INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 3, No. 10, p. 298-305, 2013

RESEARCH PAPER OPEN ACCESS

ROS and redox signaling in the response of stems of wheat durum to abiotic stress

Issaad Ghozlène*, Djebar Mohammed-Réda, Berrebbah Houria

Laboratory of Cellular Toxicology. BP.12-Annaba 2300, Algeria

Key words: Water deficit, redox signaling, hydrogen peroxide, superoxide anion.

doi: http://dx.doi.org/10.12692/ijb/3.10.298-305 Article published on October 21, 2013

Abstract

Cereals hold an important place in agricultural research programs. In Algeria, this place is more important because the country wants to achieve stable production of cereals, especially concerning wheat and barley. However, water availability is a major factor which is limiting the productivity of cereal in Algeria. This work focuses on studying the effects of water deficit on the rods of a model plant: wheat (*Triticum durum*) variety GTA. After germination, the plants were subjected to a water stress during 03, 05, 07 and 09 days. Our results showed an increase in catalase activity (CAT), ascorbate-peroxidase (APX) (specific Enzymes of cellular detoxification system) and a lesser degree of activity of Guaiacol-peroxidase (GPX). On the other hand, we also showed a stong surge in the rate of hydrogen peroxide (H_2O_2) in rods. This increase is proportional to the degree of induced stress. The stress has caused toxicity which was manifested by the production of reactive forms of oxygen, hydrogen peroxide and superoxide anion in rods of our plant model.

* **Corresponding Author:** Issaad Ghozlène ✉ semsem-dj@hotmail.fr

Introduction

Cereals hold an important place in agricultural research programs. In Algeria, this place is more important because the country wants to achieve stable production of cereals, especially concerning wheat and barley. However, water availability is a major factor which is limiting the productivity of cereal in Algeria.

Water deficit is one of the main factors which is limiting yields around the world, lack of water, which is associated with other abiotic stresses (frost, high temperature, salinity ...), and climatic variability are responsible of yield losses (Monneveux and This, 1997). Facing to these attacks, the cells synthesize a number of antioxidant enzymes capable of trapping reactive oxygen species (Flexas *et al.,* 2006, Meksem, 2007). These are at the origin of the appearance of oxidative stress (Price *et al.*, 1998 , Noctor and Foyer, 1998).

The main ROS (reactive oxygen species) which are formed are hydrogen peroxide (H_2O_2), hydroxyl radical (·OH), superoxide anion ($O_{2^{\cdot}}^{-}$) and singlet oxygen ($^1O_2$). These ROS have a strong oxidant action and can react with most biological molecules, leading to a significant changes in their physico-chemical properties with harmful consequences for the integrity of the cell. In addition, some uncharged molecules are able to diffuse through the cell walls and thus can cause multiple damages such as bases oxidation of DNA or protein oxidation (Appel and Hirt, 2004).

Oxidative stress in plants is the subject of many literature reviews (Apel and Hirt, 2004, Foyer and Noctor, 2005a, Pitzschke *et al*, 2006, Wormuth *et al*, 2007). Thus, the purpose of this section is to understand the different pro-oxidant and antioxidant processes taking place in stems cells, to understand the mechanisms leading to the generation of oxidative stress under the effect of water stress.

Material and methods

The biological material used in this work is the durum family of Poaceae more specifically it is called *Triticum durum* (Desf). The organ chosen for this study is the stem. The samples come from the Algerian office inter cereals (CATO) El Hajar, Annaba, Algeria.We used the variety of GTA hard.

Performing the test

The tests are done at the Laboratory of Cellular Toxicology of Badji Mokhtar Annaba University-Algeria and the Laboratory of Cellular and Molecular Physiology Plants of the University Pierre and Marie Curie-Paris 6. After 4 days of germination, wheat germ suffer from water stress by stopping of watering in addition wheat samples were analyzed at 3, 5, 7 and 9 th day after cessation of watering. A part continues to be watered normally and is considered as a witness. Germination was carried out at a temperature of 21 ° C day and 17-21 ° C night with artificial lighting from 6 H to 22 H.

Relative turgidity of Water in rods (RWC)

Turgor on water is determined by the percentage of water present in the excised stems and immersed in water during 2 hours, according to the method of Clarke and Mc Caig (1982) which is also used by Rascio (1988). The calculation is based on Ladiges method (1975).

