

## RELATIONSHIP BETWEEN WATER USE EFFICIENCY AND $\delta^{13}\text{C}$ ISOTOPE DISCRIMINATION OF SAFFLOWER (*Carthamustinctorius* L.) UNDER DROUGHT STRESS

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### ABSTRACT

Drought stress is one of the most limiting factors in agricultural productivity because of its highly negative effect on photosynthesis and growth of plants. The main objectives of this study were to determine the performance of four selected safflower genotypes (Remzibey, Dinçer, Balcı and TRE-ASL09/14) against drought stress. The relationship between water use efficiency (WUE) and  $\delta^{13}\text{C}$  (isotope discrimination) was investigated under well watered (60%) and drought stress (30%) irrigation in controlled conditions in a green house. The result showed that drought stress clearly reduced plant biomass, leaf area, leaf number, relative water content (RWC), specific leaf weight (SLW), WUE and  $\delta^{13}\text{C}$  in all genotypes, while chlorophyll increased under drought stress. There were significant differences between performances of all safflower genotypes in terms of response to drought stress. High WUE and low  $\delta^{13}\text{C}$  discrimination under drought stress were inversely associated with a strong regression relationship ( $R^2=0.75$ ). The analysis of  $\delta^{13}\text{C}$  revealed a substantial variation in water use efficiency among the genotypes under drought stress. It was revealed that low  $\delta^{13}\text{C}$  discrimination types had high WUE, RWC and total biomass under drought stress. Thus, the ability of the low  $\delta^{13}\text{C}$  genotypes (high water use efficiency, WUE) to maintain higher RWC may provide a good indication of the differences in drought tolerance of safflower genotypes differing in  $\delta^{13}\text{C}$ . Overall, on the basis of the consistent percentage reductions in plant heights, total dry weight, leaf area, RWC, WUE and low  $\delta^{13}\text{C}$ , the TRE-ASL09/14 new breeding line was found to be more drought tolerant when compared with the other safflower hybrids under drought stress. As a result of our study it is suggested that there is a strong relationship between WUE and lower  $\delta^{13}\text{C}$  under drought stress, indicating that it may be used as a selection criterion for developing safflower genotypes with drought tolerance.

**Key words:** Safflower, drought stress, WUE,  $\delta^{13}\text{C}$ .

**Abbreviation:** P.H: Plant Height, ChyII: Chlorophyll, TLA: Total 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,  $\delta^{13}\text{C}$ isotope: Carbon Isotope Discrimination, WUE: Water Use Efficiency, FC: Field Capacity, WW: Well Watered, DS: Drought Stress.

### INTRODUCTION

With regard to economic benefits, researchers have been impelled to search for alternative edible oilseed crops which have tolerant genotypes against changing environmental conditions in a new crop rotation system for every region. This is owing to the increase in global warming over the years, limited irrigation water and also the increasing lack of vegetable crude oil and oilseed crops in the world. Although safflower (*Carthamustinctorius* L.) is one of the oldest crops, it is traditionally grown for its seeds and used for coloring and flavoring foods and for making red and yellow dyes

(Arslan, 2003; Zohary and Hopf, 2000). It is not a popular oilseed crop compared to other oilseed crops, such as soybean, sunflower, peanut, cotton seed, and rapeseed, since it is not commonly grown globally. Much of the literature has denoted that safflower is a C3 plant tolerant to drought and salinity stress because of having deep-rooting ability (Dordas and Sioulas, 2008), water uptake from soil (Majidiet al., 2011), and different osmolyte accumulation (Bhatia et al. 1994).

Drought, which is low water availability or random and unpredictable changes in weather conditions during the period of plant growth, is considered one of the most

effective abiotic stress factors limiting production from plants. All field crops respond differently at different phenological stages to changing water status of the soil under drought stress, which means that plants are more sensitive to drought stress at some stages. For example, Blum (2005) explained that drought resistance in seedlings grown in a pot has nothing to do with drought resistance during grain filling in the field. Although the drought-resistant ideotype is still not well defined, drought resistance in its physiological context is defined according to Levitt (1972) as being determined by 'dehydration avoidance' (maintenance of water potential in tissue) and/or 'dehydration tolerance' (Levitt, 1980; Price et al., 2002). Dehydration avoidance or osmotic adjustment is defined as the plant's capacity to sustain high plant water status or cellular hydration under drought stress (Blum, 2005; Cushman, 2001).

