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Annals of Biological Research, 2013, 4 (5):11-15
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

Oxidative stress, chlorophyll content and ROS production and localization in *Triticum durum* seed

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ABSTRACT

Plants subjected to unfavorable environmental conditions (abiotic stress) and which are under stress have manifested consequences by disturbances morpho physiological. The work that we have performed concerns the effects of water stress on plant model: durum wheat (Triticum durum) variety GTA. After germination, the plants were subjected to water stress during (03, 05, 07 and 09 days). Our results showed clearly a decrease in the average content in chlorophyll and disruption of respiratory metabolism. At the cellular level, this stress has led to changes in cellular homeostasis and toxicity, which are manifested by the production of reactive forms of oxygen, superoxide anion at the roots of our plant model.

INTRODUCTION

The Environmental stress in plants involve signaling reactions which can lead to the development of defenses or trigger programmed cell death **[1- 4].**

With the exception of organisms anaerobes, oxygen is required for all animals, plants and bacteria aims to produce energy through electron transport chains such as that found in mitochondria eukaryotic cells **[5]**. During evolution, the adaptation of plants to oxygen is translated by the appearance of enzymes facilitating not only its consumption but also the detoxification of reduced metabolites such as the superoxide radical ($O_2^{\bullet -}$) and hydrogen peroxide H_2O_2 **[6]**. These species are called reactive oxygen species (ROS) because they are considerably more toxic than the oxygen **[7]**. Environmental changes are responsible of the failure of control systems and oxygen metabolites that are responsible for the phenomena of oxidative stress **[8].**

MATERIALS AND METHODS

Biological material:
The biological material used in this work is durum family Poaceae specifically *Triticum durum* (DESF). The chosen organ for this study is the root. The samples come from the interprofessional Algerian Office of cereals (CATO) El Hajar, Annaba, Algeria.We used the hard variety GTA.

Performing the test:
The tests are conducted at the Toxicology Laboratory of Cellular Badji Mokhtar Annaba University and Laboratory of Cellular and Molecular Physiology Plants of the University Pierre et Marie Curie-Paris 6.

After 4 days of germination, wheat germ suffer water stress by stopping watering and wheat samples were analyzed at 3, 5, 7 and 9 days after cessation of watering. A Part continues to be watered normally and is considered a witness.

The Germination occurs at a temperature of 21 ° C day and 17-21 ° C night with artificial lighting from 6 am to 22 H.

Extraction of chlorophyll

The extraction of chlorophyll is according to the method of Holden (1975**)**, based on a maceration of the plant in acetone. The Reading is done by two wavelengths 645 nm and 663 nm, after calibration of the apparatus with the control solution of 80% acetone **[9]**.

Monitoring of respiratory metabolism

The Respiratory metabolism was evaluated by the respiration measuring intensity (IR) of isolated roots of durum wheat. The intensity of respiration is monitored by using an oxygen electrode Clark electrode (Hansatech Ltd, Kinj's Lym, Uk). **[10,11]**.

***In situ* localization of superoxide anion**

Roots were incubated in 6 mM nitroblue tetrazolium (NBT) in 10mM Tris-HCl buffer (pH 7,) at room temperature for 30 min. Superoxide anion was visualized as deposits of dark-blue insoluble formazan compounds **[12].**

RESULTS

Average chlorophyll content:

The evolution of the average chlorophyll content (Table 01) showed a significant decrease in chlorophyll from 5 day. This decrease is proportional to the stress intensity

Table 01. Effect of water stress on mean levels chlorophyll a, b and (a + b).

