), supported the opinion of Paussioura in terms of decreasing WUE under drought stress. It can also be considered that at low WUE, photosynthetic carbon assimilation is decreased due to decreasing flow of CO_2 into mesophyll tissue and the closure of stomata (Boyers and Bowen, 1970; Chaves et al., 2003; and Flexas et al., 2004).

In this study, the leaf area of sunflower genotypes was induced by drought stress, by which the expansion and development of the transpiration surface drastically decreased under drought stress. The decrease in leaf area can be expressed in terms of smaller cells and reductions in the number of cells produced by leaf meristems. Plants also carve out a substantially defensive system by cell turgor, which plays an important role in the protection of cell growth (Tardieu et al., 2000 and Alves and Seter, 2004). In this study, leaf area was found to be strongly correlated with WUE. Especially, Sanbro had high values in terms of both WUE and leaf area under drought stress. It can be considered that a plant tends to close stomata to avoid water loss by transpiration; and therefore, CO_2 uptake is prevented. It might have indirect positive effects on WUE by controlling stomatal conductance, maintaining leaf area and facilitating water absorption (Xiong and Zhu, 2002; Chen et al., 2007). Our results also revealed that since there was a significant positive correlation between total leaf area and young fully developed leaf area, the leaf areas of all leaves on the plant don not need to be measured. It is opined that measuring a young fully developed leaf (sunlit leaf) can be enough to determine for the leaf area of sunflower genotypes. However, the determination of the young fully developed leaf must be very carefully selected by the researcher, because of the distinctive leaf forms of each sunflower genotype in each growth stage. Our results confirm that the total numbers of leaves of all sunflower genotypes slightly decreased under drought stress, similar to the finding of Pankoviç et al. (1999).

The result of SLW (specific leaf weight and a measure of leaf thickness) showed that the SLW of all genotypes was decreased under drought stress conditions, as pointed out by Pankoviç et al. (1999) and Vanaja et al. (2011). Differences in SLW among all sunflower cultivars may also be related to differences in photosynthetic capacity, because of the fact that there are strong relationships among the values of WUE, LA, TDW, RWC and Δ^{13}C isotope, in this study. In particular, our data shows that SLW had an inversely high relationship with RWC under drought stress. As a result of this, RWC which is related to cell volume and increased tissue elasticity can act synergistically on the symplastic volume, providing an increased gradient for the influx of water. It can be affected by low SLW types. This means reductions in the rigidity of leaves due to changes in the extensibility of the tissue and absorptive capacity of the cell wall.

This clearly indicates that in sunflowers, eCO_2 facilitates more maintenance of leaf area under drought stress than leaf dry weight (Vanaja et al., 2011). According to the explanation by Lambrides et al. (2004), low carbon isotope discrimination lines can be better, in terms of yield as compared to the high Δ^{13}C isotope lines, in low rainfall conditions. Generally, the Sanbro cultivar continuously showed the highest proline, WUE, RWC, total leaf area, total dry weight and the lowest Δ^{13}C isotope and SLW when compared to the other cultivars under drought stress. We found that there was a negative significant relationship between WUE and Δ^{13}C isotope under drought stress condition, similar to the previous findings of Johnson and Basset (1991), Morgan et al. (1993) and Ebdon et al. (1998) who explained that photosynthetic carbon assimilation is induced by the decreasing flow of CO_2 into mesophyll tissue and the closure of stomata. Also, Δ^{13}C isotope was decreased in parallel with the dry matter of plants under drought stress.

