



## Influence of water stress on some biochemical markers pigment contents and osmotic adjustment of durum wheat leaves induced by PEG 6000

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### Abstract

Wheat constitutes a base element in human nutrition around the world. The understanding of the mechanisms adopted by plants in constraining conditions is essential for improving wheat tolerance to abiotic stress. The objective of this investigation is to study the effect of drought imposed by Polyethylene glycol 6000 on two durum wheat varieties: Oued Znati and Guemgoum Rkhem, growing in hydroponic condition and conducted under two water regimes. Various parameters are performed such as: relative water content, osmotic adjustment, chlorophyll content (a), (b) and soluble sugar assay. The results showed that the two studied varieties differed significantly in their responses to water deficit and that stress treatment caused a decrease in the leaf relative water content, the chlorophyll content (a), as well as the chlorophyll content (b). However, we observed an increase in osmotic adjustment and soluble sugar content. As a result, both varieties follow the same tolerance or avoidance strategies for water stress, but to different degrees. From the data it was obvious that the Oued Znati variety was the most drought tolerant genotype having significantly higher osmotic adjustment, sugar accumulation, and relative water content while lower chlorophyll b content under water stress conditions. It resulted that this variety is considered a tolerant variety and can be used in further research for genetic characterization of this traits and durum wheat breeding programs.

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## Introduction

In Algeria, cereal products occupy a strategic place in the food system and in the national economy (Djermoun, 2009). Durum wheat is one of the most important crops, its national production does not respond to the needs of population, which ranks Algeria as one of the most important grain importing countries in the world (FAO, 2015). This is mainly due to climate change, desertification, salinity and drought (Abeledo *et al.*, 2008; Rao *et al.*, 2013; Rapparini and Peñuelas, 2014). Drought stress is one of the main causes of crop loss in the world and in Algeria, reducing average yields for most major crops by more than 50%, including durum wheat (Wang *et al.*, 2003; Zhengetal.,2010). Plants tolerant to drought can be acquired by applying polyethylene-glycol (PEG) or mannitol (Piwowarczyk *et al.*, 2014). In most of the cases PEG is used to induce water stress. Water stress causes fundamental problems for metabolic processes in plant cells, induces growth inhibition and reduces yield. The main effects of water deficit in cells are: reduction of water potential, increasing concentrations of compounds, osmotic adjustment, and changes in structure and conformation of macromolecules (Yadav and Srivastava, 2017). To improve durum varieties for production and yield stability, several strategies have been adopted to improve production and ensure yield stability through the creation of new varieties that are more adapted and more tolerant of unfavorable conditions (Ykhlef and Djekoun, 2000). Among these strategies, the study of the physiology and biochemistry plant mechanisms, the monitoring of the accumulation of soluble sugars and the mechanism of osmotic adjustment under water stress conditions is of great interest in the selection and improvement of durum wheat (Bartels and Salamini, 2001). Relative water content was introduced as a best criterion for plant water status, Schonfeld *et al.* (1988) showed that wheat cultivars having high RWC are more resistant against drought stress. This parameter can be used to select high yielding under water stress (Ykhlef and Djekoun, 2000). The chlorophyll content in plants is considered a favorable aspect for the plants growth. The reduction or non-

modification of the chlorophyll content of the plant under Polyethylene glycol (PEG 6000) stress condition has been observed in different plant species and its intensity depends on the intensity of the stress and its duration (Jagtap *et al.*, 1998; Moaveni, 2011), which reduces photosynthetic rate, which later effects chlorophyll-*a* and chlorophyll-*b* contents. Also osmotic adjustment is generally considered as an important element in plant tolerance to water stress (Bajji *et al.*, 2001). Plants are able to modulate their development by promoting the synthesis of osmolytes that increase the osmotic pressure and thus retain the water inside the cells (Wang *et al.*, 2003). Among its osmolytes, sugars could contribute more to the osmotic adjustment of plants subjected to stress conditions (Zhou and Yu, 2009). The maintenance of turgescence allows the plant to maintain its physiological functions despite a deterioration of the water state of the tissues. At the cellular level, several processes are involved in maintaining turgescence at low water potential of the plant including osmotic adjustment (Maury *et al.*, 2011). Therefore, the purpose of this research is to evaluate the effect of drought induced by PEG 6000 on two durum wheat varieties by physiological and biochemical indices. The selected drought tolerant genotype will then be used for crop improvement programs.