Times/Chlorophyll	3 days	5 days	7days	9 days
WS chl (a)	15,7343333	17,0943333	17,4743333	17,6636667
US chl (a)	15,469	14,8413333	14,555	13,4063333
WS chl (b)	28,795	31,2616667	31,9253333	32,2683333
US chl (b)	28,3116667	27,1793333	26,6466667	24,5613333
WS chl (a+b)	46,9813333	49,118	47,4113333	47,7133333
US chl (a+b)	46,78	45,931	44,3506667	42,3483333

***(WS**: Without Stress, **US**: Under Stress)*

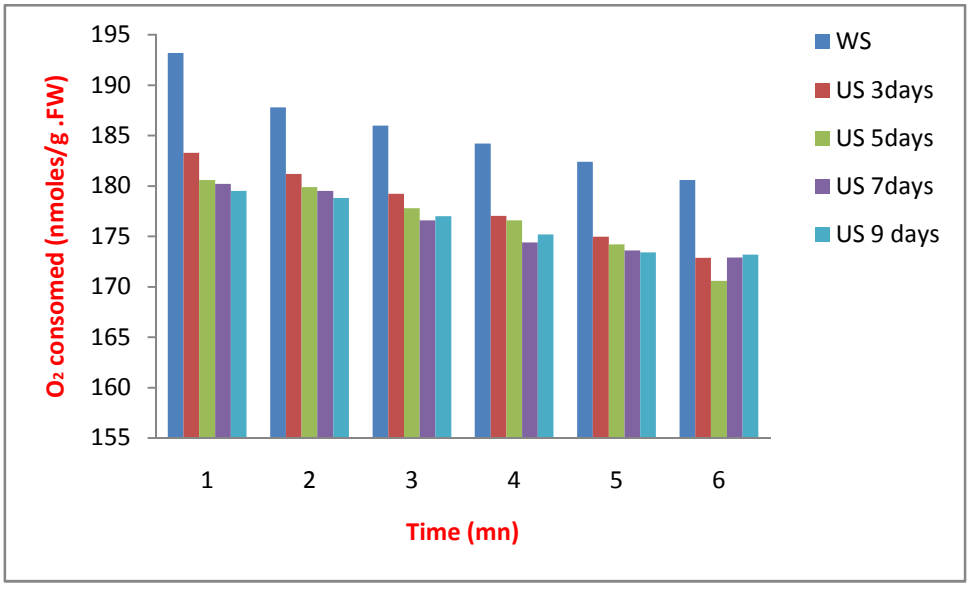

Figure 1. Oxygen Consumed at the root level (nmol / min / g. FW)
(WS: Without Stress ,**US** : Under Stress, **FW**: Frais Weight)