However, high differences in Δ^{13}C isotope were not observed among the studied sunflower genotypes under well-

watered conditions. Some researchers have explained that the lack of correlation between WUE and $\Delta^{13}C$ isotope can be explained by differences between the instantaneous measurement of gas exchange parameters and the long-term measurement of $\Delta^{13}C$ isotope in plant dry matter (Poorter and Farquhar, 1994) due to suffering small stomatal limitations. These results suggest that $\Delta^{13}C$ isotope may be an important proxy component for direct selection of drought stress in a sunflower improvement program (Ebdon and Kopp, 2004; Lambrides et al., 2004). Drought stress increased the leaf proline content, which might have contributed to osmotic adjustment allowing plants to maintain turgor pressure and adapt to limited water availability. This is conducive with previous reports on drought-induced proline accumulation (Tan et al., 2006). Our results are also in accordance with those described by Ünyayar et al. (2004) who showed that the proline content of sunflower leaf at a higher concentration acts as a solute for intercellular osmotic adjustment under abiotic stress conditions like drought and salt stress. Oncel et al. (2000) explained that differences in proline accumulation can be an adaptive behavior of plants, due to having been subjected to different environmental stresses. Our results were in agreement with those obtained by Jiang and Huang, (2002) and Ünyayar et al. (2004) who explained that decreasing leaf relative water content is an indication of a decrease in swelling pressure in plant cells, leading to a decrease in growth. This decrease has been noted to be related probably to the actual rate of photosynthetic CO_2 assimilation, stomatal conductance and CO_2 supply (Lawlor and Cornic, 2002) and osmotic adjustment. Conroy et al., (1988) explained that osmotic adjustment could delay the effects of drought on photosynthetic electron transport capacity, e.g. the slower decline in cell volume caused by the accumulation of solutes such as proline and betaine. Accumulated solutes may also ensure the maintenance of the structural integrity of the thylakoid membranes during drought. Therefore, the fact that the Sanbro sunflower cultivar had the highest proline accumulation in response to drought stress might be related to its competitive ability in a semi-arid region.

Materials and Methods

Plant material and experiment establishment

The greenhouse experiment was carried out at the research greenhouse of the Crop Science Department of the Agriculture and Horticulture Faculty in Humboldt University, Germany in 2012. The sunflower genotypes were Tarsan, TR-3080 and DKF-2525 bred by Directorate Trakya Agricultural Research Institute in Turkey and Sanbro sunflower genotype was adapted by Syngenta®. All genotypes were tested for variation under controlled drought stress and well-watered environmental conditions of approximately light/dark regime 12/12 h, at 25/15 ±3 ℃ and relative humidity 30-50%, respectively. The sunflower cultivars were planted in MItscherlich pots (30-cm deep 25 cm diameter). The plant populations were maintained as 3 plants in a pot in the greenhouse with only the natural sunlight of the summer months. Clay loam soil was used to fill pots and the cultivars were arranged completely in a randomized block design with five replications. Required amounts of chemical fertilizers were applied according to the instructions (from 1 g nitrogen from 3.70 g KAS fertilizer at the field condition) and then seeds were sown. The soil water factor included two irrigation regimes including irrigation at 30% (water deficit) and 60% (well-watered) of field capacity.

Determination of water holding capacity of soil

To determine the field capacity of soil, the field soil, which had already been taken from the field experiment area, was air-dried and ground to pass through a 5 mm sieve at room temperature. Water holding capacity was determined using a gravimetric method with five replicates as the amount of moisture (percentage). Firstly, the bottoms of 100 cm^3 cylindrical tubes were covered with paper and a plastic strap for the filter and they were taped without soil and then filled completely with soil (by compression). Each cylindrical tube with soil was weighed and settled in a tray, which was approximately as deep as the height of the cylindrical tube. The tray was fully filled with water up to the top of the cylindrical tube and 3 h were allowed for saturation. Then, all cylindrical tubes were left on the quartz soil for 2 h (for drainage and filtering). After that, all the saturated cylindrical tubes were cleaned and weighed again (wet weight). Then all the tubes were oven-dried at 105 °C 24 h and the weight of the oven-dry soil samples was measured (dry weight). The field capacity of undisturbed soil was calculated according to the following formula;

$$F.C.\% = \frac{\text{wet soil weight (saturated)} - \text{dry weight}}{\text{dry weight}} * 100$$

Drought stress treatment

To adjust for the amount of watering of the pots in terms of the 30% and 60% irrigation regimes of field capacity, the soil water content was continuously monitored and maintained by watering at 30% and 60% levels of field capacity during the experiment. Changes in the soil water of each pod were measured and checked daily by weighing each pod at the beginning and end of the removed plant. Plants were harvested 50 days after sowing when plants were at the R3 stage (bud visible).

