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Research Article

PHYSIOLOGICAL RESPONSE OF SUNFLOWER (*HELIANTHUS ANNUUS* L.) TO METHANOL FOLIAR APPLICATION UNDER DIFFERENT REGIMES OF IRRIGATION

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ABSTRACT
In order to investigate the effect of water deficit stress and methanol foliar application on yield and yield components of sunflower (*Helianthus annuus* L), an experiment was conducted in the split plot form based on Completely Randomized Block Design with three replications during growing seasons of 2011-2012. Treatments were water deficit stress in four levels: a_1: severe stress (25% FC irrigation), a_2: mild stress (50% FC irrigation), a_3: fair stress (75% FC irrigation) and a_4: normal irrigation (100% FC irrigation) and the foliar application of methanol in six levels [b_1:0, b_2:7, b_3:14, b_4:21, b_5:28 and b_6:35 (v/v)]. The analysis of variance showed significant effect of interaction between water deficit stress and methanol foliar application on chlorophyll content, stomatal resistance, number of stomata and grain size (grain length, grain width).The results showed that foliar application with 14% (v/v) methanol and normal irrigation produced 37% more chlorophyll content, 16% more number of stomata, 26% more grain length and 23% more grain width. Besides, the results also proved that 14% (v/v) methanol foliar application in 25% FC had the highest stomatal resistance.

Keywords: *Methanol, Sunflower, Water Deficit Stress*

INTRODUCTION
When plants do not receive sufficient water they are subjected to a stress called water deficit. Water deficit in the plant disrupts many cellular and whole plant functions, having a negative impact on plant growth and reproduction. Crop yields are reduced by 69% on average when plants are exposed to unfavourable conditions in the field (Bray, 2001). Water stress reduces photosynthesis via reduction in leaf area, closing stomata and reduction of carbon fixation efficiency (Abbasi *et al.*, 2014). When stomata are closed due to drought or high temperature, the available CO_2 in intercellular space (Ci) would be reduced, leading to reduced electron transport capacity and restricted assimilation potential plant (Nonomura and Benson, 1992).

On the other hand, stomata closure will result in evaluated temperatures of leaf and plant, limiting light reaction of photosynthesis (Paknejad *et al.*, 2009).

The photosynthesis permanence and maintenance of chlorophyll concentrations under stress conditions can be considered among other physiological indices of drought tolerance. Total chlorophyll concentration is reduced under drought stress (Khalilvand and Yarnia, 2013) with more retention of chlorophyll content under drought, more stability in photosynthesis (Castrillo and Trujillo, 1994).

Many Researches have done in recent years on using some compounds such as methanol, ethanol, bothanol, propanol and some amino acids like as glycine, aspartat and glutamate, to improve yields of, especially, C_3 crops (Paknejad *et al.*, 2009).

It has been shown that methanol foliar application on some crops, especially, C_3 crops, caused an increase of chlorophyll concentration in their leaves (Ramirez *et al.*, 2006). Increased turgidity of the leaves could lead to a decrease in stomatal resistance for both H_2O and CO_2 and, therefore, to an increase in CO_2 fixation (Van *et al.*, 1995).

Methanol has also been shown to have effects on stomatal resistance by its effect on removing leaf resin (Meinzier *et al.*, 1990).

Thus, the objectives of this study were to investigate the effects of methanol foliar application on Physiological response of sunflower under different regimes of irrigation

