Comparative Study of Proline Accumulation of some Varieties of Durum Wheat (*Triticum durum* Desf.) under Water Stress Conditions

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ABSTRACT

Proline accumulation, as a marker of resistance to the abiotic constraints, was evaluated on seventeen genotypes of Durum wheat (*Triticum Durum* Desf) under water stress conditions. An experiment was implemented at INRAA research station located at Baraki, Algiers. The experimental design adopted is that of split plot design with water regime (irrigated and non irrigated treatments) as main plots and varieties as sub plots and three repetitions. Proline accumulation was significantly increased under water stress ($p < 0.01$). There was differences between varieties and their interaction with water regimes ($p < 0.001$) and 04 groups were identified. When plants are subjected to water stress they increase their rate of proline, this enables to improve the capacity of the cell to maintain it turgor pressure at low water potential.

Keywords: durum wheat (*Triticum durum* Desf.), proline accumulation, resistance, water stress.

INTRODUCTION

Environmental stresses, including drought stress are one of the first factors in limiting plant growth and crop productivity (Wang *et al.*, 2003). It affects strongly the arid and semi arid zones, characterized by low and irregular rainfall and high temperatures.

In Mediterranean areas, especially in Algeria, durum wheat (*Triticum durum* Desf.) is an important crop. Wheat production is not sufficient to meet the demand of a growing population. The average consumption is estimated at more than 190 Kg/years per person, where a total requirement is around 7.3 million tons for a population more than 38.7 million. The average production is estimated at 21 million quintals, resulting in coverage of imports of around 69%. The cost of these imports amounted to more than 1.5 billion dollars. This situation affects the economical situation of the country. The primary

constraint affecting this crop production is water scarcity.

Several studies showed that under water stress, plants adopt many strategies for adaptation which differ from one species to another and that involve a large combination of morphological, physiological and biochemical factors (Monneveux & Belhassen, 1997).

To maintain a high osmotic pressure in the cell after the fall of the water potential caused by water stress (El Mourid, 1988 ; Casals, 1996), plants accumulate some osmotocums such as proline, which seems played a major role. Its accumulation in many plants under water stress was highlighted, in particular in barley by Savitskaya (1967) and in durum wheat by Tyankova (1967) and by Vlasjuk *et al.* (1968).

Proline accumulation is a common physiological response in many plants in response to a wide range to biotic and abiotic stresses.

Proline accumulation has been reported to occur after salt, drought, high temperature, low temperature, heavy metal, pathogen infection, anaerobiosis, nutrient deficiency, atmospheric pollution and UV irradiation (Hare and Cress 1997; Saradhi et al. 1995; Siripornadulsil et al. 2002.). SINGH et al. (1973) noted that in cereals,

eventually in durum wheat under water stress conditions, proline accumulation even more important than genotypes are more resistant.

The present study aims to evaluate the affect of water stress on proline accumulation in some cultivars of Durum Wheat (*Triticum durum* Desf.).

Tab. 1. List of the genotypes studied

N°	Varieties or lines	Pedigree	Origin
1	LD357E/2*TC60//JO69/3/FGO/4/GTA/5/SRN_1/6/...	CDSS04Y00755T-0TOPB-12Y-0M-06Y-1M-1Y-0B	Rép1-11
2	SORA/2*PLATA_12/3/SORA/2*PLATA_12//SOMAT_3/4/AJAIA_13/...	CDSS02B00849T-0TOPB-0Y-0M-7Y-2M-04Y-0B	Rép1-15
3	CND/VEE//CELTA/3/PATA_2/6/ARAM_7//CREX/ALLA/5/ENTE/...	CDSS02B00429S-0M-9Y-06Y-1M-1Y-0B	Rép1-21
4	MINIMUS/COMB DUCK_2//CHAM_3/3/RCOL*2/4/...	CDSS02B01108T-0TOPB-0Y-0M-5Y-4M-04Y-0B	Rép1-09
5	Beni Mestina	Témoin	Témoin
6	MINIMUS_6/PLATA_16//IMMER/3/SOOTY_9/...	CDSS02B00396S-0M-4Y-06Y-4M-1Y-0B	Rép1-03
7	PLATA_7/ILBOR_1//SOMAT_3/3/CABECA_2/PATKA_4//ZHONG ZUO/.	CDSS04Y00053S-13Y-0M-06Y-4M-1Y-0B	Rép1-16
8	MINIMUS/COMB DUCK_2//CHAM_3/3/RCOL*2/4/SOMAT_4/INTER_8	CDSS02B01108T-0TOPB-0Y-0M-5Y-4M-04Y-0B	Rép1-19
9	BCRIS/BICUM//LLARETA INIA/3/DUKEM_12/2*RASCON_21/4/...	CDSS04Y00362S-27Y-0M-06Y-4M-1Y-0B	Rép1-13
10	Sigus	Témoin	Témoin
11	LDN7D(7A)/3*ASCONCHI/3/SORA/2*PLATA_2*PLATA_12//...	CDSS04Y01137T-0TOPB-24Y-0M-06Y-1M-1Y-0B	Rép1-24
12	ALTAR84/STINT//SILVER_45/3GUANAY/4GREEN_14//YAV_10/...	CDSS04Y00341S-11Y-0M-06Y-3M-1Y-0B	Rép1-01
13	TARRO_1/2*YUAN_1//AJAIA_13/YAZI/3/SOMAT_3/PHAX_1//...	CDSS02B01143T-0TOPB-0Y-0M-7Y-4M-04Y-0B	Rép1-18
14	LLARETA INIA/4/SKEST//HUI/TUB/3/SILVER/5/LHNKE/RASCON//...	CDSS02B00574S-0M-12Y-06Y-2M-1Y-0B	Rép1-14
15	Waha	Témoin	Témoin
16	Vitron	Témoin	Témoin
17	Echams	Témoin	Témoin

MATERIALS AND METHODS

Field experiment

The experiment was carried out during the growing season of 2013-2014 at National Institute of Agronomic Research of Algeria (Long: 3°6'E, lat: 36°41'N, alt: 18m above sea level). Twelve lines selected from the international trials of CIMMYT (Mexico) and five varieties originated from Algeria were sown in pot culture (five seedling per pot) and in a greenhouse. Each pot contained 5Kg of mixture of organic matter, clay silt soil and sand (1:1:1). The characteristics of the varieties studied are presented in table 1.