Determination of relative water content (RWC)

The youngest fully expanded leaves were collected from each pot in the morning. The leaves were weighed immediately to obtain the fresh weight, and rehydrated in a water bath cap with filled water. Afterwards the leaves were rehydrated by floating for 12 h in a covered water bath cap at approximately 23 °C under conditions without light. All leaves were oven-dried for 72 h at 70 °C and RWC was calculated by dividing the amount of water in the fresh leaf tissue by the water in the leaf tissue after rehydration multiplied by 100 (Hossain et al., 2010).

$$RWC = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgit weight} - \text{Dry weight}} * 100$$

Measurement of Chlorophyll

Chlorophyll content was assessed using a chlorophyll meter (SPAD-502, Minolta) and measurements were taken at six points on both sides of young fully developed leaves (upper, middle and lower parts) two times during the experiment. Sixty readings were averaged per genotype (twelve readings of two fully developed leaves per plant five replicates) for each treatment. The average of these sixty readings was considered as the SPAD value (Markwell et al., 1995)

Measurement of the growth parameters

Plant height (PH cm) was measured weekly from on the surface to the top of plant until harvest time. Total leaf area (cm^2) was measured immediately for all leaves on a plant using a leaf area meter (Li-Cor 3100, Lambda Instruments Co., USA) after the plant was removed. Young fully developed leaf area (cm^2) was measured using a leaf area machine from two young fully developed leaves for all cultivars and treatments. Leaf dry weight, stem dry weight and total dry weight (g) were obtained after all parts of the plants were separately dried at 70 °C 72 h. All leaves on the plants were numbered before the leaf area was measured. Specific leaf weight (SLW) was calculated by dividing the total leaf area by leaf dry weight (LDW/LA) (g m^{-2}) (Lambrides et al., 2004). The water use efficiency (WUE) measurements were given in terms of the dry fresh weight per water consumed by evapotranspiration and evaluated as; (g/g plant) (Hazarlı et al., 2010).

$$WUE = \frac{\text{Total biomass}}{\text{Water consumption(the amount of irrigation (g)during the experiment}}$$

Proline analysis

The proline content of leaves was analyzed to determine its association with drought tolerance using a modified version of the method of Bates et al. (1973). The proline amount obtained in the extract was estimated spectrophotometrically using the ninhydrin method. Purfied proline was used for standardization. Before the proline analysis, when all the plants were harvested, 5 – 6 leaves fully developed leaves were collected from the middle of plant (neither young nor old leaves) for each replicate in all cultivars from both water treatments. All leaves were immediately settled in an ice box for transfer and stored at -20 °C. The frozen leaves were directly dried using the method of lyophilization, which is 5 or 10 heated shelves Ø 200 mm, freezing separately, drying outside the ice condenser chamber with CHRIST Lyophilizer GAMMA 1-16 LSC model (London, England) with 5 temperature shelves Ø 200 mm, temperature range -40 °C to +50 ° C.