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MATERIALS AND METHODS

Field Experiment

The field experiment was carried out in split plot form by Completely Randomized Block Design with three replicates at the Research Station of the Islamic Azad University, Tabriz Branch, north-western Iran, during the 2011 - 2012. The sunflower cultivar used was Recoord (a Romanian open-pollinated cultivar that is widely planted in Iran). The first factor was water deficit stress in four levels: a_1: severe stress (25% FC irrigation), a_2: mild stress (50% FC irrigation), a_3: fair stress (75% FC irrigation) and a_4: normal irrigation (100% FC irrigation). The second factor was the foliar application of methanol in six levels [b_1:0, b_2:7, b_3:14, b_4:21, b_5:28 and b_6:35 volumetric percentage (v/v)] that to prevent of methanol poisoning at light presence, 1 g lit^{-1} Glycine and 1 mg lit^{-1} Tetrahydrofolate (THF) were added to prepared solution (bayat *et al.*, 2012). In all treatments, methanol spray was applied 4 times during stages of sunflower development contain: V-8 (determined by counting the number of true leaves at least 4 cm in length), R-4 (The inflorescence begins to open), R-6 (Flowering is complete and the ray flowers are wilting) and R-7 (The back of the head has started to turn a pale yellow colour). Flooding irrigation was conducted and all of treatments were irrigated completely prior to R-4 stage. Each plot consists of 5 rows, 60 cm row spacing and 20 cm plant interval. There were 2-5 seeds beside each other and they were thinned at three leaves stage to obtain plant density of 8 plants per m^2.

Chlorophyll Content

Minolta Chlorophyll Meter (SPAD-502 Plus) used to measure chlorophyll content in leaf.

Stomatal Resistance

Prometer (Delta-T Devices Cambridge-UK) used to measure stomatal resistance in leaf.

Number of Stomata

Leaf prints were prepared from the adaxial surface of intact leaves according to the method described by Hilu and Randall (1984). Stomata were counted using a light microscope in three areas between the leaf midrib and the blade margin. The average of these three areas was considered as one replicate for each leaf.

Statistical Analysis

In order to check the normality of data, analysis of variance, and mean comparison MSTAT-C software were used. The means of the treatments were compared using the least significant difference (LSD) test at $P<0.05$.

RESULTS AND DISICUSSION

The analysis of variance showed significant effect of interaction between water deficit stress and methanol foliar application on chlorophyll content, stomatal resistance, number of stomata, grain size (grain length and grain width), (table 1).

Table 1: The analysis of variance of measured traits in experiment

S.O.V	df	Chlorophyll content	Stomatal resistance	Number of stomata	Grain length	Grain width
Rep	2	40567ns	0/017 *	0/208*	3/588**	0/161ns
WDS	3	581251**	0/706 **	1/738**	2/702**	2/596**
Error	6	42165	0/001	0/025	0/052	0/243
MFA	5	120779*	0/656**	5/176**	3/639*	9/476**
MFA×WDS	15	109082**	0/243**	0/981**	3/363**	3/414**
Error	40	42031	0/002	0/025	1/069	16/255
CV		27/41	2/6	0/89	8/16	5/83

** and ** significant at 5% &1% respectively, WDS: Water Deficit Stress, FA: Foliar application*

Chlorophyll Content

Based on the results, the highest Chl. content was related to 14% [v/v] methanol foliar application in 100% FC (52/82). The mean comparison of data showed that in 25% FC irrigation, foliar application with 0% [v/v] methanol had the lowest Chl. Content (33/24), (Table 2). Reduced chlorophyll concentrations in response to drought were also found in wheat (Paknejad *et al.*, 2007). It seems, in general, that chlorophyll concentration would be reduced certainly under severe and extended water stress, but it is possible to increase in moderate stress, showing dependency of chlorophyll concentration response to environmental conditions and genotype (Boyer *et al.*, 1987).

The observed reduction of chlorophyll in water stressed plants may be due to a reduction in the lamellar content of the light harvesting chlorophyll a/b protein. These results indicate that foliar applications of methanol can enhance chlorophyll and photosynthetic capacity for dry matter production, but higher methanol concentration can destroy chlorophyll content. Khalilvand and Yarnia (2013) and Ramirez *et al.*, (2006) stated that spraying methanol on water-deficit plants could increase chlorophyll content of their leaves, when treating well watered plants with methanol may slightly reduce their closing are among the first drought impacts on plants, which may reduce crop yield through disturbing photosynthesis processes (Paknejad *et al.*, 2009).