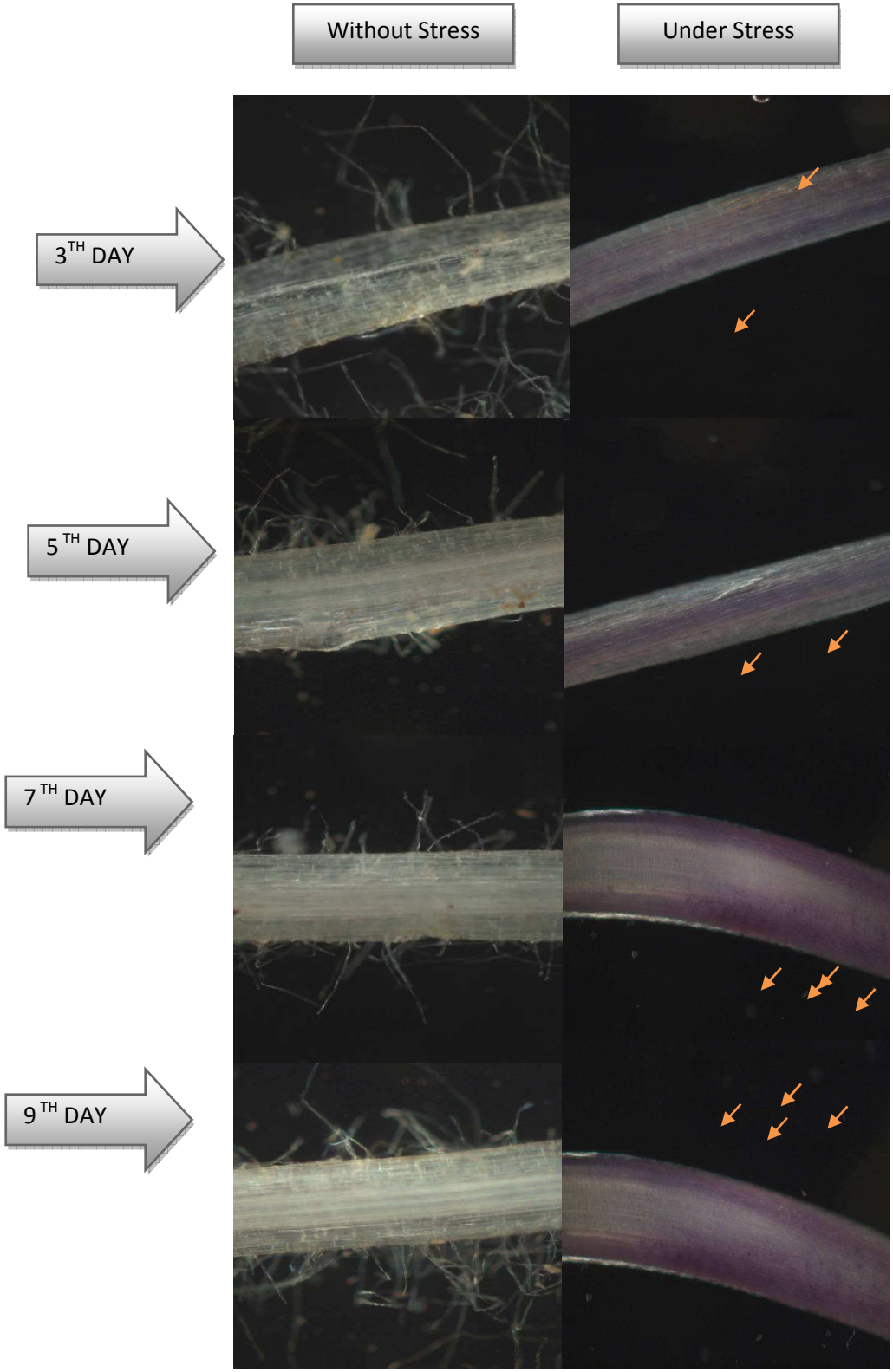

Figure 02. Using the method of Beyer and Fridovich, 1987 in highlighting the superoxide anion at the root level observed in control and stressed.

Monitoring of respiratory metabolism:

Monitoring of respiratory variations in intensity (figure 01) shows a strong inhibition in stressed roots seeds in comparison with controls. This decrease is observed according to the four time tested (3,5,7 and 9 days of stress).

Location of superoxide anion

Figure (02) shows the location of the superoxide anion in the roots of seeds subjected to stress (dark color). We find that the intensity of the violet color increases in a manner proportional to the intensity of water stress, parallel to the witnesses, no trace of $O_2^{-\cdot}$ is observed.

DISCUSSION

The water has a great importance **[13]** so its lack causes physiological and morphological disturbances and appearance of oxidative stress which is characterized by the formation of reactive oxygen species (ROS) **[14]**.

The whole of our results shows that water stress leads to a reduction of the average chlorophyll. This observation is in accordance with the work of (Havaux 1988; Djekoun and Ykhlef 1996; Kotchi, 2004 **[15-17]**.

Reduction of photosynthesis is related firstly to stomatal closure **[18-20]** and secondly to reduce the photochemical activity of photosystem (PSII) **[21]**. However, the water deficit leads to an inhibition of respiration **[22]**. The dip in our work explains the presence of large amounts of ROS, which is derived from photosynthetic and respiratory metabolism that explains the positive correlation existing between respiration rate and chlorophyll synthesis recorded in our work.

Moreover, in mitochondria (site of ROS production in root), superoxide anion (O_2^{-}) is generated at complexes I, II and III of the respiratory chain **[26-29]** due to a leak in the mitochondrial respiratory chain, specifically at the terminal acceptor: cytochrome oxidase complex IV chain electronic transport in the inner mitochondrial membrane **[28]**.