There is no consistent relationship between plant production and water use efficiency (WUE). However, Munoz et al., (1998) pointed out that high yield potential of plants under water-limited conditions is generally associated with reduced WUE mainly because of high water use. In contrast, other researchers have explained that high WUE is largely a function of reduced water use rather than a net improvement in plant production or the biochemistry of assimilation (Blum, 2005). WUE is generally equated with drought resistance and the improvement of crop yield under stress, due to variations in water use. In addition, as explained by Farquhar et al., (1989), carbon isotope distribution can reveal information about the physical, chemical, and metabolic processes involved in carbon transformations. This is because carbon isotope discrimination occurs during photosynthetic CO<sub>2</sub> uptake leading to a <sup>13</sup>C-depletion of plant organic matter. Therefore, it is not surprising that selection of high WUE using carbon isotope discrimination has resulted in earlier flowering plants that use less water over the growing season. These plants were found to be very suitable for conditions where moderated use of the given amount of stored soil moisture is crucial (Condon et al. 2002).

Maintenance of leaf turgor in the face of decreasing soil moisture has been emphasized as an important adaptation trait that contributes to drought tolerance (Hsiao et al., 1976). Tolerance to internal water deficit has been characterized by turgor loss at lower relative water content (RWC), promoting the maintenance of chloroplast functioning during dehydration (Gupta and Berkowitz, 1987; Ranney et al., 1991). The studies of dehydration tolerance in crop plants have revealed genotypic variation in plant recovery from dehydration as a measure of tolerance to be positively correlated with the plant water status (RWC) (Chaves et al., 2002). Anyia and Herzog (2004) pointed out that the high relative water content (RWC) of cowpea leaves was maintained in some of the genotypes by stomata closure and a reduction in leaf area. Many techniques and parameters such as leaf water potential, leaf osmotic potential, and canopy temperature have been used to screen drought tolerant plants in

different crops (Askahmet et al., 2007; David and Duniway, 1997). Therefore, drought resistance and its components are almost constantly being redefined to express the inventive capacity for terminology. However, none of the previous experiments attempted to identify whether  $\Delta^{13}\text{C}$  isotope is activated in safflower genotypes of any safflower cultivars under drought stress. As a result of these statements, the main aims of our study were 1) to determine selected cultivars which may be tolerant against drought stress, and 2) to investigate the relationship between plant biomass, RWC, WUE and  $\delta^{13}\text{C}$  and other traits in safflower cultivars.

## MATERIALS and METHODS

### *Plant materials and experimental conditions*

Remzibey, Balcı (brambly) and Dincer (unbrambly) safflower hybrid cultivars and the new safflower breeding line TRE-ALS09/14 (brambly), all of Turkish origin, were used to determine variation under controlled drought stress and well-watered environmental conditions. The plants were grown under light/dark regime 12/12 h, at 25/15 ± 3 °C and relative humidity of approximately 30-50% at the research greenhouse of the Crop Science department of the Agriculture and Horticulture Faculty in Humboldt University, Germany in 2012. The safflower cultivars were planted in Mitscherlich cylindrical pots (30-cm deep 25 cm dia.) in the greenhouse with only natural sunlight of the summer months. Clay loam soil was used to fill pots and all cultivars were arranged completely in a randomized block design with five replications. Required amounts of chemical fertilizers were applied and seeds were sown according to the recommendations for field conditions in the literature (1 g nitrogen from 3.70 g KAS fertilizer). Watering began immediately after sowing and once the seedlings had emerged, thinning was carried out and the plant populations were maintained (4 plants in a pot). Changes in the soil water potential of each pot were measured and checked daily by weighing each pot at the beginning and end. The soil water factor included two irrigation regimes including irrigation at 30% (drought stress) and 60% (well-watered) of field capacity.

### *Determination of water holding capacity of soil*

Field soil which had already been taken from the field experiment area was air-dried and ground to pass through a 2-3 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 five 100 cm<sup>3</sup> cylindrical tubes were covered with paper and a plastic strap as a filter, they were tared without soil and then completely filled with soil (by compression). Each cylindrical tube with soil was weighed and settled in a big tray which was approximately as deep as the height of the cylindrical tubes. The tray was fully filled with water up to the top of the cylindrical tubes and left for 3 h (saturation). Then, all cylindrical tubes were left on 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 for 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. % = wet soil weight (saturated) – dry weight / dry weight x 100. The mean of five replicates was 38.89%.

#### *Drought stress treatment*

To determine the amount of irrigation required to produce irrigation regimes at 30% and 60% of field capacity, the soil was firstly weighed to exactly 6091 g for each pot and then the net weight of each pot with filled soil was measured. Pots were initially watered with 1330 and 619 g per pot which corresponds to 60% and 30% of field capacity. The soil water content was continuously monitored and maintained at 30% and 60% of field capacity by daily irrigation during the experiment.

Plants were harvested 50 days after sowing, when the plants were at the heading stage (head visible).

#### *Determination of relative water content*

RWC (*Relative Water Content*); the youngest fully expanded leaves were collected from each pot in the morning. The leaves were weighed immediately to obtain the fresh weight. Afterwards the leaves were rehydrated by floating for 12 h in a covered water bath cap at approximately 23 °C under dark conditions. All leaves were dried for 72 h at 70 °C in an oven 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.

