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WATER STRESS EFFECT ON PHYSIOLOGICAL, MORPHOLOGICAL PARAMETERS AND THE YIELD OF FIVE SUNFLOWER CULTIVARS (*Helianthus annuus* L.) GROWN UNDER GREENHOUSE

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ABSTRACT

Sunflower (*Helianthus annuus* L.) is one of the world's major oilseeds species however, it faces the problem of summer drought which coincides with the end of its cycle inducing yield decrease. The aim of this study is to identify morpho-physiological traits that can be used as selection criteria in hybridization programs for stress tolerance. In this context, a greenhouse essay has been carried out using five oleic sunflower genotypes arranged in a randomized complete block design with two water regimes; water regime 2:100% of field capacity and water regime 1:30% of field capacity. The obtained results show that water stress affects many measured parameters such as relative water content, stomatal conductance, stem diameter and yield components (seed number per head and 100-seed weigh). Genotype effect was significant for stomatal conductance, number of leaves per plant and yield components. However, the effect of the genotype x water regime interaction was significant only for the number of leaves per plant, yield and seed number per head.

Keywords: Sunflower; *Helianthus annuus* L; drought; yield; greenhouse.

INTRODUCTION

Sunflower is one of the most important oilseed species whose production and global yield oscillate around 27 million tones and 12q/ha respectively. It is simple to manage, flexible and vigorous with low inputs, rich in oil and drought resistant [1,2]. Sunflower oil is considered a premium oil due to its pale color, high content of unsaturated fatty acids, lack of linolenic acid and trans fatty acids, neutral flavor, high resistance to oxidation, high smoke point and high diversity of fatty acid composition [1]. In addition, sunflower cropping offers a wide range of options for adaptation [3]. The importance of hybrid cultivars in sunflower has recently increased because they are more stable, very self-fertile with higher yields and more uniform at maturity [4].

However, during the summer period particularly at the end of flowering period and the beginning of grain filling stage, sunflower is confronted to high evaporative demand for the atmosphere and low availability of water in soil in dry countries which can disrupts the water balance causing water deficit which is one of the most common environmental constraints [5]. According to Flagella et al. [6], the floral button stage (R1) and other flowering stages are critical for water stress in sunflower. Water deficit affects the water relations of the plant, including relative water content and turgor. It also induces stomatal closure which limits gas exchange mainly carbon atmospheric assimilation and reduces transpiration. Thus, drought stress has negative effects on mineral nutrition in particular nutrients absorption and transport [7] which can penalize growth and photosynthesis, the main physiological functions involved in yield elaboration [5].

Drought is the most important crop production limiting factor in the changing climate scenario worldwide and in Algeria, its intensity is predicted to increase in future [8]. Because of its complexity, stress tolerance is may be the most difficult trait to be improved through conventional plant improvement. The development of stress tolerant cultivars is actually a major challenge. In this context, The present paper study the response of 5 sunflower genotypes to water stress. The objective is to identify morpho-physiological traits that can be used as selection criteria in hybridization programs for stress tolerance and that can be measured quickly.

MATERIALS AND METHODS

Plant Material

Five oleic sunflower genotypes (commercial hybrid) were used in our experiment; four of them were developed by Syngenta France in particular (Nutrasol: V4, N.K Ferti: V3, Extrasol: V5, Aurasol: V2) and one local genotype (V1).

Essay Conduct and Experimental Design

Seeds of the selected varieties were germinated in a stove at 25°C for 48 hours then they were planted manually at a depth of 2-3 cm in PVC cylinders (11 cm diameter and 60 cm height) containing a substrate made up of 40% soil, 40% compost and 20% sand as described by [16,30]. This substrate is previously sieved, air-dried and disinfected. Plants were cultured in a glass house (temperature of 25 ± 2° and 60 ± 5% relative humidity) in randomized complete block design with two water regimes; water regime 2 (WR2): 100% of field capacity and water regime 1 (WR1): 30% of field capacity. Each water regime includes three repetitions where each repetition covers the five genotypes and each genotype is represented by five plants. Plants were irrigated to the field capacity every two days from sowing to flower bud formation stage (R1), at this stage the water deficit was applied on half of plants “stressed plants”. The other plants received enough water to maintain the soil at field capacity “well irrigated plants”. In order to stimulate natural water stress similar to that of the field, we reduced progressively the irrigation from 100% to 30% of field capacity during 15 days then the stressed plants were maintained irrigated to 30% of field capacity until harvest. In both water regimes; cylinders have been weighed and water loss was carefully replaced.