## Material and methods

### *Plant material*

This work was carried out on two varieties of durum wheat (*Triticum durum* Desf) Oued Znati and Guemgoum Rkhem, the seeds were provided by the Technical Institute of Great Culture el khroub Constantine (TIGC). After pre-germination, the seedlings are transferred to a *Broughton and Dillworth* nutrient medium. The seedling development continues in a culture chamber under a photoperiod of 16 h / 8h and at a temperature of 25° C.

### *Application of osmotic stress*

Two treatments were applied: Control treatment (C) with a nutritive water alimentation and a stressed treatment (S) by the addition of PEG 6000 at an

osmotic pressure equal to - 1 MPa at the fourth leaf stage for 72 hours. Sample collection for measurements was done on the well-developed third leaf.

### Measurements

#### Relative water content (RWC %)

The relative water contents of the seedlings are determined by the calculation of fresh weight (FW) of each sample before drying in the oven at 80 °C for 48 hours. The dry weight is then determined (DW). The average water content is calculated by the following formula (Clarke and McCaig, 1982):

$$\text{RWC (\%)} = (\text{FW} - \text{DW}) \times 100 / (\text{TW} - \text{DW})$$

TW is turger weight of leaf samples after 24 hours in distilled water.

#### Osmotic adjustment (OA MPa)

The osmotic adjustment (OA) is calculated according to Ludlow *et al.*, (1983) as the maximum turgescence osmotic potential difference (PI100) between control (PI100<sup>c</sup>) and stressed (PI100<sup>s</sup>) plants. The osmotic potential at maximum turgescence is calculated according to Wilson *et al.*,(1979):

$$\text{OA (MPa)} = \text{PI100}^c - \text{PI100}^s; \text{PI100} = \text{OP} \times (\text{RWC} - \text{B}) / (100 - \text{B})$$

Where B is the apoplastic dilution which is in the wheat of the order of 15% (Gaudillère and Barcelo, 1990) and osmotic potential (OP) was measured using Osmometer (Wescor, Logan, UT).

#### Pigments content ( $\mu\text{g} / 100\text{mg FW}$ )

The pigments content are determined according to the method of Lichtenthaler (1987) and Shabala *et al.* (1998). The concentrations of chlorophyll (a) and chlorophyll (b) are calculated by the following formulas:

$$\text{Chl (a)} = 9.784 * \text{OD (662)} - 0.99 * \text{OD (644)}$$

$$\text{Chl (b)} = 21,42 * \text{OD (644)} - 4,65 * \text{OD (662)} ; \text{OD: optical density.}$$

#### Determination of soluble sugars ( $\mu\text{g} / 100\text{mg FW}$ )

The total soluble sugars are determined by the phenol method of Dubois *et al.*, (1956). Absorbance measurements are made at a wavelength of 485 nm. The concentrations are determined from a standard curve and expressed as  $\mu\text{g} / 100\text{mg}$  fresh weight.

#### Statistical analysis

The data were statistically analyzed using XL stat 2014. The analysis of variance (ANOVA) and the correlation study were performed using three replicates per treatment for all studied parameters.

## Results and discussion

### Relative water content

Our results show that at the control level, the two studied varieties have the best RWC values that vary from (90,83  $\pm$  1,19%) % in the OZ variety and (88,97  $\pm$  1,57) % in the GGR variety (Table.1). Under the effect of the water deficit, the two varieties show RWC values close to each other of the order of (76,35  $\pm$  2,00) to (66,45  $\pm$  5,55)% recorded successively in the varieties OZ and GGR (Table.1).