RWC = Fresh weight – Dry weight / Turgid weight – Dry weight x 100

#### *Measurement of Chlorophyll*

Chlorophyll content was assessed using a chlorophyll meter (SPAD-502, Minolta) and measurements were taken at two points on both sides of young fully developed sunlit leaves (upper, middle and lower parts) two times during the experiment (Days 30. and 40. of the experiment after sowing). Forty readings were averaged per genotype (twelve readings of two fully developed leaves per plant five replicates) for each treatment. The average of these forty readings was considered to be the SPAD value for one cultivar under one condition.

#### *Measurement of the growth parameters*

Plant height (PH cm) was measured from the soil surface to the top of plant 3 times between emergence and harvest time. Total leaf area (cm<sup>2</sup>) was measured immediately for all leaves on a plant using a leaf area meter (Li-Cor 3000, Lambda Instruments Co., USA) after the plant was removed. Leaf and stem dry weights were obtained after all parts of the plant were separately dried at 70 °C for 72 h. The total dry matter production per plant (g plant<sup>-1</sup>) was obtained with the summation of dry weight of all plant parts and was expressed on a per plant basis. All leaves on a plant were numbered before the leaf area was measured. After that the plant was removed. Specific leaf weight (SLW) was calculated by dividing the

total leaf area by leaf dry weight (LDW/LA) (g m<sup>-2</sup>). The water use efficiency (WUE) measurements were completed in order to note another important index to estimate the water productivity over time. WUE was given in terms of the dry fresh weight per water consumed by evapotranspiration and evaluated as (g/g plant);

WUE = Total biomass / Water consumption (amount of irrigation (g) during the experiment).

#### *Analyses of carbon isotope discrimination ( $\delta^{13}C$ )*

Carbon isotope discrimination was analyzed from the same leaves (young fully expanded leaves) which were kept at -20 °C. The leaves were dried at 60 °C for 72 h and ground through a 0.1 mm screen to produce a flour for carbon isotope analysis. <sup>13</sup>C analyses were performed by 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:  $\delta^{13}C$  (‰) = [(R sample/R reference – 1) × 1000], with R being the <sup>13</sup>C/<sup>12</sup>C ratio. Carbon isotope discrimination ( $\Delta$ ) was calculated using the following formula (Farquhar *et al.* 1989):  $\delta^{13}C$  (‰) = [( $\delta a - \delta p$ ) / (1 +  $\delta p$ )], where  $\delta p$  is the  $\delta^{13}C$  of the leaves and  $\delta a$  is the  $\delta^{13}C$  of the atmospheric CO<sub>2</sub> (-8‰).

#### *Statistical analysis*

To determine the effect of drought stress on the four safflower hybrid cultivars, the samples were analyzed statistically as a randomized block design with five replications. ANOVA was applied to analyze the variance of drought stress on safflower hybrid cultivars and the interaction of drought and cultivars. The ANOVA (analyses of variance) of this study and correlation coefficients among the traits are given as the mean of genotypes under each condition. Significant differences between the means of replications were tested using Fisher's least squares difference (LSD) method. All differences referred to in the text were significant at 0.05. Regression analyses were computed using Microsoft Excel office program y diagram to assess the relationship between  $\delta^{13}C$  and WUE only under drought stress conditions.

## **RESULTS**

The variance analyses clearly showed the significant effects of drought stress statistically on the physiological and morphological traits measured in the safflower genotypes during the experiment (Table 1). Drought stress x cultivar interaction was also statistically significant in terms of  $\delta^{13}C$ , WUE, plant height, leaf number and chlorophyll of safflowers in Table 1. The correlation coefficients obtained from our study shown in Table 2 indicate that drought stress was considerably negatively correlated with total leaf area, relative water content, total dry weight, specific leaf weight,  $\delta^{13}C$  and water use efficiency, while it was positively correlated with chlorophyll. Also a significant negative correlation coefficient of -0.46\*\* was found between RWC and  $\delta^{13}C$ . Total leaf area had a significantly positive relationship with WUE,  $\delta^{13}C$  and leaf number under drought stress

conditions (Table 2). Total dry weight had a significantly positive relationship with plant height, leaf number, total leaf areas,  $\delta^{13}\text{C}$  and water use efficiency (WUE) (Table 2).

It appears that SLW was positively correlated with plant height, total leaf area,  $\delta^{13}\text{C}$ , RWC and slightly less with WUE (Table 2).

**Table 1.** The result of variance analyses for all components measured of safflower under the well watered and drought stress conditions.

