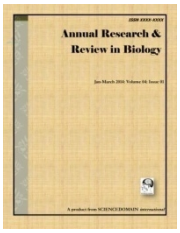

Annual Research & Review in Biology
4(8): 1241-1249, 2014

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ROS and Antioxidant System of *Triticum durum* after Water Stress

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Authors' contributions

This work was carried out in collaboration between all authors. Author IG carried out trials and managed the literature searches. Author RR designed the study, wrote the protocol, and wrote the first draft of the manuscript, translated it from French to English language. Authors DMR and BH followed and supervised this study. All authors read and approved the final manuscript.

Original Research Article

Received 14th June 2013
Accepted 17th October 2013
Published 2nd January 2014

ABSTRACT

Aim: Water stress is the cause of many disturbances in plants. This work focuses on the effects of water stress on the roots and seeds of a durum wheat (*Triticum durum*) of the GTA variety.
Study Design: After germination, the plants were submitted to water stress, and many enzymatic tests are followed to evaluate the response of durum wheat plant to this stress.
Place and Duration of Study: Laboratory of cellular toxicology, department of biology, faculty of sciences, Annaba university, and laboratory of Cellular and Molecular Physiology of Plants of the Pierre and Marie Curie, Paris VI, France University.
Methodology: Seeding was carried out in plastic vessels. After 4 days of germination, the seeds of wheat were submitted to water stress by stopping irrigation and wheat samples were analyzed at 3rd, 5th, 7th and 9th day after interruption of watering. Controls were watered normally.
Germination took place at a Day temperature of 21ºC and 17-21ºC at Night with artificial lighting from 6 am to 10 pm.
Results: The results showed an increase in catalase activity (CAT), ascorbate peroxidase

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(APX) (specific enzymes o cellular detoxification system); this increase was less observed in the activity of guaiacol peroxidase (GPX).
On the other hand, as ever increase in the level of hydrogen peroxide (H_2O_2) in the roots was observed. This increase is proportional to the degree of induced stress. The observation under a fluorescence microscope was revealed that the produced hydrogen peroxide was localized generally in the cytoplasm and the cell wall.
Conclusion: It is clear and possible that water stress induced many plant disturbances and the increase of ROS production.

Keywords: Water stress; water state; catalase; ascorbate peroxidase; guaiacol peroxidase; hydrogen peroxide; reactive oxygen species.

1. INTRODUCTION

Oxidative stress in plants is the subject of numerous studies in recent years [1-4]. Plants are constantly exposed to environmental changes or a biotic stresses such as lack, cold, high temperatures, orbiotic stress types (of the many xenobiotics used to fight against pests or other infections). These stresses are responsible for cell disruption, causing the release of free oxygen radicals in the cytoplasm [5,6], including the superoxide (O^{2-}), hydroxyl radicals (OH^-),radicals and peroxides(RO)and non-radical forms such as hydrogen peroxide (H_2O_2).These elements are produced in the reduction of oxygen by cytochrome respiratory chain. The chloroplast is the compartment of photosynthesis associated with high-energy electron transport, and thus a supply of oxygen, which can cause the formation of ROS (Reactive Oxygen Species) [7]. In case of excess, the increase off reeoxygen radical sincells causes irreversible cellular damage, such as lipid peroxidation. These ROS at high concentrations can be fatal to cells by impairing the functions and cellular structures. Plants, however, have developed a system of defense to self-protect against oxidative stress. So, cells had been developed an antioxidants enzyme systems to cope with cascades of reaction striggered by oxidative stress at the cellular level. Catalase, ascorbate peroxidase and guaiacol peroxidase are among the most involved detoxification enzymes [8]. In this work, we propose to evaluate the resilience of a plant model (durum wheat, *Triticum durum*), subjected to water stress by monitoring the activity of not only the antioxidant enzymes (CAT, APX, GPX) but also by localization of produced hydrogen peroxide(H_2O_2) in the medium.

2. MATERIALS AND METHODS

2.1 Biological Material

Biological material used in this work was the roots and seeds of the hard wheat *Triticum durum Desf* GTA variety. The samples were received from the International Office of Algerian Cereals (CATO) El Hadjar, Annaba, Algeria.

2.2 Conduct of the Trial

The trials were led at the Cellular Toxicology Laboratory, University of Annaba, and the laboratory of Plants Cellular and Molecular Physiology, Pierre and Marie Curie, Paris VI, France University.

Seeding was carried out in plastic pots. After 4days of germination, the seeds of wheat are submitted to water stress by stopping irrigation and wheat samples were analyzed at 3rd, 5th, 7th and 9th day after interruption of watering. Controls were watering normally.

Germination took place at day temperature of 21ºC and 17-21ºC at Night with artificial lighting from 6 am to 10 pm.