Dry plant leaves were ground in a Retsch ball milling machine (Germany). 80 mg dry samples were homogenized with 1500 µl of 3% sulfosalicylic acid in a mortar. Samples were centrifuged at 0 °C at 11000 U/min for 30 min. 300 µl solution was taken and transferred from the top of the remaining solution in the tube to another tube. To 300 µl upper aqueous phase, 300 µl of acid ninhydrin and 300 µl of glacial acid were added in a test tube and mixed by vibration stirrer for 10-12 sec., and the reaction mixture tubes were incubated at 90 °C for 1 h. Then all test tubes were left in an ice cap for 5 minutes. The reaction mixture was added to 900 µl toluene and mixed vigorously with a test tube stirrer for 10-12 sec. All tubes were centrifuged at 0 °C temperature at 11000 U/min for 10 min. The remaining solution from the top of the solution mixed in the tubes was transferred to special reading tubes in a spectrophotometer. The absorbance 520 nm wavelength was used on a spectrophotometer. Proline content was determined from a standard curve calculated as µg g^{-1} according to the following formula. Six standard curves were constructed from a dilution series of 0 – 20 µl (0, 2, 5, 10, 15, 20 µl) of proline in increments of 0 – 300 µl (0, 30, 75, 150, 225, 300 µl) and sulfosalicylic acid was added in increments of 300 – 0 µl (300, 290, 225, 150, 75, 0 µl) respectively.

$$\text{Prolin µg g}^{-1} = \frac{\text{concentration value (reading)} * \text{extraction volume (1.5 ml)}}{\text{dry sample weight (g)}}$$

All samples for both water treatments were taken as 2 duplicates.

The concentration value was calculated by Microsoft Excel office program y diagram, which was obtained from the value of the standard curve.

The analyses of carbon isotope discrimination **($\Delta^{13}C$)**

Carbon isotope discrimination was analyzed from the same leaves (young fully expanded sunlit leaf), which were kept at -20 °C and used for proline analysis. The leaves were dried at 60 °C 72 h and ground on a 0.1 mm screen like flour for carbon isotope analysis. $\Delta^{13}C$ analyses were performed in Prof. Dr. K.D. Wutzke, Research Laboratory, University of Rostock, Germany, measured by isotope ratio mass spectrometry with the Tracer mass 20-20, SerCon, Crewe, UK and calculated as:

$\delta^{13}C_{(‰)} = \left[\left(\frac{R\ sample}{R\ referance} - 1\right) * 1000\right]$, with R being the $^{13}C/^{12}C$ ratio. Carbon isotope discrimination (Δ) was calculated using the following formula (Farquhar et al., 1989b): $_{(‰)} = \left[\frac{(\delta a - \delta p)}{(1+\delta p)}\right]$, where, δp is the $\delta^{13}C$ of the leaves and δa is the $\delta^{13}C$ of the atmospheric CO_2 (–8‰).

Statistical analysis

To determine the effect of drought stress on the four sunflower cultivars, the samples were analyzed statistically in a randomized block design with five replications. ANOVA was applied to analyze the variance of drought stress on sunflower cultivars and the interaction of drought and cultivars. The ANOVA (analyses of variance) of this study and correlation coefficients among the traits were shown as the mean of genotypes in each condition. The analysis was conducted using the SPSS and Tarist (Açıkgöz et al., 1994) statistical computer program. Significant differences between the means of replications were tested using Fisher's least squares difference (LSD) method. All differences referred to in the test were significant at 0.05. Regression analyses were computed using Microsoft Excel office program y diagram to assess the relationship between RWC and SLW, and also $\Delta^{13}C$ isotope discrimination and WUE under only drought stress conditions.

Conclusions

The results of the experiment indicated a significant and wide genetic variability for several traits, in terms of response to drought stress. Differential responses of sunflower genotypes to drought stress were observed due to variation in a number of biochemical or physiological traits which are associated with the processes related to the mechanism of drought stress. Our study also suggested that the Sanbro genotype, which has high proline, WUE, RWC and low $\Delta^{13}C$ isotope, SLW, can be selected and used as a parent in order to discover more tolerant plants against drought stress in breeding programs. Our experiment confirmed that under drought stress condition, the WUE was negatively relationship with $\Delta^{13}C$ isotope and was no significant relationship under well-watered. However, the relationship among parameters showed differences under both conditions. It was also revealed that the effect of drought stress is a very complex

matrix by creating multiple interactions among many traits in sunflower. Therefore, strategies for the improvement of relationship WUE and $\Delta^{13}C$ isotope in sunflower tissues under drought stress can provide an effectively selection criteria for plant responses to stress as well as for stress adaptation in future breeding program.