Measuring of the enzyme activity

The appropriate method to obtain the enzymatic extract of the stems of durum wheat is to Loggni *et al.*,1999.The extract is used for measuring the Catalase activity (CAT) which is performed according to the method of Boscoloa *et al.*, 2003, ascorbate peroxidase-(APX) according to the method of (Manivannan *et al.*, 2007) and Guaiacol peroxidase-(GPX) according to the method of (Hiner *et al.,* 2002).

The fresh stems (1g) are crushed cold in a mortar with a phosphate buffer (50ml Na K, pH 7.2) at a rate of 1 ml buffer per 1 g of rod. The homogenate is then filtered and centrifuged cold 12000xg for 20 mn

(Centrifuge Sigma 3-16 K). The supernatant obtained will be used for the determination of various enzymatic extracts.

Measuring the production of H_2O_2

The rods are incubated in 250 µl of buffer solution potassium phosphate (20 mM, pH 6) containing 5µM of scopoletin (Sigma) and 1U/ml (final concentration) horseradish peroxydase at 25 ° C in the darkness using a stirrer as described by (Schopfer *et al.*,2001). The production of H_2O_2 is measured by the decrease in fluorescence (excitation 346 nm, emission 455 nm) of the environment of incubation and was converted into a molar concentration of average of three replicates and are expressed as mg fresh weight of stems.

Location of hydrogen peroxide H_2O_2

Intercellular ROS production was visualized using DCFH-DA (Molecular Probe) with a fluorescence microscope. The rod is incubated in a buffer for 20 mM potassium phosphate (pH 6) containing 50 microns DCFH-DA for 60 min at 20 ° in the dark. The samples were rinsed 3 times in buffer .The images are taken (excitation: 480 nm, emission: 525 nm) with a fluorescence microscope using a digital lens * 05. To compare the density of DCFH-DA, samples of different experimental conditions are set at the same time and analyzed under the fluorescence microscope using the same experimental parameters (Oracz *et al.,* 2009).

Location of superoxide anion $O_2^{\cdot-}$

The rods are incubated in nitroblue tetrazolium 6 mM (NBT) in 10 mM Tris-HCl (pH 7.4) at room temperature for 30 mn. Superoxide anion is displayed in the form of dark blue deposits of insoluble formazan (Beyer and Fridovich, 1987).

Results

Relative Water turgor (RWC)

Figure (01) shows the relative water turgor (RWC), determined by the percentage of water which is present in the stems. We find a strong and high significant decrease in stems subjected to water stress; according to the witness.

Table 1. Effects of water stress on the activities of catalase (CAT), ascorbate-peroxidase (APX) and guaiacol peroxidase (GPX) in the GTA hard variety subjected to a water stress (WS :Without Stress ,US : Under Stress).

Times (Days)	3D	5D	7D	9D
WS (APX)	0,04435581	0,04842756	0,03756345	0,03296641
US (APX)	0,05305535 P > 0,05	0,05531245 P > 0,05	0,06163034 P <0,05	0,16380848 P < 0,01
WS (CAT)	0,00209596	0,00197406	0,00203046	0,00153465
US (CAT)	0,00306521 P ≤ 0,001	0,00520419 P ≤ 0,001	0,00673824 P ≤ 0,001	0,01026853 P ≤ 0,001
WS (GPX)	0,01549042	0,01590525	0,0161675	0,01555897
US (GPX)	0,02270164 P ≤ 0,001	0,01707614 P ≤ 0,001	0,01445492 P ≤ 0,001	0,015062 P ≤ 0,001

Enzymatic activities.

The development in activities of Catalase, ascorbate peroxidase and guaiacol-peroxidase in seeds subjected to water stress (Table 01), shows that there is a positive correlation between the severity of water stress and the enzymatic activity from the 3rd day. However the rise of the catalase activity and guaiacol-peroxidase are both very highly significant within the 3rd day according to the witness ,by another hand; the graph presents a non- significant raise of ascorbate peroxidase activity from the 3rd day to which followed by a significant increase until the 7th day .

Measurement of Hydrogen Peroxide (H_2O_2) producted

The evolution of the average content H_2O_2 in rods (Figure 02) reveals a very high and significant production of Hydrogen Peroxide from the 3 rd day tends to increase gradually when the stress level increases.