**Table 1.** The measurements of different parameters of two varieties of durum wheat under water stress represented in the averages and the standards deviations (SD) of at least three independent replicates.

Varieties	Averages $\pm$ standard deviation			
	Oued Znati		Guemgoum Rkhem	
Parameters Treatment	-PEG	+PEG	-PEG	+PEG
Relative water content(%)	90,83 $\pm$ 1,19	76,35 $\pm$ 2,00	88,97 $\pm$ 1,57	66,45 $\pm$ 5,55
Chlorophyll a content ( $\mu\text{g}/100 \text{ mg FW}$ )	61,16 $\pm$ 9,57	36,53 $\pm$ 6,83	64,55 $\pm$ 7,90	18,37 $\pm$ 1,14
Chlorophyll b content ( $\mu\text{g}/100 \text{ mg FW}$ )	19,74 $\pm$ 2,99	11,15 $\pm$ 2,56	15,73 $\pm$ 3,09	4,88 $\pm$ 0,37
Sugars content ( $\mu\text{g}/100 \text{ mg FW}$ )	35,22 $\pm$ 4,26	63,81 $\pm$ 4,73	30,16 $\pm$ 6,74	89,88 $\pm$ 4,01
Osmotic adjustment (MPa)	/	0,37 $\pm$ 0,06	/	0,63 $\pm$ 0,05

+PEG : water stress (-1MPa) ; -PEG : without water stress (Control).

There is a significant decrease in this parameter under stress conditions with -25,31% in the GGR variety and -15,93% in the OZ variety (Fig. 1a). This decrease is negatively correlated with stress ( $r = 0,97$ ).

The variance analysis of the RWC revealed a very highly significant difference for the water stress effect and a significant difference for the varieties effect (Table. 2).

**Table 2.** Variance Analysis for relative water content (RWC), chlorophyll a and b content (chl a, chlb) under control and water stress conditions.

Source	Parameters					
	RWC		Chl a		Chl b	
Treatment	F	Pr	F	Pr	F	Pr
Water conditions (W)	105,96	< 0,0001	74,37	< 0,0001	44,89	0,000
Varieties (V)	10,71	0,011	3,24	0,110	12,53	0,008
Interaction (VxW)	05,02	0,055	6,89	0,030	0,61	0,457

chl a :chlorophyll a content; chlb :chlorophyll b content; F of Fisher; P : probability ; ( $P > 0.05$ ) no significant; ( $P < 0.05$ ) significant; ( $P < 0.01$ ) highly significant; ( $P < 0.0001$ ) very highly significant.

According to the results obtained, it is found that the water stress causes a decrease of the RWC in the two genotypes studied, this decrease does not exceed 50% because of the low intensity and the short duration of the stress applied. According to Ykhlef *et al.*, (1998) during low intensity stress, some wheat varieties have the ability to save a relatively large amount of water in their leaves. This is explained by a partial closure of the stomata as soon as the water deficit appears (Ykhlef and Djekoun, 2000). Keyvan (2010) show that, increasing the intensity of water stress on

cultivated wheat varieties causes a decrease in water, which confirms our results. These same results have been demonstrated in beans by Korir *et al.* (2006). Much work has been done on the variation of the relative water content of leaves of different species grown under water stress, their conclusions confirm that RWC is affected by water stress and decreases (Nayyar and Gupta, 2006). According to Geravandia *et al.* (2011) plant species that maintain high relative leaf-water levels are considered to be drought-tolerant species.

**Table 3.** Variance Analysis for leaf sugars content and osmotic adjustment (OA) under control and water stress conditions.