Acknowledgements

We would like to thank the Trakya Agricultural Research Institute of Turkey for providing the sunflower cultivars and Mr. Heiko Vogel for his assistance during establishment of the experiment.

References

Açıkgöz N, Akba ME, Moghaddam A, Özcan K (1994) Turkish data based statistics programmer for PC. Turkey Field Crops Congress, Ege University Press, pp. 264-267.

Alves AAC, Setter TL (2004) Response of cassava leaf area expansion to water deficit: Cell proliferation, cell expansion and delayed development. Ann Bot (London). 94: 605–613.

Andrich G, Balzini S, Zinnai A, Silvestri S, Galoppini C (1996) The effect of drought stress on some characteristics of sunflower seeds. Agricoltura Mediterranea. 126: 285–291.

Bajji M, Lutts S, Kinet JM (2001) Water deficit effects on solute contribution to osmotic adjustment as a function of leaf ageing in three durum wheat (*Triticum durum* Desf.) cultivars performing differently in arid conditions. Plant Sci. 160: 669-681.

Bates L, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil. 39: 205-207.

Beeflink WG, Rozema J, Huiskes AEL (1985) Ecology of Coastal Vegetation. 2nd Edn., W. Junk Publication. USA. ISBN: 9061935318, pp: 640.

Boyers B, Bowen L (1970) Inhibition of oxygen evolution in chloroplasts isolated from leaves at low water potentials. Plant Physiol. 45: 612-615.

Canavar Ö, Ellmer F, Chmielewski FM (2010) Investigation of yield and yield components of sunflower (*Helianthus annuus* L.) cultivars in the ecological conditions of Berlin (GERMANY). Helia. 33: 117-130.

Cechin I, Rossi SC, Oliveira VC, Fumis TF (2006) Photosynthetic responses and proline content of mature and young leaves of sunflower plants under water deficit. Photosynthetica. 44: 143-146.

Cechin I, Corniani N, Fumis TF, Cataneo AC (2010) Differential responses between mature and young leaves of sunflower plants to oxidative stress caused by water deficit. Cienc Rural. 40: 1290-1294.

Chaves MM, Maroco JP, Pereira J (2003) Understanding plant responses to drought– from genes to the whole plant. Funct Plant Biol. 30: 239–264.

Chen S, Bai Y, Lin G, Huang J, Han X (2007) Isotopic carbon composition and related characters of dominant species along an environmental gradient in Inner Mongolia, China. J Arid Environ. 71: 12–28.

Condon AG, Richards RA (1992) Broad sense heritability and genotype × environment interaction for carbon isotope discrimination in field-grown wheat. Aust J Agri Res. 43: 921–934.

Condon AG, Richards RA, Rebetzke GJ, Farquhar GD (2004) Breeding for high water use efficiency. J Exp Bot. 55: 2447–2460.

Conroy JP, Virgona JM, Smillie RM, Barlow EW (1988). Influence of drought acclimation and CO_2 enrichment on osmotic adjustment and chlorophyll a fluorescence of sunflower during drought. Plant Physiol. 86: 1108-1115.

Ebdon JS, Petrovic AM, Dawson TE (1998) Relationship between carbon isotope discrimination, water use efficiency, and evapotranspiration in Kentucky bluegrass. Crop Sci. 38: 157–162.

Ebdon JS, Kopp KL (2004) Relationships between water use efficiency, carbon isotope discrimination, and turf performance in genotypes of Kentucky bluegrass during Drought. Crop Sci. 44: 1754-1762.

Farquhar GD, Hubick KT, Condon AG, Richards RA (1989a) Carbon isotope fractionation and plant water use efficiency. p. 220–240. *In* P.W. Rundell et al. (ed.) Stable isotopes in ecological research. Springer-Verlag, New York.

Farquhar GD, Ehleringer JR, Hubick KT (1989b) Carbon isotope discrimination and photosynthesis. Annu Rev Plant Phys. 40: 503–537.