Measurement Stage and Studied Parameters

Physiological traits

Physiological measurements were carried out on the youngest and fully developed leaf and were focused mainly on:

Relative water content (RWC)

Measured following Barrs method [9]; leaves were weighed directly (FW, fresh weight) then immersed in test tubes filled with distilled water. The tubes were placed in dark and cool place during 24 hours and the saturated leaves weigh was recorded

(TW, turgid weight). Finally, the sample is dried at 85°C stove and weighed after 48 hours (DW, dry weight). The RWC is evaluated following the formula:

RWC (%) = (FW-DW) / (TW-DW) * 100.

Chlorophyll content

Determined according to Linchtenthaler and Welburn [10]. 100 mg of leaves were crushed in 8 ml of diluted acetone (80%). The crushed material was filtered in a test tube then the tube volume was completed to 10 ml by adding diluted acetone (80%). The absorbances were read with a spectrophotometer at 645 nm, 663 nm wavelengths. Chlorophyll content determination was realized according to the formula:

Chl t (mg.g-1 FW) = 17,32 x DO645 + 7,18 x DO663.

Stomatal conductance

Measured by an AP4 DELATA-T DEVICES CAMBRIDGE UK automatic porometer.

Morphological parameters

Morphological measurements concerned leaves number per plant, plant height (cm), stem diameter (mm) and head diameter (cm). Physiological and morphological measurements were carried out for both well irrigated and stressed plants at the end of flowering stage. Counting of seed number per head, weighing of 100 seed (g) and seed yield per plant estimation (g/plant) were carried out at the harvest.

Statistical Analysis

Two-factorial ANOVA was carried out to determine the effect of water regime on the physiological and morphological parameters and yield components using SPSS statistical program (version 16).

RESULTS

Physiological Parameters

Analysis of variance reveals a significant effect of water regime on relative water content (RWC). However, the difference between genotypes and genotype x water regime interaction was not significant (Table 1). Relative water content decreases with increased water stress (Fig. 1a). In addition, water regime, genotype and the interaction between these two factors do not have a significant effect on chlorophyll content (Table 1).

The results illustrated in Table 1 show that genotype and water regime has a significant influence on stomatal conductance whereas, genotype x water regime interaction has not revealed any significant differences. The most important value of stomatal conductance is recorded in genotype 5 (20.12 $mM.m^{-2}.s^{-1}$) in stressed plants while the lowest value is reported in genotype 3 (2.83 $mM.m^{-2}.s^{-1}$) in well irrigated plants (Fig. 1c).

Morphological parameters

Our results show that water regime has a significant effect on stem diameter, on head weight and diameter (Table 1). Stem diameter of stressed plants is lower than that of unstressed plants with values ranging between 11.3 and 14.1 mm for WR2, 11.3 and 12.0 mm for WR1 (Fig. 2d). Thus, head weight and diameter have experienced a remarkable reduction under water stressed conditions (Fig. 2e and f).

Table 1. Results of variance analysis of morphological traits, seed yield and yield components of sunflower genotypes under two water regimes in greenhouse conditions

SV	RWC	CHT	SC	NLP	HP	SD	HD	HW	NSH	100SW	SY
Gen	1,015$^{n.s}$	0,173$^{n.s}$	6,913***	2,773*	1,611$^{n..s}$	2,715*	24,84***	2,29$^{n.s}$	30,009***	6,845***	5,427***
WR	12,718***	0,093$^{n.s}$	17,781***	1,943$^{n.s}$	0,549$^{n.s}$	3,749*	53,243***	55,171***	186,142***	14,718***	173,441***
interaction	2,429$^{n.s}$	0,613$^{n.s}$	0,903$^{n.s}$	2,565*	1,36$^{n.s}$	4,642**	3,681**	4,518**	5,443***	0,103$^{n.s}$	3,641**

*SV: source of variation, RWC: relative water content, CHT: chlorophyll content, SC: stomatal conductance, NLP: number of leaves per plant, HP: plant height , SD: stem diameter, HD: head diameter, HW: head weight, NSH: number of seeds per head, 100SW: weight of 100 seeds, SY: seed yield per plant, Gen: genotype, WR: water regime, n.s: not significant, *: significant, **: highly significant, ***: very highly significant*

