# **Biochemical Screening for Osmotic Adjustment of Wheat Genotypes under Drought Stress**

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### Summary

The aim of this work is to study the effect of water scarcity on some physiological and biochemical markers of durum wheat genotypes. Two genotypes differing in their origin were tested. Measurements of drought effects were carried out after ten days of exposure to gradual levels, and allowed us to examine if there exists a differential response of synthesis, accumulation and transport between leaves and roots regarding total proteins, proline and soluble sugars, with respect of membrane stability and water content, to reveal any patterns of discrimination between genotypes. It seems that the two genotypes develop the same strategies under drought conditions with a significant difference in the rate of osmoticums synthesis and accumulation. This difference concerns mainly proline accumulation which appears to be strongly correlated with genotypic variability. Indeed, there is a large accumulation of proline in the local genotype Oued Zenati compared to the genotype Acsad 289 although both genotypes showed an ability to synthesize them leading to adapt drought conditions. The parameters studied in the present investigation could be very useful for screening of wheat genotypes resistant to drought. Considering our results, the exploitation of local genotype Oued Zenati could constitute a basis of selection for agriculture in arid and semiarid regions.

#### Résumé

#### Criblage biochimique de l'ajustement osmotique chez quelques génotypes de blé soumis à la sécheresse

Le but de ce travail est d'étudier l'effet du déficit hydrique sur certains marqueurs biochimiques et physiologiques des génotypes de blé dur. Deux génotypes différant dans leur origine ont été testés. Les mesures des effets du déficit hydrique ont été effectuées après dix jours d'exposition à des niveaux progressifs, et nous ont permis d'examiner l'éventuelle existence d'une réponse différente de la synthèse, de l'accumulation et du transport entre les feuilles et les racines en ce qui concerne les protéines totales, la proline et les sucres solubles en relation avec la stabilité membranaire et à la teneur en eau. afin de trouver des facteurs de discrimination entre les deux génotypes. Il semble que les deux génotypes utilisent les mêmes stratégies en conditions de sécheresse avec une différence significative dans le taux de synthèse et d'accumulation des différents osmolytes. Cette différence concerne essentiellement la proline qui semble être fortement corrélée avec la variabilité génotypique. En effet, on constate une forte accumulation de proline chez le génotype local Oued Zenati comparativement à l'Acsad 289 bien que les deux génotypes ont montré une capacité de synthèse leur permettant de s'adapter à la sécheresse. Les paramètres étudiés dans le présent travail peuvent s'avérer très utiles pour le criblage des génotypes de blé résistants à la sécheresse. Au vu des résultats obtenus, l'exploitation du génotype local Oued Zenati pourrait constituer une base de sélection pour l'agriculture en zones arides et semi-arides.

#### Introduction

The scarcity of rainfall and the high evaporative demand of the atmosphere in the arid and semiarid regions of the world induce frequent drought leading also to osmotic stress. Where available water is considered the most limiting factor for crop production, wheat represents the principal food crop for human consumption but increased resistance to drought appears critical to keep yields at a sufficient level (27). The screening of such cultivars, based on their productivity, is a long and tedious task that would undoubtedly be improved if traits that could be reliably related to water deficit were well known (3). Osmotic adjustment is considered to be an important component of drought tolerance mechanisms in plants (15). Compatible solutes of low molecular weight which include amino acids, betaine and soluble sugars are accumulated in plants under drought (11). In addition to these organic substances, some inorganic solutes are also a significant fraction of the osmotically active solutes present in plant cells (14).

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Significant differences have been reported between species, cultivars or landraces in terms of osmotic adjustment capacity and with respect to the nature of the major solutes contributing to osmotic potential (25). In wheat, osmotic adjustment was suggested to be an important factor explaining differences in yield or yield stability (28).

Proline and soluble sugars frequently have been shown to increase under water stress and are potentially important contributors to osmotic adjustment. Among studies devoted to osmotic adjustment in wheat, durum wheat (*Triticum durum* Desf.) has been less investigated than bread wheat (*Triticum aestivum* L.) (18).