Variance Source	d.f	Calculated of Mean Square						
		PH1	PH2	PH3	LDW	SDW	TDW	LN
D.S	1	83.377**	152.881**	2061.809**	1.895**	7.043**	15.952**	172.225**
C	3	2.619**	18.614**	156.340**	0.102**	0.432**	0.888**	38.425**
D.S x C	3	3.004**	32.426**	18.074**	0.012ns	0.055ns	0.083ns	8.425**
		TLA	SLW	ChyIII	ChyII2	WUE	RWC	$\delta^{13}\text{C}$
D.S	1	34686.901**	1578.541**	577.600**	1037.852**	0.047**	0.014**	156.816**
C	3	2935.079ns	119.807*	15.479ns	13.974**	0.012**	0.003ns	7.381**
D.S x C	3	867.342ns	20.449ns	9.259ns	21.870**	0.001ns	0.001ns	3.637**

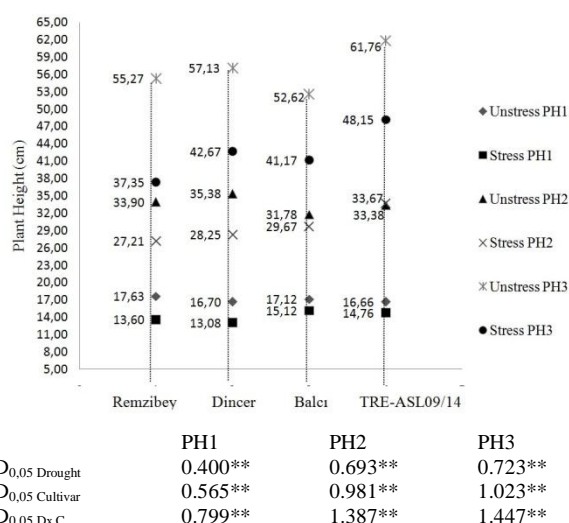
D.S: Drought Stress, C: Cultivars, d.f.: Degree of Freedom, ns: non-significant; \*P<0.05; \*\*P<0.01, P.H.: Plant Height, LDW: Leaf Dry Weight, SDW: Stem Dry Matter, TDW: Total Dry Weight, LN: Leaf Number, TLA: Total Leaf Area, SLW: Specific Leaf Weight, Chyll: Chlorophyll, WUE: Water Use Efficiency, RWC: Relative Water Content,  $\delta^{13}\text{C}$  discrimination

**Table 2.** Correlation coefficients between condition and traits of Safflower.

	Condition	LA	TDW	Chyll2	SLW	RWC	WUE	$\delta^{13}\text{C}$	PHU	LN
Condition	-	-0.87**	-0.70**	0.95**	-0.68**	-0.46**	-0.70**	-0.89**	-0.89**	-0.71**
LA	-	-	0.84**	-0.82**	-0.58**	-0.35*	0.69**	0.74**	0.81**	0.74**
TDW	-	-	-	-0.66**	ns	0.37*	0.80**	0.55**	0.75**	0.69**
Chyll2	-	-	-	-	-0.57**	-0.46**	-0.69**	-0.77**	-0.87**	-0.81
SLW	-	-	-	-	-	ns	0.39*	0.67**	0.55**	ns
RWC	-	-	-	-	-	-	0.32*	-0.46**	0.48**	0.55**
WUE	-	-	-	-	-	-	-	-0.43**	0.86**	0.46**
$\Delta^{13}\text{C}$	-	-	-	-	-	-	-	-	0.66**	0.44**
PHU	-	-	-	-	-	-	-	-	-	0.75**
LN	-	-	-	-	-	-	-	-	-	-

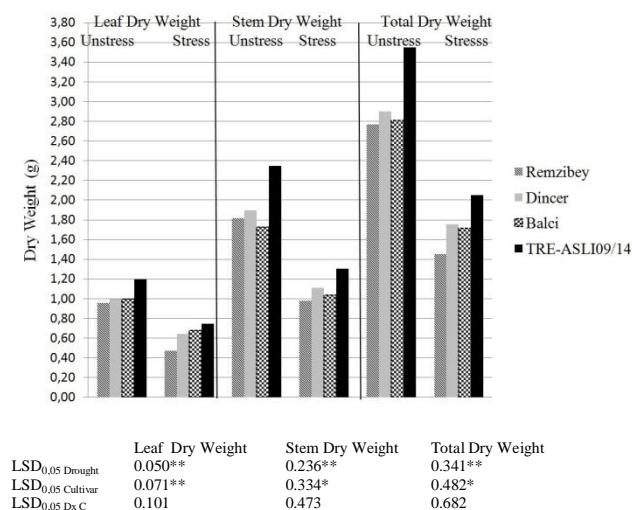
Chyll: Chlorophyll, LA: Total Leaf Area, RWC: Relative Water Content, TDW: Total Dry Weight, SLW: Specific Leaf Weight,  $\delta^{13}\text{C}$  discrimination, WUE: Water Use Efficiency, LN: Leaf Number, PHU. Plant Height3.