Location of hydrogen peroxide and superoxide anion

Figure (03) confirmed the presence of H_2O_2 (black darts) in stems of seeds subjected to stress, visible as fluorescent granule. Thus the figure shows the location of the superoxide anion (blue dart).In controls we observed no trace of H_2O_2 or $O_2^{\cdot-}$

Discussion

Plants are constantly subjected to changes in their environment, requiring them to alter their metabolism to maintain a balance between production and consumption of energy. This balance depends largely on a signaling network that coordinates three important processes in the life of plants: photosynthesis, respiration and photorespiration. (Noctor *et al.*, 2007, Foyer and Noctor, 2009, Foyer *et al.*, 2009 ; Pfannschmidt *et al.*, 2009). However drought causes morphophysiological changes: this is one of the major factors that limit plant growth under natural conditions (Cornic and Massacci, 1996). Disruption of plant growth under stress is due to a decrease in leaf growth responsible of a reduction of the amount of the absorbed light by the plants. The assimilation of CO_2 decreases when the stress persists (Baxter *et al.*, 2007, Takahashi and Murata, 2008). This diminution is mainly due to the closure of stomata (Brodribb and Holbrook, 2003), which the opening limits the diffusion of CO_2 from the air to the sites of carboxylation. Stomatal closure also mitigates the cavitation for maintaining the hydraulic conductivity of the xylem. When plants lose a significant amount of their water that may occur damages which are probably due to the passive concentration of reactive oxygen species (ROS) (Buchanan and Blamer ,2005, Moller and Sweetlove, 2010).

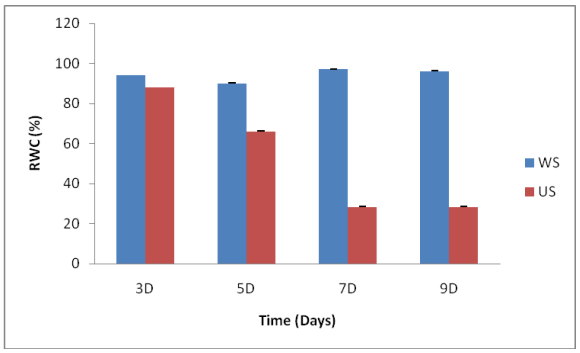

Fig. 1. Turgor Relative Water (RWC) at the stem (WS: without stress, US: under stress).

Deal with this stress, plants synthesize detoxification enzymes including catalase, guaiacol peroxidase and ascorbate peroxidase, involved in the maintain of cellular structures by the detoxification of ROS, which are then frequently produced, and responsible of the appearance of an oxidative stress (Flexas *et al.,* 2006, Meksem, 2007).

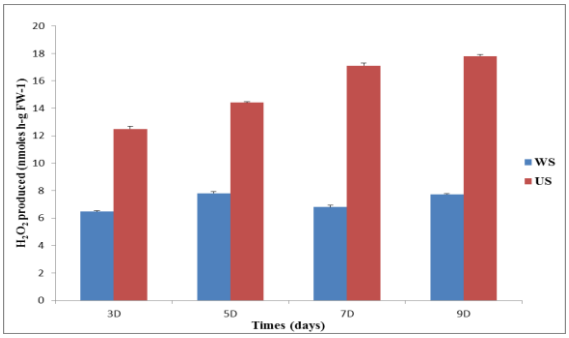

Fig. 2. Average content in hydrogen peroxide produced in durum wheat stalks.

According (Foyer and Noctor, 2003, Edreva, 2005, Asada, 2006) chloroplasts and mitochondria are the main sources of ROS in plants. However, the chlorophyll cells, due to their production of $O_2^{\cdot-}$ during the photosynthetic process, are particularly vulnerable to the generation of ROS. This process is responsible for the direct formation of $^1O_2$ and $O_2^{\cdot-}$ H_2O_2 and indirect (by dismutation of $O_2^{\cdot-}$) and $\cdot OH$ .There is still some time, photosystem I (PSI) was considered as the main source of ROS chloroplast. Under normal physiological conditions, the electron flow of PSI is directed to the NADP which is reduced to NADPH by NADP reductase ferredoxin (FNR). During stress, the electron chain may saturate. Part of the electron flow can be derived by ferredoxin to

oxygen (Figure 04). In addition to ferredoxin, other electron acceptors of PSI have a redox potential sufficiently negative to reduce dioxygen to superoxide anion (Mehler, 1951, Asada *et al.*, 1974). In recent years, it became clear that the PSI was also a site of generation of $O_2^{\cdot -}$ (Cleland and Grace, 1999, Dat *et al.*, 2000). Quinones A and B of PSI are involved in the loss of electrons leading to the reduction of O_2 (Figure 04). Some authors have even suggested that the production of superoxide anions from chloroplast is mainly the result of the loss of electrons from PSII, in particular at the level of quinone B (Zhang *et al.*, 2003). Chlorophyll a (Chl a) of PSII also has the ability to transmit an electron directly to oxygen in case of saturation of the electron transport chain (ETC) (Hippeli *et al.*, 1999). Once superoxide anions are produced in the stroma, they are quickly taken care of by superoxide dismutase (SOD), responsible of the dismutation of H_2O_2 (Foyer *et al.*, 1994, Edreva, 2005). (Figure 04).