Source	Parameters			
	sugars		OA	
Treatment	F	Pr	F	Pr
Water conditions (W)	228,87	< 0,0001	/	/
Varieties (V)	12,95	0,007	33,947	0,004
Interaction (VxW)	28,43	0,001	/	/

F of Fisher; P : probability ; ( $P > 0.05$ ) no significant; ( $P < 0.05$ ) significant; ( $P < 0.01$ ) highly significant; ( $P < 0.0001$ ) very highly significant.

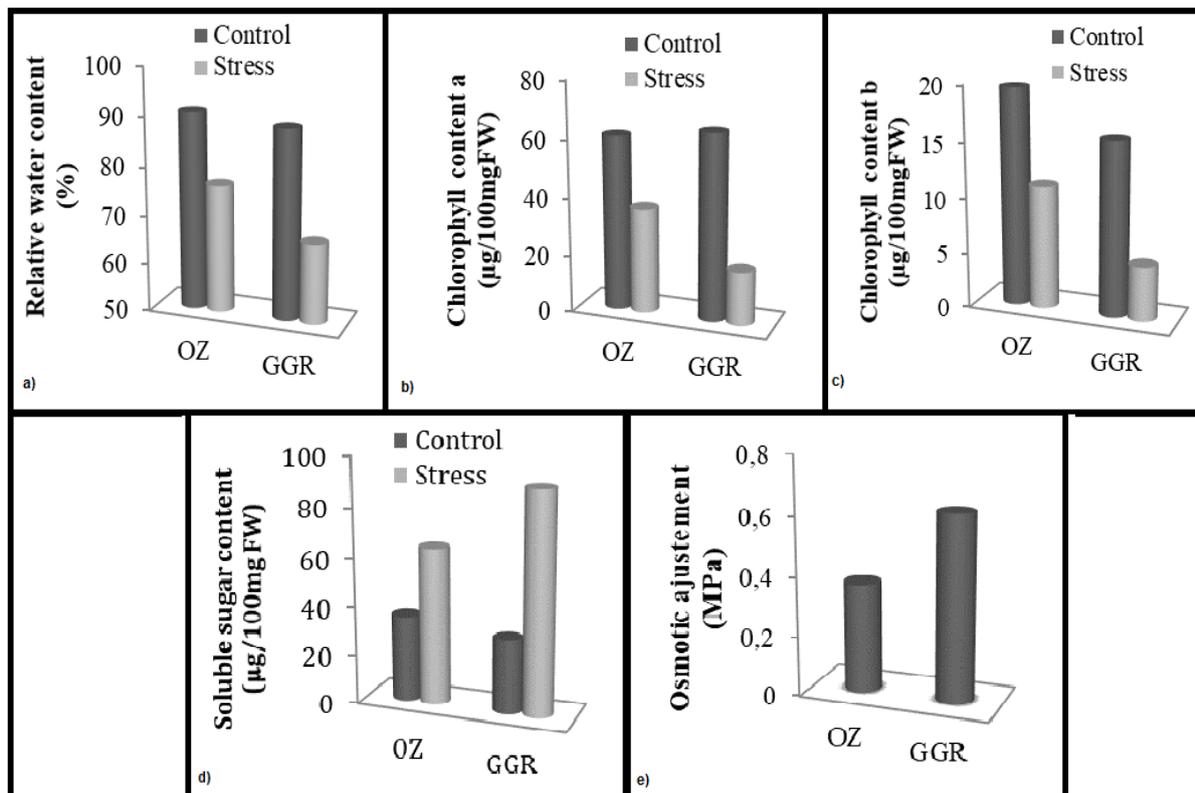
#### Osmotic adjustment

From our results, we observe that there is a large variation in the osmotic adjustment between the two varieties studied, the addition of PEG6000 (-1MPa) increases the osmotic adjustment values (Fig.1e).