As all plants were exposed to drought stress, all safflower genotypes responded to the drought stress by decreasing the plant height measured three times (Figure 1). There were statistical differences among the genotypes in terms of plant height under both well-watered and drought stress conditions. For example, although the TRE-ASL09/14 genotype was not statistically in the first group at the 1<sup>st</sup> and 2<sup>nd</sup> measuring times, it was statistically in the first group with regard to plant height at the 3<sup>rd</sup> measuring time under both well-watered and drought stress conditions. Also, the percentage decrease in the plant height of TRE-ASL09/14 was generally the lowest when compared with the other cultivars at all measuring times under drought stress (Figure 1). This study identified differences among the plant heights of safflower genotypes in response to drought stress markedly change during the growth process (at 3<sup>rd</sup> measuring time), illustrated in Figure 1, because of the increasing percentage decrease in plant height of all safflowers during drought stress. The greatest reaction can be observed in the 17.8 cm decrease in the plant height of the Remzibey safflower genotype at the last measuring time under drought stress (Figure 1).



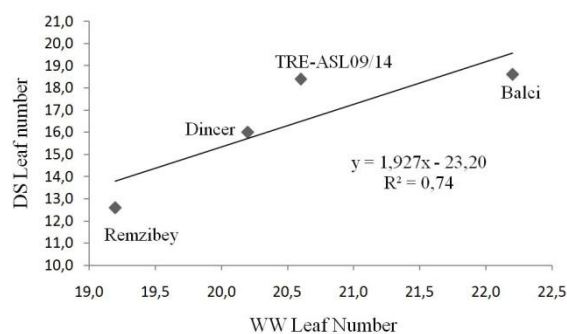
**Figure 1.** Plant heights of safflower genotypes under the well watered (WW) and drought stress condition (DS). (PH1: first measuring; PH2: second measuring; PH3: third measuring time)

The results indicate that increasing drought, decreased leaf, stem and total dry weight were significant in all safflower genotypes (Figure 2). It was determined that the highest percentage of decrease in terms of total dry weight was determined in the TRE-ASL09/14 safflower genotype under drought stress conditions (Figure 2). The highest mean leaf, stem and total dry weight was observed in the TRE-ASL09/14 safflower genotype under both conditions (Figure 2). It was determined in this study that the greatest plant weight component was the plant stem, as it was 65% of the average of all safflower genotypes under both conditions in Figure 2 (not shown in Figure).



**Figure 2.** Leaf, stem and total dry weight of safflower genotypes under the well watered (WW) and drought stress conditions (DS).

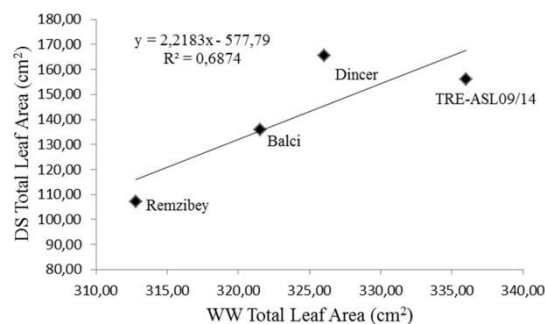
Drought stress statistically reduced the leaf number of all safflower genotypes, shown in Figure 3. There were also significant differences in terms of leaf number among all the safflower genotypes under both the well-watered and drought stress conditions (Figure 3). However, it was revealed that although the Balci genotype was in the highest statistical group under both conditions, the leaf number of TRE-ASL09/14 was more stable as there was a smaller reduction than in the other cultivars under drought stress (Figure 3).



LSD<sub>0,05</sub>Drought: 0.564\*\*, LSD<sub>0,05</sub> Cultivar: 0.797\*\*, LSD<sub>0,05</sub> Dx C: 1.127\*\*

**Figure 3.** Relationship between total leaf number of safflower genotypes under drought stress (DS) and well watered conditions (WW) condition.

Our regression analyses, shown in Figure 3, indicate there was a significant relationship between the leaf number of unstressed plants and the leaf number of stressed plants with  $R^2=0.74$ . Drought stress significantly reduced the total leaf area of all safflower genotypes (Figure 4). Although all sunflower genotypes did not show statistically different reactions under well-watered and drought stress conditions, there was a difference of 53 cm<sup>2</sup> in terms of total leaf area between Dincer and Remzibey cultivars under drought stress (Figure 4) (not shown in Figure). The largest leaf area was obtained from Dincer and TRE-ASL09/14 under well-watered and drought stress conditions (Figure 4). Data obtained from the study shows that the percentage of reduction in terms of leaf area was lowest in the Dincer safflower cultivar under the effects of drought stress (Figure 4). It was revealed that there was significant relationship, with  $R^2=0.68$ , between the total leaf area of stressed plants and the total leaf area of unstressed plants (Figure 4).