Genetic improvement of crops for drought resistance requires a research for possible physiological and biochemical components of drought resistance and the exploration of their genetic variation (10). One approach is to establish a single drought resistance character, which will benefit growth and yield under water limited conditions, and then to incorporate it into the existing breeding program (10). Consequently, there have been many suggestions that improvement in plant growth and yield could be achieved by identifying physiological characteristics or traits which could be included in a set of selection criteria by plant breeders (1). Several strategies have been devised to overcome the problem of drought stress; drought screening tests have been identified for use in breeding programs. The study of genotypeenvironment interaction has also been an important technique for selecting a resistance genotype against certain stress. Genotype-environment interaction is an important concern to all plant breeders in developing improved varieties. It is preferable when comparing the physiology and biochemistry of genotypes against drought tolerance that the genetic backgrounds be similar so as to eliminate differences that are unrelated to drought tolerance (16).

Varietal differences in drought have been reported in wheat (13), which can be exploited further by breeding programs to develop new varieties. The aim of this work was to study the effect of drought stress on some physiological and biochemical characteristics of durum wheat to examine the potential for osmotic adjustment and survival of wheat genotypes under different levels of water deficit. In this work, we have used two genotypes of wheat differing in their origin. Measurements of the effects of drought were carried out after ten days of exposure, and allowed us (a) to examine if there exists a differential accumulation and transport between leaves and roots of total soluble proteins, proline and soluble sugars with respect of water statue and membrane stability to reveal any patterns of discrimination between wheat genotypes, (c) to evaluate the elemental distribution in roots and leaves of these genotypes and (d) to investigate whether drought stress could induce differential proline and carbohydrate accumulation.

## Material and methods

The experiment was conducted under greenhouse controlled conditions. Diurnal and nocturnal temperatures were 24-27 °C and 16-19 °C respectively with 14 hours/day photoperiod. The relative humidity was about to 70%. Two genotypes of wheat (Triticum durum Desf.), the local Algerian genotype Oued Zenati (O.Z) and the introduced genotype Acsad 289 (Acs) from the Arabian Centre for Studies of Arid Zones and Drylands (ACSAD, Syria), were tested in this study under gradual drought levels. Wheat seeds were surface sterilized by dipping the seeds in 1% mercuric chloride solution for 2 min and rinsed thoroughly with sterilized distilled water. Seeds were pre-germinated in Petri dishes. After the emergence of the first leaf, the seedlings were grown in PVC cylinders of 50 cm height and 10 cm diameter filled with a mixture of sand, soil and organic dry matter (8:1:1). Seedlings were irrigated by sufficient water each two days with an equivalent of 80% of the field capacity. After complete four full expanded leaves, different water treatments were applied for ten days. Plant controls were conducted fully irrigated at 100% of field capacity (F.C). For the other deficient treatments, plants received 75%, 50%, 25% and 0% of their field capacity, respectively.

The levels of water content were maintained constant for each treatment. Evaporation was minimized by covering the containers with polystyrene. Pots were weighed every day and the water lost through evaporation and transpiration was replaced by adding water or nutrient solution equivalent to the loss by these factors. Reference pots were used to determine incremental water required for treatments by weighing both control and experimental pots. Such increments were observed as plants grew in size.

Leaf relative water content was estimated according to the method of Weatherley (30). Membrane stability was determined as per the method of Premchandra *et al.* (23) modified by Sairam (26). Protein content was measured as described by Lowry *et al.* (17), with minor modifications, to minimize the absorbance of interfering substances. Proline content was determined according the method described by Bates *et al.* (4). Total soluble sugars content was measured following the method described by Yemm and Willis (31).

The variance of homogeneity of the data was assessed and conformed to the model which would permit analysis of variance (ANOVA) on the data set. Results were analyzed using the General Linear Model (GLM) procedure implemented in the statistical software SPSS 16.0 (SPSS Inc, Chicago, USA) by ANOVA analysis. Treatment means were separated by different letters significantly different following Student-Newman-Keuls mean separation test. The term significant indicates differences for which P< 0.05 under the confidence level  $\alpha$ = 95%. Collected data were used also for calculation of correlation coefficient among examined traits.

## **Results and discussion**

Statistical analysis showed significant differences between the two genotypes tested, applied water deficit levels and interactions Genotype x treatment for all examined variables (P < 0.05). To better understand the factor that contributes mainly in the total variation of measured variables, the sum of squares percentage for each variable relative to the total variation was calculated. Relative water content is a good indicator of water and physiological statue under drought stress in wheat (22).