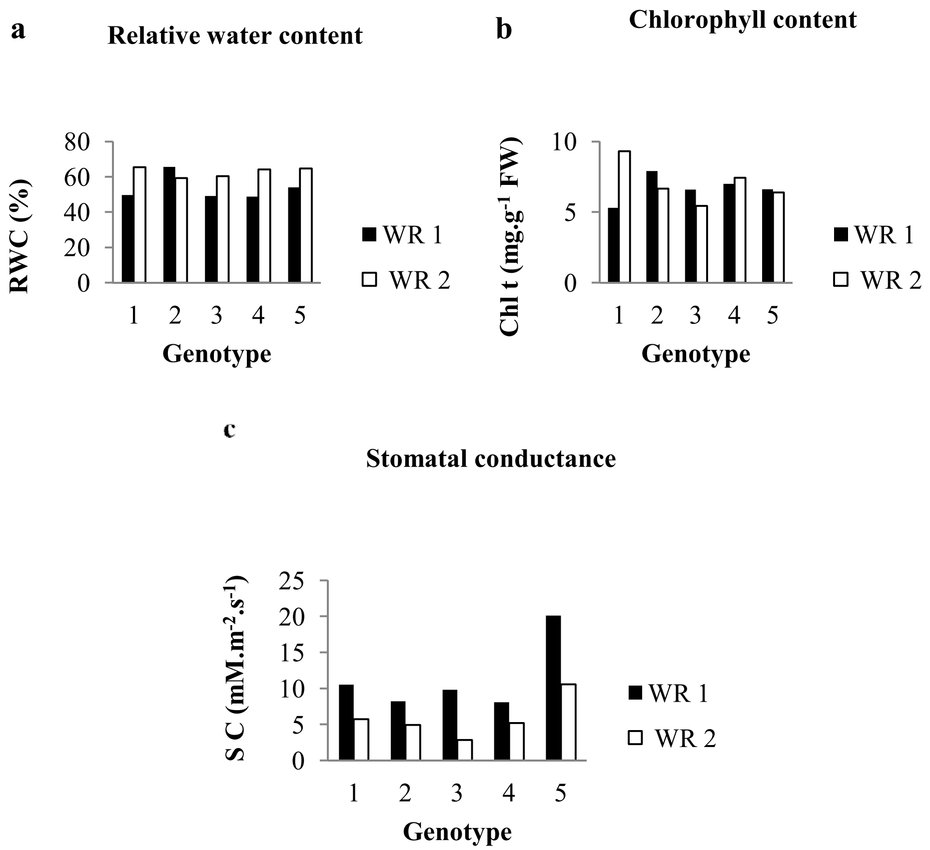
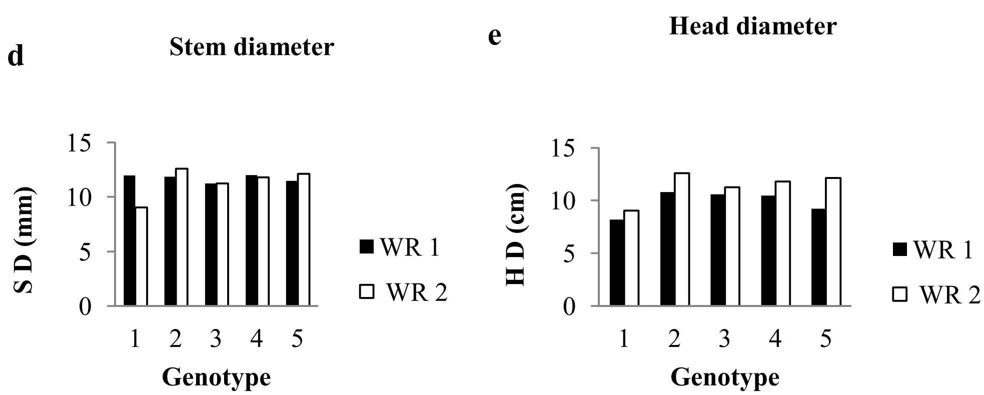


Fig. 1. histogram of the effect of water regime and genotype on physiological parameters of sunflower (*Helianthus annuus* L.): a relative water content (%), b chlorophyll content (mg.g^{-1} FW), c stomatal conductance (mM.m^{-2}.s^{-1})