Fig. 3. The demonstration of hydrogen peroxide and superoxide anion level in stems observed in controls and stressed. (Darts black: Represents the granules of the hydrogen peroxide (Darts blue: represent the superoxide anion).

Thus, mitochondria are a major source of ROS in non- chlorophyllous cells, whereas they represent only a small part of the chlorophyll cells (such as stem) (Foyer and Noctor, 2003). Under normal physiological conditions, approximately 2% to 6% of the total oxygen consumed by mitochondria is converted into ROS (Boveris and Chance, 1973). In the same way as the (ETC) of chloroplasts it is a saturation of the mitochondrial (ETC) seems to be the cause of the loss of electrons to O_2 (Figure 04). The intermediates radical of ubiquinones formed by the reduction of the pool of ubiquinones by complexes I and III of the ETC are the main electron donating to oxygen (Moller, 2001, Rhoads *et al.*, 2006). However, flavoproteins of complex II may also contribute to the production of $O_2^{\cdot -}$ (Young *et al.*, 2002) ROS production near the (ETC) may cause damages to the membrane, inducing a further decrease in the efficiency of the (ETC) and a greater loss of electrons. The result is a rise in the generation of ROS decreasing the efficiency of (ETC). During major stress, the initial generation of ROS can lead to deleterious cycle of production of these species which are toxic to the body. This cycle of mitochondrial ROS production is regarded as one of the main causes of cellular aging (Loeb *et al.*, 2005).

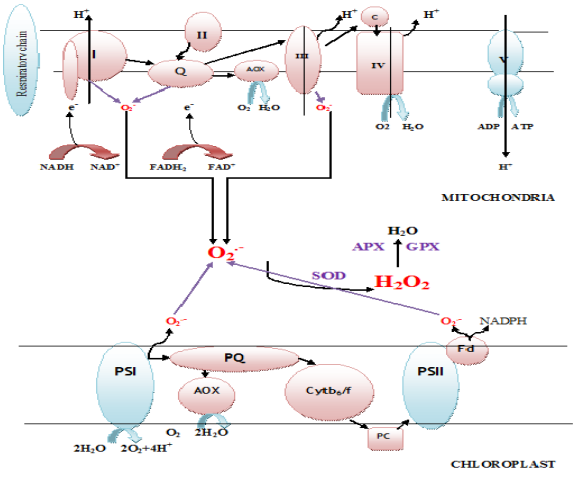

Fig. 4. Representation of the formation of superoxide anion and hydrogen peroxide in the mitochondria and chloroplasts of wheat stem hard "*Triticum durum* Desf".

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 ,APX: ascorbate peroxidase, GPX: guaiacol peroxidase, SOD: superoxide dismutase, Cytb6 / f: Cytochrome b6 / f, Fd: ferredoxin, PC: plastocyanin, PQ plastoquinone, Q: quinones.

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 and measurement of ROS.

References

Apel K, Hirt H. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annual Review of Plant Biology **55,** 373-399. http://dx.doi.org/10.1146/nnev.arplant.55.031903.11701

Asada K. 2006. Production and Scavenging of Reactive Oxygen Species in Chloroplasts and Their Functions. Plant Physiology **141(2)**, 391-396.

Asada K, Kiso K, Yoshikawa K. 1974 Univalent Reduction of Molecular Oxygen by Spinach Chloroplasts on Illumination. Journal of Biological Chemestry **249(7),** 2175-2181.

Baxter CJ, Redestig H, Schauer N. 2007. The metabolic reponse of heterotrophic Arabidopsis cells to oxidative stress. Plant Physiology **143,** 312-325. http://dx.doi.org/10.1104/pp.106.090431

Beyer WF, Fridovich I. 1987. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Analytical Biochemistry **161,** 559-566. http://dx.doi.org/10.1016/0003-2697(87)90489-1

Boscoloa P, Menossib M, Renato Jorgea A. 2003. Aluminium-induced oxidative stress in maize. Phytochemistry **62,** 181-189. http://dx.doi.org/10.1016/S0031%209422(02)00723-9