Comparing genotypes revealed that changes in leaf water status were different depending on the genotype (P< 0.01\*\*). Changes in RWC induced by water restriction were higher in O.Z ( $r^2 = 0.98^{**}$ ) than in Acs (r<sup>2</sup>= 0.92\*\*). Sum of squares percentage analysis showed that 90% of the total variability is due to drought constraint. Under irrigated conditions, O.Z maintained the highest relative water content. The RWC decreased significantly with increasing drought levels in both genotypes and the reduction was higher in O.Z than Acs, particularly, at the unwatered treatment (Table 1). The higher RWC in O.Z would be a difference in the amount of bound water in relation to cell wall composition which was demonstrated to be a heritable trait in durum wheat whose expression could be influenced in water stress conditions (24).

Under environmental stresses plant membranes are subject to changes often associated with the increases in permeability and loss of integrity (5). Water statue of both genotypes was closely dependent to membrane stability indices ( $r^2$ = 0.91\*\*). Genotype O.Z presents almost high membrane stability and the decrease was more pronounced in genotype Acs mostly under treatments watered below 50% F.C. Recorded reduction in membrane stability under drought conditions have been also reported in other genotypes of wheat (7).

Dhanda *et al.* (10) opined that because of genetic advances great benefit from selection can be expected

for the membrane stability of leaf segments in wheat. Compatible solutes have a key role in drought tolerance, they can protect plants from stress through different mechanisms including cellular osmotic adjustment, detoxification of reactive oxygen species, protection of membrane integrity, and stabilization of proteins-enzymes (2). Concentration of total proteins content was increased significantly in leaves stressed plants similarly in both genotypes ( $r^2 = 0.89^{**}$ ), proteins accumulation reached fourfold the control equally under treatments watered at 50% and 25% F.C. The highest accumulation was recorded by the unwatered genotype O.Z. Increased rate of total proteins content in roots was slightly and almost linear for the genotype Acs by a difference around 30% between each treatment ( $r^2 = 0.9^{**}$ ). Instead, the genotype O.Z accumulated higher proteins level under treatments watered at 50% and 25% F.C to reach a maximum accumulation under the unwatered treatment (r<sup>2</sup>= 0.98\*\*) (Table 2). The maximum difference of accumulation between the two genotypes roots was recorded under the unwatered treatment (245 µg.mg<sup>-1</sup> DM). In order to elucidate origin of protein content variability, sum of squares percentage indicated that 98% of the whole variability was due to drought in root while in leaves, drought contributed by 60% as the genotypic difference provided 17%.

Proteins ratio leaves/roots was decreased significantly under water stress (P< 0.01<sup>\*\*</sup>) expressing either low leaves accumulation or high root accumulation or there is directed translocation from leaves to roots. The amino acids are known to occur widely in higher plants and normally accumulate in large quantities in response to environmental stresses including drought stress (6). Osmotic adjustment in wheat seems governed by a gene conditioning primarily differences in potassium accumulation, with amino acid accumulation as a secondary dependent response (8).

Table 1
Water statue and membrane stability of O.Z and Acs genotypes subjected to
different water treatments during ten days

Genotypes and treatments (% F.C)		Relative water content (%)	Membrane stability index (%)
Oued Zenati	100	96,69 ± 0,46a	94,90 ± 0,26a
	75	95,01 ± 0,16b	94,50 ± 0,30a
	50	93,45 ± 0,14c	92,53 ± 0,49b
	25	92,28 ± 0,31d	90,77 ± 0,25c
	0	89,39 ± 0,76e	$88,10 \pm 0,46d$
Acsad 289	100	95,13 ± 0,33a	93,77 ± 0,47a
	75	93,98 ± 0,29b	93,10 ± 0,20a
	50	94,14 ± 0,27b	91,13 ± 0,31b
	25	90,64 ± 0,69c	87,87 ± 0,35c
	0	90,33 ± 0,15c	85,27 ± 0,85d

Data are the mean ± SE (n= 5). Different letters a column indicate significant difference (P< 0.05, Student-Newman-Keuls test).