LSD<sub>0,05</sub> D: 34.367\*\*, LSD<sub>0,05</sub> C: 48.603, LSD<sub>0,05</sub> DXC: 68.735

**Figure 4.** Relationship between total leaf area of safflower genotypes under drought stress (DS) and well watered conditions (WW).

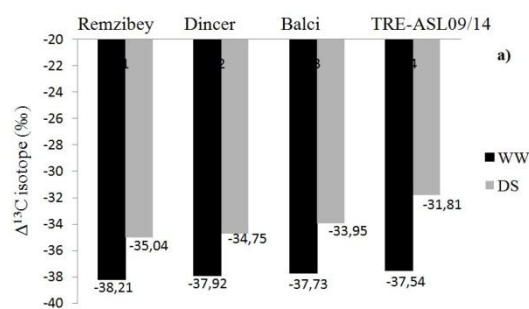
As shown in Figure 5a, the data obtained from both conditions indicated that  $\delta^{13}C$  (‰) in all safflower genotypes significantly ( $P<0.01$ ) decreased as they were exposed to drought stress. The highest reduction in  $\delta^{13}C$  isotope percentage was determined from TRE-ASL09/14 with a 6.09% decrease under drought stress (Figure 5a). It was also observed that the lowest  $\delta^{13}C$  was obtained from TRE-ASL09/14 under drought stress conditions. However, the highest WUE was determined in the TRE-ASL09/14 genotype under drought stress. There were significant differences in terms of  $\delta^{13}C$  among all safflower genotypes in both well-watered and drought stress conditions (Table 1 and Figure 5a). In particular, significant differences in  $\delta^{13}C$  among all safflowers could be observed under the effects of drought stress (Figure 5a). Drought stress reduced the WUE of all safflower genotypes (Figure 5b). There were also statistically significant differences in terms of WUE among all the safflower genotypes (Table 1 and Figure 5b). The highest reduction in WUE under drought stress conditions was observed in TRE-ASL09/14 and Remzibey with decreases of 0.5 and 0.6 g g.plant<sup>-1</sup> because of having high WUE under well watered conditions (Figure 5b). The highest WUE was determined in the TRE-ASL09/14 genotype in



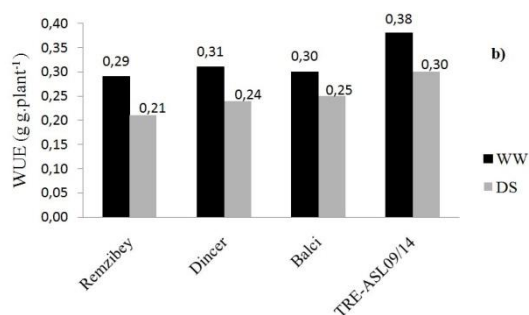
both well-watered and drought stress conditions (Figure 5b). Dincer and Balci safflower genotypes showed the lowest reduction in terms of WUE with decreases of 0.2 g g.plant<sup>-1</sup> under drought stress (Figure 5b).

The decrease in the specific leaf weight (SLW) of all safflower genotypes under drought stress can be seen in Figure 5c. There were significant differences in terms of SLW among all safflower genotypes under both conditions. The SLW changes observed in all genotypes in response to drought stress indicated that the Balci safflower genotype was the most sensitive genotype to drought stress (Figure 5c). The highest SLW under drought stress conditions was observed in the Balci and TRE-ASL09/14 safflower genotypes (Figure 5c). It is evident from our study that the relative water content (RWC) of the four safflower genotypes decreased significantly under drought stress (Figure 5d). On average, the mean RWC of the genotypes showed a reduction of 3.62% under drought stress. The result indicates there were no statistical differences in the value of RWC among all safflower genotypes (Table 1 and Figure 5d). In particular, the RWC values of all genotypes were observed to be quite close under well-watered conditions, while the differences in these values among all safflower genotypes appeared to be more marked under drought stress (Figure 5d). Remzibey had the highest reduction of 0.6% in terms of RWC under drought stress. Figure 5d shows that although there were no statistically significant differences among the genotypes and drought x cultivar interactions, the lowest reductions in the mean RWC were observed in the Balci and TRE-ASL09/14 genotypes under drought stress.