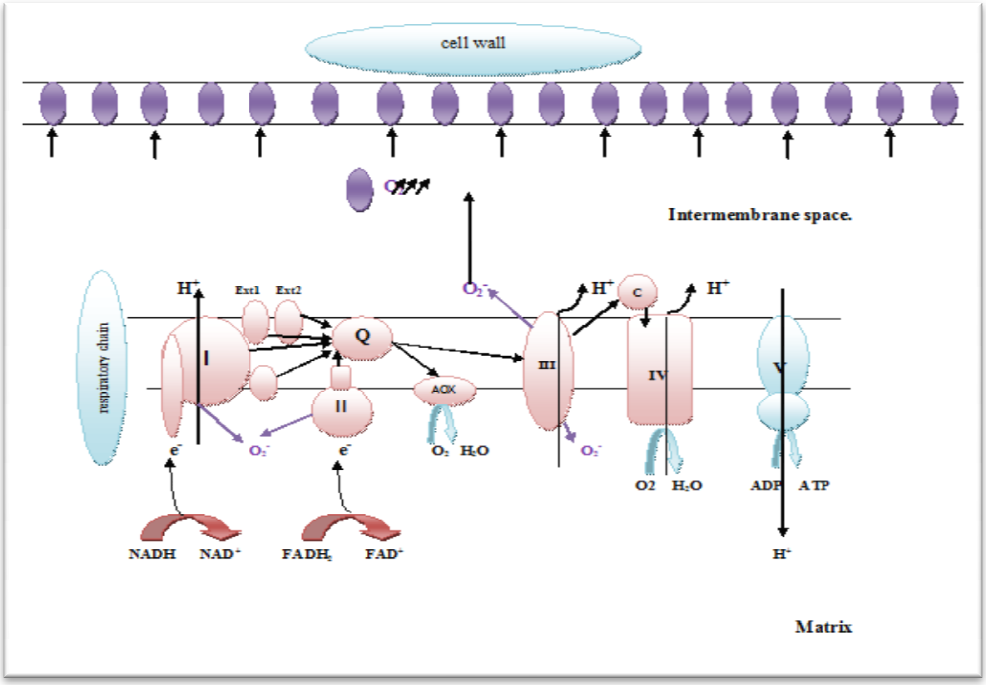

Figure.03 Representation of the formation of superoxide anion at the mitochondrial respiratory chain of durum wheat roots « *Triticum durum* DESF »

The purple arrows represent "leakage" of electrons to O_2 to form superoxide anions. Complex I: NADH dehydrogenase, complex II: Succinate dehydrogenase, complex III: Cytochrome bc1 complex IV: Cytochrome c oxidase , Complex V: ATPase, Q: ubiquinone pool, AOX: alternative oxidase, C: cytochrome C, Ext1 and Ext2 : NAD (P) H dehydrogenases external.

And oxygen is partially reduced 2 to 3% of the oxygen is reduced at single electronic ubiquinone **[29, 30]**. The amount of ROS produced in mitochondria may be at least partially explained by the presence of alternative airway (AOX), which constitutes an emergency route for the diminution of O_2 does not involve complex III **[31,32]**. The efficiency of the antioxidant activity of ubiquinone (Q) depends of the fate of the semiquinone (SQH) that can perform any activity contrary (that is to say pro-oxidant) via the formation of superoxide anion (figure 03). Thus, complexes I and II reduced coenzyme Q using electrons donated by NADH and $FADH_2$. Coenzyme Q transfers the electrons to complex III which transferred to cytochrome C. Complex IV uses electrons from cytochrome C to

reduce oxygen to water. Complexes I, II and III through the superoxide anion incomplete reduction of oxygen. Complexes I and II produce the superoxide anion in the matrix and the complex III in the matrix and the inter-membrane.

CONCLUSION

The results obtained in our study confirm the toxicity of water stress on wheat (*Triticum durum* DESF) variety GTA. Indeed deficiency water causes an inhibition of the synthesis of chlorophyll and metabolic disorder breathing. These two basic metabolisms are essential for the growth and development of the plant and their disruption causes a state of oxidative stress through increased production of reactive oxygen species such as superoxide anion, we have highlighted the roots.

Acknowledgements
The authors wish to thank Professor Christophe Bailly Laboratory of Cellular Physiology and Plant Molecular University Pierre and Marie Curie-Paris 6 for the illustration of the location of ROS.