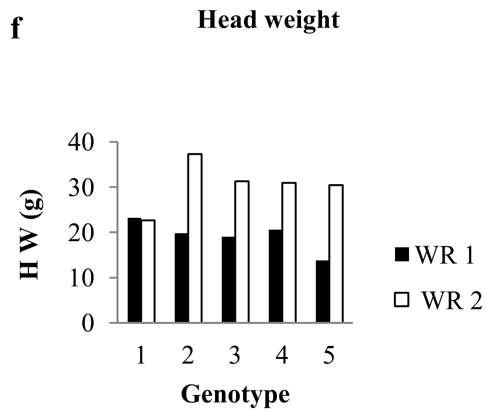


Fig. 2 histogram of the effect of water regime and genotype on some morphological parameters of sunflower ((*Helianthus annuus* L): d stem diameter (mm), e head diameter (cm), f head weight (g)

Plant height and leaves number per plant were not influenced by water regime. The deference between genotypes was significant for number of leaves per plant, stem diameter and head diameter. The interaction between the two factors genotype x water regime had a significant effect on the number of leaves per plant, stem diameter, head diameter and head weight (Table 1).

Seed yield and Yield Components

The difference between the two water treatments WR1 and WR2 is highly significant for yield and yield components (number of seeds per head and 100 seed weight) for all genotypes (Table 1). The interaction genotype x water treatment is also responsible for variation in yield and number of seeds per head (Table 1).

The number of seeds per head varied between 154 and 295 in both genotypes 1 and 3 in WR1, in WR2 it varied between 217 and 537 in the same genotypes (Fig. 3g). In addition, 100 seeds weight ranged between 2.54 and 5 g in genotypes 4 and 1 in WR1 and between 3.61 and 5.3g for the same genotypes in WR2 (Fig. 3h). Water regime 2 produces the highest yield with a value of 18.9 g/plant recorded in genotype 3 while the lowest yield is produced by water regime 1 with a value of 5.98 g/plant recorded in genotype 4 (Fig. 3k).

DISCUSSION

Water stress is one of the leading causes of crop loss worldwide [11] affecting plant establishment, growth and development by disturbing various physiological, biochemical and morphological processes [12].

Relative water content (RWC) is the most trait commonly used to evaluate water status of plants [13]. The Different levels of water stress reduce RWC leading to a progressive and significant reduction in stomatal conductance and photosynthesis [13]. In our experiment, water stress reduces RWC which is in agreement with the results found by Andrade et al, Darvishzadeh et al. Poormohammad Kiani et al. [14,15,16].