Stomatal Resistance

Table 2: Mean comparison of interaction between methanol foliar application and deferent regimes of irrigation

WDS	MFA	Chlorophyll content	Stomatal resistance(Sm$^{-2)}$	Number of stomata	Grain length (mm)	Grain width (mm)
25% FC	0	33/24	1/243	16/09	10/69	6/80
	7	33/28	1/323	17/52	12/25	6/05
	14	35/23	2/730	17/52	12/10	6/60
	21	38/42	2/428	18/62	11/63	6/775
	28	34/23	2/147	17/54	11/98	6/175
	35	34/34	1/750	18/13	11/40	6/05
50% FC	0	34/42	1/547	18/73	13/70	6/25
	7	35/13	1/533	17/84	13/63	7/00
	14	36/85	2/150	18/24	12/38	6/00
	21	39/52	1/813	16/96	12/00	6/50
	28	38/12	1/847	17/65	12/70	6/50
	35	37/59	1/643	18/33	12/80	6/00
75% FC	0	43/27	1/583	17/24	11/32	6/30
	7	44/13	1/710	17/54	14/50	7/60
	14	45/65	1/743	18/14	13/35	7/30
	21	44/22	1/623	19/22	13/10	6/49
	28	43/16	1/540	19/26	12/30	6/30
	35	40/15	1/412	18/53	11/50	6/32
100% FC	0	49/37	1/477	18/18	12/02	7/02
	7	50/14	1/460	17/37	13/81	7/02
	14	52/82	1/440	19/34	14/63	7/85
	21	49/14	1/537	17/55	13/88	7/50
	28	45/23	1/360	17/22	13/73	6/36
	35	40/72	1/327	16/63	12/60	6
LSD5%		0/2858	0/0738	0/2609	1/478	1/357

WDS: Water Deficit Stress, MFA: Methanol Foliar application

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Our results also showed that the highest stomatal resistance was related to 14 % [v/v] methanol in 25% FC (2/73 Sm^{-2}). In addition, 35 % [v/v] methanol in 100% FC had the lowest stomatal resistance (1/ 327 Sm^{-2}), (Table 2). Meinzier *et al.,* (1990) showed that methanol has effects on stomatal resistance by its effect on removing leaf resin.

Number of Stomata

Moreover, it is clear from the data shown in the Table 2 that the number of stomata per mm^2 at the abaxial leaf surface increased significantly after application of 14 % [v/v] methanol in 100% FC (19/34). Also, 0% [v/v] methanol foliar application in 25% FC irrigation had the lowest number of stomata (16/09), (Table 2). Ramadan and Omran (2005) showed that 30 % methanol increased significantly the number of stomata of developing leaves at the first application time (shoot length:20-30 cm) while 10, 30, 40 and 50 % methanol solutions were more effective at the second application date (pre-bloom).

Grain Size

Grain Length and Grain Width

The mean comparison of data showed that in 100% FC irrigation, foliar application with 14% [v/v] methanol had the highest grain length (14/63 mm) and grain width (10/69 mm),(Table 2). Furthermore, the lowest grain length (7/85 mm) and grain width (6 mm) related to 0 % and 35 % [v/v] methanol foliar application in 25% FC, respectively. Paknejad *et al.,* (2012) found that drought stress during seed development decreased yield shortens the grain-filling stage and lowers final seed size and in severe stress, the seeds are very wrinkled and ill-formed.

It seems foliar application of methanol on sunflower plants can reduce negative impacts of water deficit stress and improve plant potential to withstand prevailing harsh and dry climate in arid areas. Sunflower physiological characteristics are affected by methanol spraying and under water deficit stress, methanol application somewhat can reduce destructive effects of drought and prevent of yield loss. Based on the results, spraying of methanol with 14% (v/v) had positive effects on physiological characteristics.

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