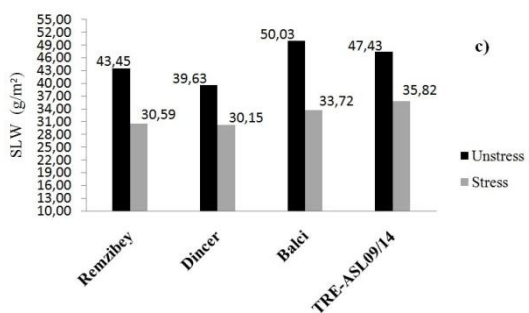
Our results show that there was a significant relationship ( $R^2=0.75$ ) between water use efficiency and  $\delta^{13}C$  under drought stress (Figure 6). The chlorophyll data obtained from SPAD twice showed that drought stress had significant effects on the chlorophyll of young fully developed leaves in all genotypes. Statistically the chlorophyll of all genotypes was increased by drought stress (Figure 7). On average, the increasing tendency of 44.08 obtained at the first measuring time and 47.13 obtained at the second measuring time under well-watered conditions were statistically different by 7.03 and 10.05 at  $P < 0.01$  with respect to chlorophyll under drought stress (Figure 7). Our study also revealed that the chlorophyll of all genotypes showed changes depending on drought duration. There were significant differences between 1<sup>st</sup> and 2<sup>nd</sup> measuring times in all safflower genotypes under both conditions (Figure 7). In particular, at the second measuring time although the chlorophyll of TRE-ASL09/14 safflower under unstressed conditions was the highest when compared with the other genotypes, it was in the lowest group under stressed conditions.



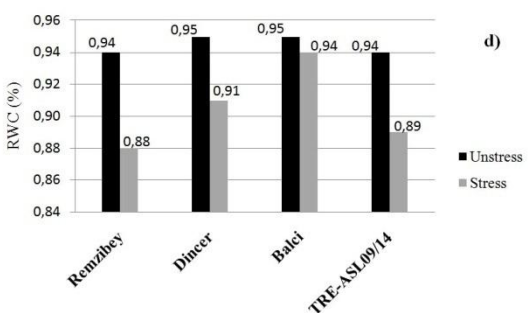
LSD<sub>0,05</sub> Drought: 0.284 \*\*, LSD<sub>0,05</sub> Cultivar: 0.402 \*\*, LSD<sub>0,05</sub> DxC: 0.568\*\*



LSD<sub>0,05</sub> Drought: 0.015 \*\*, LSD<sub>0,05</sub> Cultivar: 0.021 \*\*, LSD<sub>0,05</sub> DxC: 0.029ns

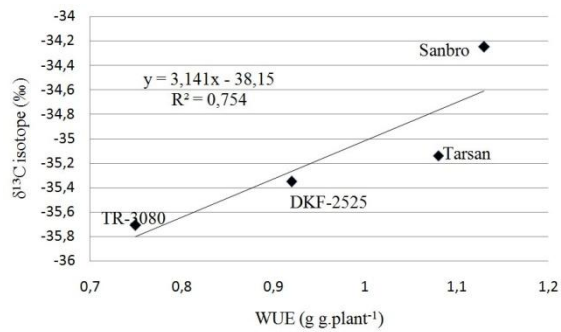


LSD<sub>0,05</sub> Drought: 3.322 \*\*, LSD<sub>0,05</sub> Cultivar: 4.698 \*\*, LSD<sub>0,05</sub> DxC: 6.644ns

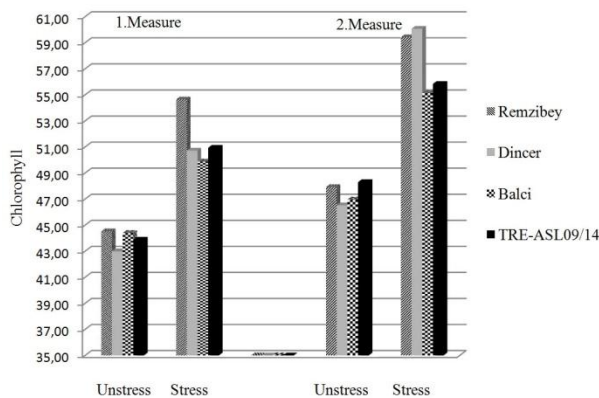


LSD<sub>0,05</sub> Drought: 0.024 \*\*, LSD<sub>0,05</sub> Cultivar: 0.033ns, LSD<sub>0,05</sub> DxC: 0.047ns

**Figure 5.**  $\delta^{13}C$  isotope (‰) (a), water use efficiency (g g.plant<sup>-1</sup>) (b), specific leaf weight (g/m<sup>2</sup>) (c) and relative water content (%) (d) of safflower cultivars under the well watered (WW) and drought stress (DS).



**Figure 6.** Relationship between plant water use efficiency and  $\delta^{13}\text{C}$  isotope for four safflower genotypes under the drought stress condition.



**Figure 7.** Chlorophyll content measured by SPAD in two times of safflower genotypes under well watered (WW) and drought stress conditions (DS).