Flexas J, Bota J, Loreta F, Cornic G, Sharkey TD (2004) Difusive and metabolic limitaton to photosynthesis under drought and salinity in C plants. Plant Biol. 6: 269–279.

Hare PD, Cress WA (1997) Metabolic implications of stress-induced proline accumulation in plants. Plant Growth Regul. 21: 79-102.

Hazarlı H, Zardashti MR, Darvishzadeh R, Najafi S (2010) The Effect of water stress and polymer on water use effciency, yield and several morphological traits of sunflower under greenhouse condition. Not Sci Biol. 2: 53-58.

Hossain MI, Khatun A, Talukder MSA, Dewan MMR, Uddin MS (2010) Effect of drought o physiology and yield contributing characters of sunflower. Bangladesh J Agril Res. 35: 113-124.

Jiang Y, Huang B (2002) Protein alterations in tall fescue in response to drought stress and abscisic acid. Crop Sci. 42: 202-207.

Johnson RC, Basset LM (1991) Carbon isotope discrimination and water use efficiency in four cool-season grasses. Crop Sci. 31: 157–162.

Kiani SP, Maury P, Sarrafi A, Grieu P (2008) QTL analysis of chlorophyll fluorescence parameters in sunflower (*Helianthus annuus* L.) under well-watered and water-stressed conditions. Plant Sci. 175: 565–573.

Kraus TE, Mckersie BD, Fletcher RA (1995) Paclobutrazole induced tolerance of wheat leaves to paraquat may involve antioxidant enzyme activity. J Plant Physiol. 145: 570–576.

Krizmanic M, Liovic I, Mijic A, Bilandzic M, Krizmanic G (2003) Genetic potential of OS sunflower hybrids in different agroecological conditions. Sjemenarstvo. 20: 237–245.

Lambrides CJ, Chapman SC, Shorter R (2004) Surveys of carbon isotope discrimination in sunflower reveal considerable genetic variation, a strong association with transpiration efficiency and evidence of cytoplasmic inheritance. Crop Sci. 44: 1642–1653.

Lawlor DW, Cornic G (2002) Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant Cell Environ. 25: 275–294.

Markwell J, Osterman JC, Mitchell JL (1995) Calibration of the Minolta SPAD-502 leaf chlorophyll meter. Photosynth Res. 46: 467-472.

Massacci A, Nabiev SM, Pietrosanti L, Nematov SK, Chernikova TN, Thor K, Leipner J (2008) Response of the photosynthetic apparatus of cotton (*Gossypium hirsutum*) to the onset of drought stress under field conditions studied by