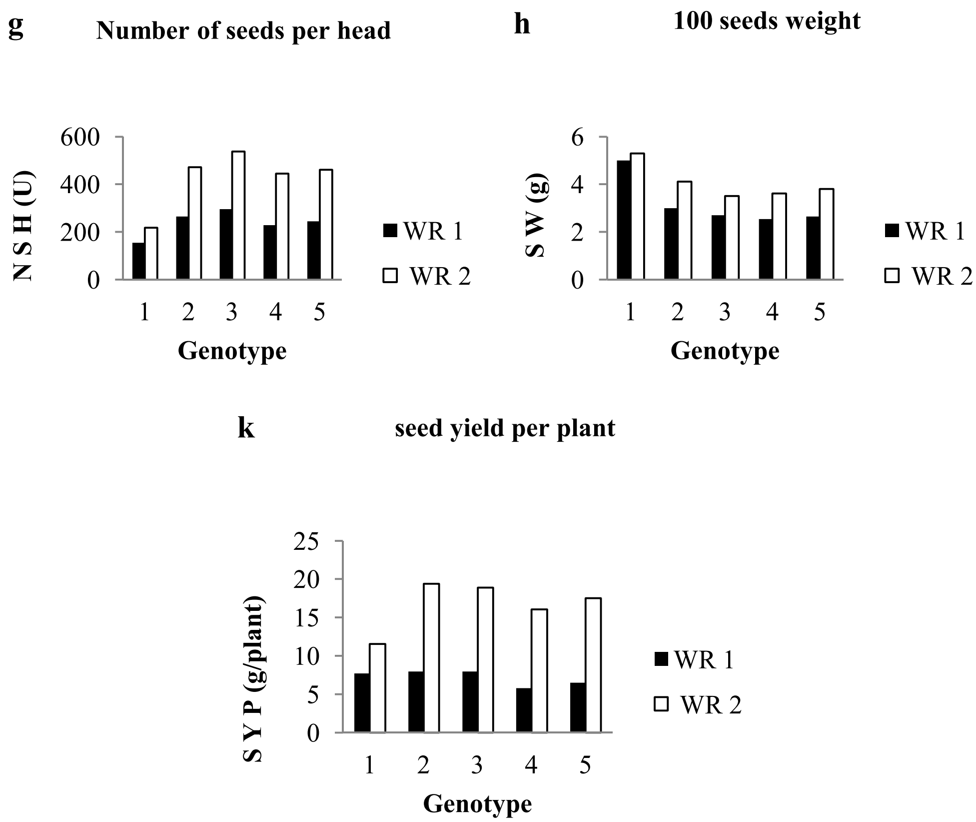


Fig. 3. Histogram of the effect of water regime and genotype on seed yield and yield components of sunflower (*Helianthus annuus* L.): g number of seeds per head, h 100 seeds weight (g), k seed yield per plant (g/plant)

Also, in our experiment we found that the difference in chlorophyll content between both water regimes was not significant. This result is confirmed by Nezami et al. [17]. However, Andrade et al. [14] found that severe water stress conditions induce significant reduction in chlorophyll content in sunflower. In addition; according to Manivannan et al. [18] drought stress lowered the total chlorophyll content significantly.

Stomatal conductance measurement informs us about the degree of stomata closure [19] which is one of the early responses to water deficit [20]. Its purpose is to protect the plant against dehydration but at the same time causes a decrease in carbon assimilation which disrupts photosynthesis [21]. Our results are consistent with the results of Moraes et al. [22] and Maury et al. [23] who found that stomatal conductance was negatively affected by water stress.

According to Andrade et al. [14], morphological traits analysis is useful for studying plant adaptation to environmental stress such as water deficit. Unlike the results found in most experiments where water deficit induced a reduction in plant height, obtaining similar heights of stressed and well-irrigated plants can be explained by the growing conditions under a greenhouse that is characterized by a short height which prevent the development of stems in length masking the water stress effect. Our results are contrary to the result of Manivannan et al. [18].

According to Hall [24], leaves number is a trait fixed genetically and slightly affected by environmental factors. According to our results, leaves number has not changed between stressed and unstressed plants which are consistent with the results of Darvishzadeh et al. Poormohammad Kiani et al. [15,25]. However, it has varied from genotype to another which is confirmed by the same authors. The interaction between the two factors; genotype and water regime were significant which is confirmed by [15].

According to Thakur and Rai [26], water deficit causes a delay in plant growth resulting a reduction in plant height and stem diameter. In this context, our results are confirmed by those of Nezami et al. Poormohammad Kiani et al. [17,25].

Based on our experiment, water regime, genotype and interaction of these two factors have negatively influenced the head diameter and weight which is in agreement with the results of Darvishzadeh et al, Nezami et al. and Poormohammad Kiani et al. [15,17,25].

The processes involved in crop yield elaboration are influenced by two types of factors; genetic factors (intrinsic to the plant) and environmental factors. Genotype-environment interactions play also an important role. Among the morphological characteristics involved in yield elaboration, we cite biomass production and yield components [27]. According to Fahad et al. [28]; significant yield losses have been reported in major field crops due to drought stress. In addition; the problem of low yields which due to water stress in sunflower was also set off by different authors in particular [8, 29,30,31].

The reduction in seed number per head due to water stress observed during our experiment can be explained on one hand by the decrease of carbon amount transferred to the head and on the other hand by the direct effect of low water potential on ovarian metabolism and the modifications in hormonal balance of flowers [32]. This result is confirmed by Flagella et al, Nezami et al, Poormohammad Kiani et al and Buriro et al. [6,17,30,33].

The reduction in weight of seeds under water stress conditions is confirmed by Pekcan et al, Flenet et al, Chimenti et al and Elsheikh et al. [31,34,35,36]. Based on our results, the yield is influenced by genotype and genotype x water regime interaction, which is affirmed by the works of Darvishzadeh et al, Poormohammad Kiani et al. [15,25].

CONCLUSION

Our results indicate that the application of severe water stress on a greenhouse sunflower culture at the reproductive stage induces a very highly significant effect on some physiological parameters including relative water content and stomatal conductance. By the same, the effect is significant on morphological parameters such as stem diameter, head weight and head diameter. Water stress also implies a lowering of yield components. According to our results, genotypic effect was remarkable for stomatal conductance and yield components. Genotype x water regime interaction was significant only for yield and seed number per head.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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