Osmotic adjustment are positively correlated with stress condition ( $r = 0,94$ ). The GGR variety represents the highest value of the osmotic adjustment with a mean of ( $0,63 \pm 0,04$ ) MPa, while the OZ variety rank the lowest value with an average

of  $(0,36\pm 0,06)$  MPa (Table 1). Analysis of variance reveals the existence of a highly significant variety effect (Table 3). These results are confirmed by Ashraf and Foolad (2007) which show that the decrease in osmotic potential is one of the pronounced responses to water stress in many plants. The same results

obtained by other researchers, Fu *et al.* (2010) indicate that, the osmotic potential decreases under drought conditions. This decrease is due to the effect of dehydration, as the lowest water content coincides with the lowest osmotic potential (Ogawa and Yamauchi, 2006).



**Fig. 1.** Variation of studied parameters: relative water content (a), chlorophyll a (b), chlorophyll b (c), soluble sugars content (d) and osmotic adjustment (e) of leaves of two *durum wheat* varieties Oued Znati (OZ) and Guemgoum Rkhem (GGR) after three days of PEG treatment. The measurements represent the averages of at least three independent replicates.

This requires an increase in osmolyte levels in the cytoplasm either by synthesis of solutes (compatible with cellular metabolism) or by their uptake of the soil solution (Cixin He, 2005). Osmotic adjustment is recognized as an effective mechanism of tolerance to water stress, several advantages are conferred on it, the most important being the maintenance of cellular pressure (Chen and Gallie, 2004). Osmotic adjustment capacities are variable in the plant and depend on the variety (Maury *et al.*, 2011).

#### Pigments chlorophyll content

The same behavior is noted in both genotypes tested under both conditions. The pigments chlorophyll

content (a) and (b) were negatively correlated with the stress applied in the two varieties studied (Fig.1b, c). We note high chlorophyll content (a) in GGR variety of  $(64,55\pm 7,90)$   $\mu\text{g}/100$  mg FW in control condition (Table.1). On the other hand stress reduces the chlorophyll content (a) by -71,53%, in the same variety with a content of  $(18,37\pm 1,14)$   $\mu\text{g}/100$  mg FW (Table.1).

The same remark is noted with the OZ variety, there is a decrease of -40,26% in the chlorophyll content (a) under stress conditions, the chlorophyll content values (a) recorded are  $(61,16\pm 9,57)$  and  $(36,53\pm 6,83)$   $\mu\text{g}/100$  mg FW for the stress and control lot (Table.1).

The correlation coefficient is  $r = 0,96$ . ANOVA shows a very highly significant for water conditions and significant effect for interaction (varieties x water conditions) (Table.2).

There is a decrease in chlorophyll (b) recorded in GGR variety ranging from  $(15,73 \pm 3,09) \mu\text{g}/100 \text{ mg FW}$  to  $(4,88 \pm 0,37) \mu\text{g}/100 \text{ mg FW}$  (Table.1), this decrease is -46,76%. While, the OZ variety shows a slight decrease in chlorophyll content (b) under stress conditions with a -43,50 % level compared to the control (Fig.1c). Chlorophyll content values (b) ranged from  $(19,74 \pm 2,99) \mu\text{g}/100 \text{ mg FW}$  in the control group to  $(11,15 \pm 2,56) \mu\text{g}/100 \text{ mg FW}$  in the stress group (Table.1).

The correlation coefficient is  $r = 0,93$ . The results for ANOVA analysis are shown in Table 2, there is a very highly significant different in water conditions factor and varieties factor. These results confirm other results from other studies, where they mentioned that lack of water causes a drop in leaf chlorophyll content (Malik and Ashraf, 2012). Decreased chlorophyll content in stressed plants is reported by many authors as one of the main causes of productivity and growth reduction (Hikosaka *et al.*, 2006; Caglar *et al.*, 2011). According to Tambussi *et al.*, (2007) a decrease in chlorophyll levels disrupts the photosynthetic mechanism of the upper part of the leaves and affects the grain yield.