gas-exchange analysis and chlorophyll fluorescence imaging. Plant Physiol Bioch. 46: 189–195.
Mensah JK, Obadoni BO, Eroutor PG, Onome-Irieguna F (2006) Simulated flooding and drought effects on germination, growth and yield parameters of Sesame (*Seasamum indicum* L.). Afr J Biotechnol. 5: 1249-1253.
Morgan JA, LeCain DR, McCaig TN, Quick JS (1993) Gas exchange, carbon isotope discrimination and productivity in winter wheat. Crop Sci. 33: 178–186.
Munns R, Passioura JB, Guo J, Chazen O, Cramer GR (2000) Water relations and leaf expansion: importance of time scale. J Exp Bot. 51: 1495-1504.
Nagarathna TK, Shadakshari YG, Ramakrishna Parama VR, Jagadish KS, Puttarangaswamy KT (2012) Examination of root characters, isotope discrimination, physiological and morphological traits and their relationship used to identify the drought tolerant sunflower (*Helianthus annuus* l.) genotypes. Helia. 56: 1-8.
Oncel I, Keles Y, Ustun AS (2000) Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings. Environ Pollut. 107: 315-320.
Oukarroum A, Madidi SE, Schansker G, Strasser RJ (2007) Probing the responses of barley cultivars (*Hordeum vulgare* L.) by chlorophyll a fluorescence OLKJIP under drought stress and re-watering. Environ Exp Bot. 60: 438–446.
Pankoviç D, Sakac Z, Kevres S, Plesnicar M (1999) Acclimation to long-term water deficit in the leaves of two sunflower hybrids: photosynthesis, electron transport and carbon metabolism. J Exp Bot. 50: 127–138.
Paussioura JB (1986) Resistance to drought and salinity: Avenues for improvement. Aust J Plant Physiol. 13: 191-201.
Poorter HH, Farquhar GD (1994) Transpiration, intercellular carbon dioxide concentration and carbon-isotope discrimination of 24 wild species differing in relative growth rate. Aust J Plant Physiol. 21: 507–516.
Rahman MU, Gul S, Ahmad I (2004) Effects of water stress on growth and photosynthetic pigments of corn (*Zea mays* L.) cultivars. Int J Agric Biol. 6: 652-655.
Rauf S (2008) Breeding sunflower (*Helianthus annuus* L.) for drought tolerance. Commun Biometry Crop Sci. 3: 29–44.
Ristic Z, Bukovnik U, Prasad PVV (2007) Correlation between heat stability of thylakoid membranes and loss of chlorophyll in winter wheat under heat stress. Crop Sci. 47: 2067–2073.
Ritchie SW, Nguyan HT, Holaday AS (1990) Leaf water content and gas exchange parameters of two wheat genotypes differing in drought resistance. Crop Sci. 30: 105-111.
Shahbaz M, Ashraf M, Akram NA, Hanif A, Hameed S, Joham S, Rehman R (2011) Salt-induced modulation in growth, photosynthetic capacity, proline content and ion accumulation in sunflower (*Helianthus annuus* L.). Acta Physiol Plant. 33: 1113–1122.
Sinclair TR, Ludlow MM (1986) Influence of soil water supply on the plant water balance of four tropical grain legumes. Aust J Plant Physiol. 13: 329–341.
Tahkokorpi M, Taulavuori K, Laine K, Taulavuori E (2007) Aftereffects of drought-related winter stress in previous and current year stems of *Vaccinium myrtillus* L. Environ Exp Bot. 61: 85–93.
Tan Y, Liang ZS, HongBo S (2006) Effect of water deficits on the activity of anti-oxidative enzymes and osmoregulation among 3 different genotypes of Radix Astragali at seeding stage. Colloids Surface B. 49: 60–65
Tardieu F, Reymond M, Hamard P, Granier C, Muller B (2000) Spatal distributon of expansion rate, cell division rate and cell size in maize leaves: A synthesis of the efects of soil waterstatus, evaporatve demand and temperature. J Exp Bot. 51: 1505–1514.
Ünyayar S, Keles Y, Ünal E (2009) Proline and ABA levels in two sunflower genotypes subjected to water stress. Bulg J Physiol. 30: 34-47.
Vanaja M, Yadav SK, Archana G, Jyothi Lakshmi N, Ram Reddy PR, Vagheera P, Abdul Razak SK, Maheswari M, Venkateswarlu B (2011) Response of C4 (maize) and C3 (sunflower) crop plants to drought stress and enhanced carbon dioxide concentration. Plant Soil Environ. 57: 207–215.
Yordanov I, Velikova V, Tsonev T (2000) Plant responses to drought, acclimation, and stress tolerance. Photosynthetica 38: 171–186.
Zengin FK, Kirbağ S (2007) Effects of copper on chlorophyll, proline, protein and abscisic acid level of sunflower (*Helianthus annuus* L.) seedlings. J Environ Biol. 28: 561-566.
Zobayed S, Afreen F, Kozai T (2005) Temperature stress can alter the photosynthetic efficiency and secondary metabolite concentrations in St. John's Wort. Plant Physiol Bioch. 43: 977–984.
Xiong L, Zhu JK (2002) Molecular and genetic aspects of plant responses to osmotic stress. Plant Cell and Environ. 25: 131–139.