The experimental design adopted, in this experiment, is that of a split plot design with two water regimes as main plot and 17 varieties as sub plots and three replications. The water stress treatment was imposed when the 6th leaf started to appear by stopping the irrigation, followed by a hydration period. The control plants were irrigated every two days.

Proline determination. Proline colorimetric determination proceeded according to Troll and Lindsley (1955) method modified by Magne and Laher (1992). Leaves were collected and etuvé at 65°C for 48 Hours. 50mg of Dry weight were homogenized with 1ml of distilled water and kept in water bath for 30mn. Then centrifuged at 13000xg for 15mn and supernatant was separated. One millilitre of the extract was taken

and 2ml of acid ninhydrine reagent were added and placed in a water bath at 100°C for 30mn. The chromophore was extracted with 3 ml of toluene and its absorbance at 520 nm was determined in a spectrophotometer (SHIMADZU). The proline content is expressed in µg/mg of dry weight basis. The method was calibrated for each determination with standard proline solutions.

Statistical analysis: The results were analyzed by using GenStat Discovery, version4, ANOVA two-way (in randomized blocks).

RESULTS AND DISCUSSION

The results show an increase of proline accumulation under stress conditions,

While under well watered conditions (control), proline content in different genotypes remains low (Fig. 1).

The behavior of different genotypes under water stress showed variability in the accumulation of this amino acid „proline“. We have classified the cultivars according to their aptitude to accumulate proline; 04 groups were identified (Fig. 2).

1) The variety N5 = Beni Mestina was characterized by high accumulation of proline 133.7 µg/mg of dry matter and with a percentage increase of 288 % under water stress conditions.

2) Genotypes characterized by an average accumulation of proline which were between

Fig. 1. The proline content of last leave under watred and stress conditions on some genotypes of Durum Wheat (*Triticum durum Desf.*).

Fig. 2. The percentage increase of proline content under water stress conditions, on some genotypes of Durum Wheat (*Triticum durum* Desf.).

100 µg/mg and 200 µg/mg are: 1, 8, 11, 12 and 15.

3) An intermediate group was identified according to their aptitude to accumulated proline and which were between 6 µg/mg and 100 µg/mg are: 3, 4, 6, 9, 10, 14, 16, 2 and 13.

4) Genotypes characterized by very reduced accumulation of proline (<6 µg/mg) under water stress conditions, was about 110.4 µg/mg (5.14 %) and 123.2 µg/mg (0.57 %) respectively for PLATA_1//SOMAT_3/3CABECA_2/PATKA_4//ZHONG ZUO/. Line and Echams variety.

The results showed that variability within cultivars ($p < 0.003$) and differences between treatments ($p < 0.001$) were significant. Under stress conditions, proline accumulation was highest. Proline accumulation which is often considered as an excellent marker of resistance to stress hydrique, increased significantly ($p < 0.01$) under water stress conditions (Tab. 2). The results showed that Beni Mestina variety is highly

resistant with a production rate of 133.7 µg /mg (288%) while PLATA_1 // SOMAT_3 / 3CABECA_2 / PATKA_4 // ZHONG ZUO / . line and the Echams variety are sensitive with rate of accumulation proline respectively 110.4 µg /mg (5.14%) and 123.2 µg /mg (0.57%) under water stress conditions. The others cultivars of germoplasm studied are resistant.

Proline accumulation has been demonstrated in many species and in different situations of stress (osmotic, water and heat) as osmolyte involved in protective mechanisms during abiotic stress (Al-Rumaih and Al-Rumaih, 2008).

Increasing the amount of proline is positively related to the fall of the water level in the plants. This result is confirmed by several scientific research, mentioning those of Monneveux et Nemmar (1986), Bellinger *et al.* (1991) et Gorham (1993).

Many studies report that proline accumulated in plants under unfavorable conditions (Sivaramakrishnan *et al.*, 1988) which reflects the character of resistance to water stress (Greenway

Tab. 2. Analysis of variance for proline determination.

	Effect of genotypes		Effect of water		Interaction Gen*Water	
	F	Pr	F	Pr	F	Pr
Proline content	324.45	0.003	3.41	<.001	3.49	<.001

and Munns , 1988). According Quien et al. (2001), accumulation proline under water stress contributes to the development of resistance by maintaining cell turgor, created by osmotic adjustment.

CONCLUSION

At conclusion, the study showed that our germoplasm of durum wheat is resistant with the exception of two genotypes PLATA_1 SOMAT_3/3CABECA_4//ZHONG ZUO/. Line and Echams variety are sensitive. All genotypes have developed the same strategy which is the proline accumulation under water stress, except that they differ by the production rate of this amino acid. Proline accumulation can be a real biochemical mechanism of resistance to water deficit in durum wheat.

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