## DISCUSSION

Our data shows that the plant height, leaf, stem and total dry weight of all safflower genotypes decreased significantly under drought stress because plants respond to drought stress by improving root traits (Price et al., 2002) and, possibly diminishing cell expansion and cell division under drought stress (Munnset al., 2000; O'Neill, 1983). In parallel, the leaf area and leaf numbers of all genotypes also decreased to reduce the evaporative surface (leaf area) under drought stress, similar to the findings of Mitchell et al. (1998). This is because of reduced water loss through reduced epidermal conductance and reduced radiation absorption, as in the results of Farooq et al. (2009). The correlations found in our study, which were significantly positive between WUE and total dry matter (0.80\*\*), further support this theory. Reduced leaf area results in reduced transpiration surface (Namirembe et al., 2008) and may be a drought avoidance strategy for plants. On the other hand, the reduction in leaf area limits photosynthesis and further decreases biomass production, consistent with the positive correlation between total leaf area and biomass production. Also Harbet et al. (2010) pointed out in studies on *Arabidopsis thaliana* under drought stress that the reductions in dry matter accumulation and leaf expansion were dependent on the developmental stage of the plants and can be managed by many genes (explained in Figure 11 in the review of Harbet et al., 2010).

The WUE values of all safflower genotypes markedly decreased under drought stress. The reason for the lower WUE under drought stress might be due to a greater effect on biomass production than on water use, or it might be that lower conductance of  $\text{CO}_2$  and the activity of photosynthetic enzymes decreased photosynthesis and biomass, reducing WUE (Singh and Singh, 2003). The important relationship between WUE and  $\delta^{13}\text{C}$  obtained from regression analyses, with  $R^2=0.75$  under drought stress, showed that cultivars with high water-use efficiency can be selected using low carbon isotope discrimination under drought stress as presented by Condon et al. (2002). Thus, greater biomass production under stress has been associated with relatively greater WUE as pointed out by Condon et al. (2004), who also explained that the basic unit of production could be the moles of carbon gained by photosynthesis in exchange for water used in transpiration.

The RWC values of both unstressed and stressed plants in the present experiments were higher than those previously reported for safflowers in field experiments by Eslam (2011). One possible explanation for decreasing RWC in safflower leaves could be the limitation of carbohydrate supply caused by water stress. Lawlor and Cornic (2002) and Lawlor (2002) noted photosynthetic carbon assimilation and associated metabolism in relation to RWC in plants and distinguished two general types of relationship between photosynthetic potential and RWC. Our study determined that Balci and TRE-ASL09/14 were sturdy safflower genotypes in terms of stable water retention in leaf tissue when compared with the other genotypes under drought stress. Francaet al. (2000) explained that genotypes characterized by better drought tolerance mechanisms and higher tissue water retention capacity lead to better growth in beans under water deficits. Thus, avoiding desiccation by maintaining leaf water status at a high level could be considered. Since drought stress decreased RWC, it seems that these indices could reflect the effect of stress that occurred during the vegetative stage (till head visible).

The results of this study indicate that the Balci and TRE-ASL09/14 safflower genotypes were better than the other genotypes in terms of maintaining SLW under drought stress. According to O'Neill (1983), stressed leaves had a lower SLW, suggesting that these leaves were thicker or had more densely packed mesophyll cells with less intracellular air space. These alterations in leaf anatomy could also result from an inhibition of cell expansion. The possible relationship between specific leaf weight and WUE is based on the fact that SLW could be an indicator of leaf photosynthetic capacity. The increase in SLW could be due to carbohydrates and variation in mesophyll tissue density or leaf thickness, as proposed by Arauset al. (1986). Results of a study revealed that safflower genotypes with greater SLW provided more photosynthetic protein per unit ground area, as explained by Wells et al. (1986).

## CONCLUSION

Our study suggested that using high relationship  $R^2$ : 0.75 regression between WUE and  $\delta^{13}\text{C}$  (carbon isotope discrimination) under drought stress can be used to selection criteria in response to drought stress for new developing safflower breeding programs. Also, TRE-ASL09/14 new safflower breeding line can be used for evaluation under drought stress, because of its high WUE, RWC, SLW, biomass and  $\delta^{13}\text{C}$  against drought stress. Plants manifest a wide range of behaviors varying in response to drought stress. These results indicated that the observed variety of physiological and biochemical responses at cellular and whole-organism levels such as inducing the many biomass traits,  $\delta^{13}\text{C}$ , WUE, RWC and high SLW were triggered directly by the imposed drought stress treatment, thus making it a complex phenomenon. Hence, it is important to make comparisons among all the safflower genotypes against drought stress conditions using bilateral and multilateral relations of traits for further breeding selection tests. Therefore, understanding the biological processes involved in the response of plants to drought is very useful for further study.

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