REFERENCES

[1] C.A. Lamb, R.A . Dixon, *Annu Rev Plant Physiol Plant Mol Biol*., **1997**, **48**.251-275.
[2] H. Mamoudou, *Selected Works*., **2005**, 561-567**.**
[3] M.F. Kasraoui , M. Braham ,M. Denden ,H. Mehri ,M. Garcia ,T. Lamaze ,F Attia **,** Science direct.,**2006**, 329. 98-105.
[4] M. El Fakhrir, S.Mahboub, M. Benchekroun, *Nature &Tchnol*ogie., **2010**, 6-12.
[5] I.E .Scheffler, Wiley-Liss.,**1999.**
[6] B. Halliwell, R. Aeschbach, J. Loliger, O.I Aruoma, *Food Chem Toxicol*.,**1995**, 33 (7). 601-617.
[7] G.P. Novelli, *J.Physiol. Pharmacol*., **1997**., **48**. 517-527.
[8] C.Parent, N. Capelli, J. Dat, *C. R. Biologies*., **2008,** 331.
[9] M. Holden, *Edition Academic Press New York*. ,**1975**,133.
[10] M.R Djebar, H. Djebar**,** *Synthèse*., **2000**, 23 - 25.
[11] L.Meksem, PhD thesis , Badji Mokhtar University, (Annaba, Algeria) **, 2007.**
[12] W.F. Beyer, I. Fridovich, *Anal. Biochem*., **1987**, **161.** 559-566.
[13] D. Soltner, *Coll. Sciences et Techniques agricoles*., **1990**, **464.**
[14] R.Mittler, *Trends Plant Sci*., **2002**, **7**(9), 405-410.
[15] M. Havaux, *Plant Physiol. Biochem*.,1**988**, **26.** 245-251.
[16] A. Djekoun, N. Ykhlef **,** 3éme Reunion du réseau SEWANA, de blé dur, IAV Hassan II, 6-7 Décembre ., **1996.**
[17] S.O. Kotchi, *EPFL*., **2004,115**
[18] R.C. Johnson, H.T. Nguyen, L.I. Croy**,** *Crop Sci*., **1984**, 24. 957-962.
[19] T. Guissou , B.A. Amadou, C. Plenchette, S.Guinko, R. Dupon**nois,** *Science et changements planétaires*.,**2001**, 12.
[20] A. Mahhou, M.Theodore, S.Ken, *CIRAD,EDP Sciences*.,**2005**, 55-68.
[21] G.D. Farquhar, T.D. Sharkey, *Ann. Rev. Plant Physiol*,**1982**, **33**,317 – 345.
[22] N.Vartanian, M. Chauveau, *Plant and soil*.,**1986**, **92 ,**255-264.
[23] J.A. Imlay, *Annu Rev Microbiol*., **2003**, **57,**395-418.
[24] E. Herrero, J. Ros, G. Belli, E. Cabiscol, *Biochim Biophys Acta* .,**2008** (11), 1217-1235.
[25] M.P. Murphy, *Biochem J*., **2009**, **417**(1): 1-13.
[26] R.B. Hamanaka, N.S .Chandel**,** *Trends Biochem Sci*., **2010, 10**.
[27] D. Han, E.Williams, E.Cadenas, *Biochem. J*., **2001**,***353*, 411-416**.
[28] E.Cadenas, J.A. Davies, *Free Radical. Biol. Med*., **2000**, *29*, 222-230.
[29] L.A. Rio, F.J. Corpas, L.M. Sandalio, J.M. Palma, M. Gomez, J.B. Barroso, *J Exp Bot.*, **2002**, 53(372), 1255-1272.
[30] K. Oracz, H . El-Maarouf Bouteau, J.M. Farrant, K. Cooper, M. Belghazi, C. Job, D. Job, F Corbineau, C. Bailly,*Plant J.*, **2007**,**50**(3), 452-465.
[31] D.P. Maxwell, , Y. Wang, L. McIntosh**,** *Proc Natl Acad Sci U S* A.,**1999, 96**, 8271-8276.
[32] A.M.Wagner, *FEBS Lett*., **1995 368**. 339-342.