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Genotypes and		Ē	otal proteins			Proline		Sc	oluble sugars	
treatments (% F.C)		Leaves (µg.mg¹ DM)	Roots (µg.mg <sup>-1</sup> DM)	Leaves/ Roots	Leaves (µg.mg <sup>-1</sup> DM)	Roots (µg.mg <sup>-1</sup> DM)	Leaves/ Roots	Leaves (µg.mg <sup>-1</sup> DM)	Roots (µg.mg <sup>-1</sup> DM)	Leaves/ Roots
Oued Zenati	100	6,31±0,66a	16,04±0,51a	0,39a	8,93±0,13a	13,00±1,00a	0,69a	18,69±0,76a	25,14±0,94a	0,74a
	75	12,34±0,56b	55,05±0,82b	0,22b	13,15±0,59b	97,81±52,75b	0,13b	26,30±0,25b	71,88±1,24b	0,37b
	50	22,64±0,63c	132,27±1,61c	0,17c	29,80±0,96c	111,41±0,95b	0,27c	33,57±0,73c	103,16±0,64c	0,33c
	25	22,80±0,47c	127,76±1,17d	0,18c	33,35±0,70d	118,58±0,41b	0,28c	35,27±0,67c	155,78±0,80d	0,23d
	0	65,63±1,28d	352,49±1,25e	0,19c	159,36±0,67e	125,44±1,02b	1,27d	42,11±0,89d	168,12±1,75e	0,25d
Acsad 289	100	7,85±0,75a	10,26±1,45a	0,77a	4,23±0,33a	7,68±0,61a	0,55a	11,68±0,87a	15,85±0,81a	0,74a
	75	11,44±0,30b	38,67±1,89b	0,30b	6,08±0,39b	13,02±0,62b	0,47b	22,75±1,34b	46,85±1,27b	0,49b
	50	22,23±0,63c	58,39±1,27c	0,38c	8,24±0,80c	16,25±0,73c	0,51c	29,06±0,44c	62,32±1,87c	0,47b
	25	25,30±0,43d	71,03±1,80d	0,36c	10,00±0,39d	51,40±1,61d	0,19d	31,57±0,75d	92,55±1,19d	0,34c
	0	57,73±1,05e	106,93±4,67e	0,54d	14,34±0,57e	61,42±1,40e	0,23e	35,81±1,48e	165,39±3,70e	0,22d
Data are the mean ±	SE (n=5). [	Different letters a colur	mn indicate signific	ant difference (	>< 0.05. Student-New	/man-Keuls test).				

Proline was one of the minor free amino acids in control plants, but increased proportionally in response to water deficit both in leaves ( $r^2 = 0.55^{**}$ ) and roots ( $r^2 = 0.58^{**}$ ). A higher proportion of proline in total amino acid content of stressed wheat plants  $(r^2 = 0.8^{**})$  was also recorded by Mattioni *et al.* (18). Drought caused an increase in proline accumulation in both genotypes organs (r<sup>2</sup>= 0.55\*\*) (Table 2). Genotype O.Z showed more proline content than genotype Acs under all drought treatments. Proline accumulated almost equally in leaves of the genotype Acs under all drought treatments, although in genotype O.Z, proline accumulation rises 30% in leaves likewise under both 50% and 25% F.C treatments then accumulation rises at 150% under the unwatered treatment. In durum wheat, de novo synthesis seems to be the main mechanism of proline accumulation under drought conditions, while the other amino acids made very small or no contribution to osmotic adjustment as reported by Kameli and Lösel (15).

Following drought stress, the proline concentration was much higher in the roots of the genotype O.Z whilst concentration increased slightly in the roots of the genotype Acs. Proline content increased fivefold in the roots of the genotype O.Z under treatment of 75% F.C comparing with control plants to reach higher value under the unwatered treatment. Proline content increased significantly in genotype Acs when soil humidity decrease below 50% F.C to reach its maximum under dry conditions. Maximum differences between two genotypes tested were observed under the treatment watered by 50% F.C for roots (95 µg.mg<sup>-1</sup> DM) and under the unwaterd treatment for leaves (145 µg.mg<sup>-1</sup> DM). Proline ratio leaves/roots decreased linearly in the genotype Acs. This ratio fall sharply in the genotype O.Z under the first deficient treatment to rises again slightly but not significantly thereafter until reaching an extremely high value under the unwatered treatment.

The effect of water deficit is most notable (44%) compared to genotypic variability (20%) in the expression of proline accumulation in leaves, the genotypic difference contributed over than 43% for the expression of this trait in roots. Proline accumulation in plants under drought is a result of the reciprocal regulation of two pathways: increased expression of proline synthetic enzymes and repressed activity of proline degradation. This leads to a "proline cycle", the homeostasis of which depends on the physiological state of tissue (19). Evidence for the transport of proline to the root tip, where is accumulates during stress, has been reported (29). The increase of proline accumulation in our experimental drought conditions was consistent with the previous results reported. The data indicate that plants may have evolved a mechanism to coordinate synthesis, catabolism and transport activities for proline accumulation.