Boveris A, Chance B. 1973. The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. Biochemical Journal **134(3),** 707-716 http://dx.doi.org/10.1172/JCI116700

Brodribb TJ, Holbrook NM. 2003. Stomatal closure during leaf dehydration, correlation with other leaf physiological traits. Plant Physiol **132,** 2166-2173 http://dx.doi.org/10.1104/pp.103.023879

Buchanan BB, Blamer Y. 2005. Redox regulation: a broadening horizon. Annual Review of Plant Biology **56,** 187- 220. http://dx.doi.org/10.1146/annurev.arplant.56.032604.144246

Clarke JM, Mc Caig JN. 1982. Evaluation of techniques for screening for drought resistance in wheat. Crop Science **22,** 503.

Cleland RE, Grace SC. 1999. Voltammetric detection of superoxide production by photosystem II. Febs Letters **457(3),** 348-352. http://dx.doi.org/10.1016/S0014-5793(99)01067-4

Dat J, Vandenabeele S, Vranová E, Van Montagu M, Inzé D, Van Breusegem F. 2000. Dual action of the active oxygen species during plant stress responses. Cellular and Molecular Life Sciences **57(5),** 779-795. http://dx.doi.org/10.1007/s000180050041

Edreva A. 2005. Generation and scavenging of reactive oxygen species in chloroplasts: a submolecular approach. Agriculture, Ecosystems and Environment **106(2-3),** 119-133. http://dx.doi.org/10.1089/152308603321223531

Flexas J, Bota J, Galmés J, Medrano H , Ribas-Carbó M. 2006. Keeping a positive carbon balance

under adverse conditions: responses of photosynthesis and respiration to water stress. Physiologia Plantarum **127,** 343-352. http://dx.doi.org/10.1111/j.1399-3054.2006.00621.x

Foyer CH, Bloom AJ, Queva G, Noctor G. 2009. Photorespiratory metabolism: genes, mutants, energetics, and redox signaling. Annual review of plant biology **60,** 455-484 http://dx.doi.org/10.1146/annurev.arplant.043008.091948

Foyer CH, Lelandais M, Kunert KJ. 1994. Photooxidative stress in plants. Physiologia Plantarum **92(4),** 696-717. http://dx.doi.org/10.1111/j.1399-3054

Foyer CH, Noctor G. 2003. Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Physiologia Plantarum **119,** 355-364. http://dx.doi.org/10.1034/j.1399 3054.2003.00223.x

Foyer CH, Noctor G. 2005a. Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. Plant, Cell and Environment **28,** 1056- 1071. http://dx.doi.org/10.1111/j.1365-3040.2005.01327.x

Foyer CH, Noctor G. 2009. Redox regulation in photosyntheticorganisms : singaling, acclimation, and practical implications. Antioxidants and Redox Singnaling **11,** 861-905. http://dx.doi.org/10.1089/ars.2008.2177

Hiner A, Ruiz J, Lopez JN, Arnao MB, Raven EI, Canovas FG, Ascota M. 2002. Kinetic study of the Ascorbate-peroxidase by hydrogen peroxide Biochemical Journal **348,** 321-328.

Hippeli S, Heiser I, Elstner EF. 1999. Activated oxygen and free oxygen radicals in pathology: New insights and analogies between animals and plants. Plant Physiology and Biochemistry **37(3),** 167-178. http://dx.doi.org/10.1016/S0981-9428(99)80031-X

Ladigues PY. 1975. Some aspect of tissue water relation in three populations of *Eucalyptus viminalis* Labill. New Phytologist **75,** 53-62. http://dx.doi.org/10.1111/j.1469-8137

Loeb LA, Wallace DC, Martin, GM. 2005. The mitochondrial theory of aging and its relationship to reactive oxygen species damage and somatic mtDNA mutations. Proceedings of the National Academy of Sciences **102(52),** 18769-18770. http://dx.doi.org/10.1073/pnas.0508886102

Loggni B, Scartazza A, Brugnoli E , Navari-Izzo F.1999. Antioxydative defence system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. Plant physiology **119,** 1091-1099.

Manivannan P, Abdullaleel C, Kishocekumar A, Saukar B, Somasundaram R, Sridharam R, Panneersel R. 2007. Changes in antioxidant metabolism of vigna ungui culata (L.). walp by propiconazole under water deficit stress. Colloides and surfaces . Bio interfaces **57,** 69-74. http://dx.doi.org/10.1016/j.colsurfb.2007.01.004

Mehler A. 1951. Studies on reactions of illuminated chloroplasts. I. Mechanism of the reduction of oxygen and other Hill reagents. Archives of Biochemestry **33(1),** 65-77. http://dx.doi.org/10.1016/0003-9861(51)90082-3

Meksem L. 2007. Etude des effets de deux fongicides: Le Flammenco SC et le Tilt 250 EC sur la physiologie, la croissance et le métabolisme énergétique des racines isolées de *Triticum durum* DESF. PhD, university of Badji Mokhtar, annaba, p. 136-137.