In the same context, the work of Marwood and Greenberg (1996) indicates that, the reduction of chlorophyll (b) is due to a more selective destruction of chlorophyll biosynthesis (b) or to the degradation of precursors of chlorophyll (b). Fotovat *et al.* (2007) found that by exerting severe drought stress on wheat, the leaf chlorophyll content (a) significantly decreased. Chlorophyll is very important in the process of photosynthesis (Silva *et al.*, 2007). The modification of the composition and the pigment contents would therefore be a character of adaptation to the environment. Khayatnezhad and Gholamin (2012) reported that, resistant cultivars have more chlorophyll.

#### *Soluble sugar content*

The second figure shows a significant increase in the soluble sugar content of the leaves of the two studied varieties under water stress condition. This increase is positively correlated with water stress with a correlation coefficient ( $r = 0,97$ ). In the control conditions, both varieties have low levels of soluble sugars compared to those recorded under stressed conditions, these values range from  $(35,2 \pm 24,26) \mu\text{g}/100 \text{ mg FW}$  in the OZ variety to  $(30,16 \pm 6,74) \mu\text{g}/100 \text{ mg FW}$  in the GGR variety (Fig. 1d). Under stress conditions, we observed an increase in the content of soluble sugars of 197,95% in the GGR variety and 81,17 in the OZ variety compared with their controls, the obtained values are spread between  $(89,88 \pm 4,01) \mu\text{g}/100 \text{ mg FW}$  (GGR) and  $(63,81 \pm 4,73) \mu\text{g}/100 \text{ mg FW}$  (OZ) (Table.1). Analysis of variance of soluble sugar content and shows that there is a very highly significant difference between the two conditions, the (variety x water conditions) interaction and a highly significant difference between the two varieties (Table.3).

From the obtained results, it is found that under a water deficit the studied genotypes react by increasing the content of soluble sugars in the leaf tissues, this increase is different according to the genetic inheritance of each genotype. Our results are consistent with those of (Mekliche *et al.*, 2003) who confirmed that the water deficit caused a significant accumulation of soluble sugars in the leaves.

In addition, it has been observed that under water stress, the starchy stores are gradually used following their rapid conversion to sucrose, which may be associated with an inhibition of starch synthesis (Bensari *et al.*, 1990; Ohashi *et al.*, 2009).

The accumulation of soluble sugars is a means adopted by plants in the event of stress, in order to resist the constraints of the environment (Prado *et al.*, 2000). According to Kameli and Losel (1993) the accumulation of sugars plays a critical role in the osmotic adjustment of plants. Arabzadeh (2012) reports an accumulation of soluble sugars in the

leaves of plants during desiccation to help maintain their turgidity and prevent dehydration of cell membranes. Soluble sugar content proved to be a better marker for selecting improvement of drought tolerance in durum wheat (*Triticum durum* Desf.) (Al hakimi *et al.*, 1995).

### Conclusion

In conclusion, the statistical analysis of the variance shows the existence of significant differences between the control plants and the stressed plants in all the parameters analyzed. Water stress significantly reduces the relative water content of cells, degradation of chlorophyll (a) and (b) molecule. In addition, a strong accumulation of soluble sugars is prevented in both varieties followed by a high osmotic adjustment. However, OZ variety is remarkable with chlorophyll content (a) and (b) more interesting in water stress conditions. A very highly significant positive correlation ( $r = 0.918$ ,  $r = 0.891$ ) between relative water content and chlorophyll content (a) and (b), a very highly significant negative correlation ( $r = -0.950$ ) between relative water content and sugars accumulation, these results indicate that the variety that keeps its water supply is the same variety that protects its chlorophyll pigments and accumulate less sugars, Also, the results of this investigation represented, in drought stress conditions that have more relative water content and chlorophyll content are more tolerance to drought stress, it concerned the OZ variety, that seems to be more tolerant and could be useful for durum wheat improvement.

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### Abbreviations

Chl: chlorophyll; GGR: GuemgoumRkhem; OA: osmotic adjustment; OZ: OuedZnati; PEG 6000: Polyethylene glycol 6000; r: Correlation coefficient; RWC: relative water content.

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