The mechanisms of proline action are not fully

understood, but it has been suggested that in addition to its role as an osmolyte during osmotic regulation, an osmo-protectant role was also recorded. Proline contributes to stabilizing sub-cellular structures such as membranes and proteins ( $r^2$ = -0.68<sup>\*\*</sup> in roots,  $r^2$ = -0.8<sup>\*\*</sup> in leaves), scavenging free radicals, and buffering cellular redox potential under stress conditions (2). Proline accumulation under drought stress has been correlated with stress tolerance, and its concentration has been shown to be higher in stress-tolerant plants (20). However, other researchers suggested that the accumulation of proline was a symptom of stress injury rather than an indication of stress tolerance in plants (9).

Carbohydrates are a major category of compatible solutes that include hexoses (mostly fructose and glucose), disaccharides (sucrose and trehalose), sugar alcohols (inositol and mannitol), and complex sugars (raffinose and stachyose), all of which accumulate during stress. Usually the magnitude of proline accumulation is relatively dependant toward carbohydrates contents (12). There was an increase in total soluble sugars content in both genotypes under drought stress treatments (Table 2).

Larher et al. (12) mentioned that soluble sugars have a positive effect on proline accumulation. Total soluble sugars content was much higher in O.Z genotype than in Acs genotype under all drought treatments. Increased concentrations of carbohydrates content in response to drought stress have been also reported by Kameli and Lösel (15). Soluble sugars content have been shown to increase in wheat leaves (r<sup>2</sup>= 0.92\*\*) and roots (r<sup>2</sup>= 0.94\*\*) under water scarcity noted that the increase was more pronounced in roots than in leaves, similar results to Kameli and Lösel (15). These authors suggest that soluble sugars are the main organic solutes contributing to osmotic adjustment in wheat species, especially in leaves as a sink, and an accumulation of sugars at high level may result from a reduction in the utilization of assimilates induced by water deficit in relation to an inhibition of sucrose synthase or invertase activities in one hand, and deterioration of translocation from sources to sink from the other hand (3). The magnitude of difference in total soluble sugars content in O.Z genotype over Acs genotype elevate in roots with increase in drought levels but both genotypes reach the same value under the unwatered treatment (166 µg.mg<sup>-1</sup> DM). Differences in soluble sugars accumulation in both organs are due mainly to water scarcity (90%). Soluble sugars content ratio leaves/roots was decreased almost linearly in both genotypes expressing either low rate of synthesis in leaves or high rate degradation and/or accumulation in roots. Otherwise, there was a directed active translocation of soluble sugars from leaves to roots. Osmotic adjustment is usually defined as a decrease in cell sap osmotic potential resulting from the net increase in intracellular solutes rather than from the loss of cell water. This osmotic adjustment allowed the maintenance of turgor pressure for cell elongation and several metabolic functions, although the complex relationships between turgor maintenance, growth and osmotic adjustment have been debated by some authors on the basis of stress-induced modifications of cell wall properties (21). These results suggest that osmotic adjustment represent an important part of the drought resistance mechanisms developed by durum wheat which could be exploited in breeding programs for improved drought tolerance (3).

## Conclusion

Wheat crop responds to water deficit in the form of changes in various physiological and biochemical processes. The physiological changes observed could be consequences of deleterious effects of drought on important metabolic processes as well as responses of various defense mechanisms adapted by the plant under drought conditions.

It seems to be a quantitative more than qualitative difference between these genotypes. The two genotypes develop the same strategies under drought conditions with a significant difference in the rate of osmoticums synthesis and accumulation. This difference concerns mainly proline accumulation which appears to be strongly correlated with genotypic variability. Indeed, there is a large accumulation of proline in the local genotype Oued Zenati compared to the genotype Acsad 289 although both genotypes showed an ability to synthesize them leading to adapt drought conditions.

The parameters studied in the present investigation could be very useful for screening of wheat genotypes resistant to drought. Considering our results, the exploitation of local genotype Oued Zenati could constitute a basis of selection for agriculture in arid and semiarid regions.

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