Moller IM. 2001. Plant mitochondria and oxidative stress: Electron Transport, NADPH Turnover, and Metabolism of Reactive Oxygen Species. Annual Review of Plant Physiology and Plant Molecular Biology **52(1),** 561-591. http://dx.doi.org/10.1146/annurev.arplant.52.1.561

Moller IM, Sweetlove LJ. 2010. ROS signaling-specificity is required. Trends in plant science **15,** 370-374.
http://dx.doi.org/10.1093/aobpla/pls014

Monneveux P, This D. 1997. La génétique face au problème de la tolérance des plantes cultivées à la sécheresse : espoirs et difficultés. Science et changements planétaires / Sécheresse **1,** 29-37.

Noctor G, De Paepe R, Foyer CH. 2007. Mitochondrial redox biology and homeostasis in plants. Trends in plants science **12,** 125-134.
http://dx.doi.org/10.1016/j.tplants.2007.01.005

Noctor G, Foyer CH. 1998. Ascorbate and glutathione: keeping active oxygen under control. Annual Review of Plant physiology and Plant Molecular Biology **49,** 249-279.
http://dx.doi.org/10.1146/annurev.arplant.49.1.249

Oracz K, El-Maarouf-Bouteau H, Kranner L, Bogatek R, Corbineau F, Bailly C. 2009. The mechanism involved in seed dormancy alleviation by hydrogen cyanide unravel the role of reactive oxygen species as key factors of cellular signaling during germination. Plant Physiology **150,** 494-505.
http://dx.doi.org/10.1104/pp.109.138107

Pfannschmidt T, Brautigam K, Wagner R, Dietzel L, Schroter Y, Steiner S, Nykytenko A. 2009. Potential regulation of gene expression in photosynthetic cells by redox and energy state: approaches towards better understanding. Annals of Botany **103,** 602-609.
http://dx.doi.org/10.1093/aob/mcn081

Price A, Hendry G. 1997. The signification of the tocopherols in stress survival in plant. In: Evans CR, ed. Free Radicals, Oxidant Stress and Drug Action, Richelieu Press, 443-450 p.

Rascio A. 1988. Several mechanisms of water stress adaptation in durum wheat Gen. Agraria **42,** 90.

Rhoads DM, Umbach AL, Subbaiah CC , Siedow JN. 2006. Mitochondrial Reactive Oxygen Species. Contribution to Oxidative Stress and Interorganellar Signaling. Plant Physiology **141(2),** 357-366.
http://dx.doi.org/10.1104/pp.106.079129

Schopfer P, Plachy C, Frahry G. 2001. Release of reactive oxygen intermediates (superoxide radicals, hydrogen peroxide, and hydroxyl radicals) and peroxidase in germinating radish seeds controlled by light, gibberellins,and abscisic acid. Plant Physiology. **125,** 1591-1602.
http://dx.doi.org/10.1104/pp.125.4.1591

Takahashi S, Murata N. 2008. How do environmental stresses accelerate photoinhibition? Plant Science **13,** 178-182.
http://dx.doi.org/10.1016/j.tplants.2008.01.005

Wormuth D, Heiber I, Shaikali J, Kandlbinder A, Baier M, Dietz KJ. 2007. Redox regulation and antioxidative defence in Arabidopsis leaves viewed from a systems biology perspective. Journal of Biotechnology **129(2),** 229-248
http://dx.doi.org/10.1016/j.jbiotec.2006.12

Young TA, Cunningham CC, Bailey SM. 2002. Reactive oxygen species production by the mitochondrial respiratory chain in isolated rat hepatocytes and liver mitochondria: studies using myxothiazol. Archives of Biochemistry and Biophysics **405(1),** 65-72.
http://dx.doi.org/10.1016/S0003-9861(02)00338-7

Zhang S, Weng J, Pan J, Tu T, Yao S, Xu C. 2003. Study on the photo-generation of superoxide radicals in Photosystem II with EPR spin trapping techniques. Photosynthesis Research **75(1),** 41-48.
http://dx.doi.org/10.1023/A:1022439009587