2.3 Roots Relative Turgidity in Water(RWT)

The relative turgidity in water was determined by the percentage of water present in the excised roots and immersed in water for 2 hours, depending on the method of Clark and Mc Caig [9], which was used by Rascio [10]. The calculation is done according to Ladigues [11].

2.4 Measurement of the Enzyme Activity

To obtain the enzyme extract of the durum wheat roots Loggni et al. [12] method was followed. The extract was used for the measurement of catalase activity (CAT) according to the method of Boscoloa et al. [13], as corbate-peroxidase (APX) according to the method of Manivannan et al. [14] and guaiacol-peroxidase (GPX) according to the method of Hiner et al. [15].

Fresh roots (1g) were ground with cold mortar in a phosphate buffer (50mlNaK, pH7.2) at a rate of 1ml buffer per1g of roots. The homogenate was then filtered and centrifuged cold 12000xg for 20 min (CentrifugeSigma3-16K). The obtained super natant was used for the determination of various enzymatic activities.

2.5 Localization of Hydrogen Peroxide H_2O_2

Intercellular ROS production was visualized using DCFH-DA (Molecular probe) [16] with a fluorescence microscope. The root was incubated in a buffer of 20 mM potassium phosphate (pH6) containing 50 microns DCFH-DA for 60min at 20ºC in the dark. The samples were washed three times in the buffer solution. The images were taken (excitation: 480nm, emission: 525 nm) with a fluorescence microscope using a 05* numerical target. To compare the density of DCFH-DA, samples from different experimental conditions were set at the same time and analyze dunder a fluorescence microscope using the same experimental parameters.

2.6 Measurement of the Hydrogen Peroxide H_2O_2 Production

The roots were incubated in 250ml of buffer solution of potassium phosphate (20mM,pH6) containing 5µ Mscopoletin (Sigma) and1U/ml (final concentration) horse radish peroxidase at 25ºC in the dark using a haker as described by Schopfer et al. [17]. H_2O_2 production was assessed by the decrease in fluorescence (excitation 346nm, emission 455nm) o the incubation medium and was converted into a molar concentration of H_2O_2 using a linear calibration curve. The results were expressed as mg fresh weight of roots.

3. RESULTS

3.1 Roots Relative Turgidity in Water (RWT)

Fig. 1 represents Roots Relative turgidity in Water (RWT), determined by the percentage of water present in the roots. A significant decrease in roots subjected to water stress was observed.

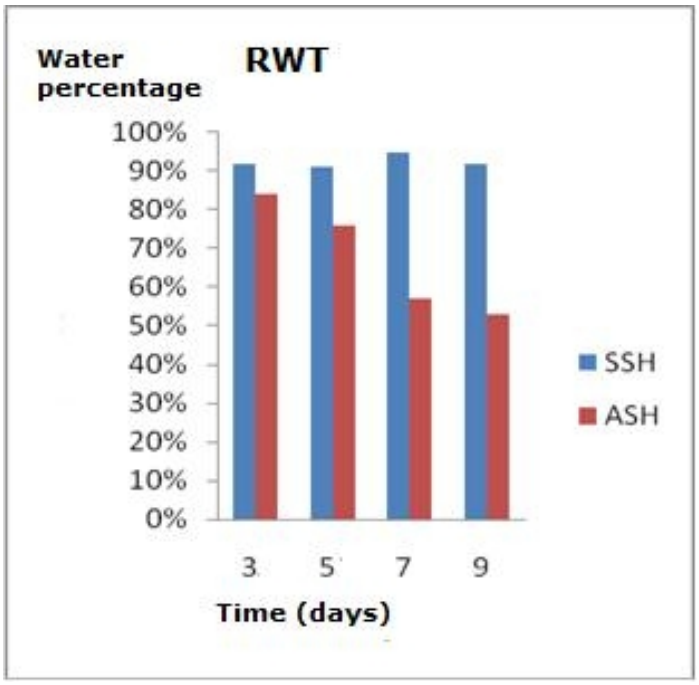

Fig. 1. Roots relative turgidity in water (%)
SSH without water stress. ASH: with water stress

3.2 Enzymatic Activities

The activities of catalase, as orbate peroxidase and guaiacol-peroxidase in seeds subjected to water stress (Table 1) showed a positive correlation between these verity of water stress and enzyme activity from the 3rd day.

Table 1. Effects of water stress on the activities of catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) in the hard variety GTA seeds subjected to water stress

Time (days)	3d	5d	7d	9d
SSH CAT	0.00679326	0.00656357	0.00637979	0.00661052
ASH CAT	0.00543	0.01263091	0.02009793	0.0263769
SSH APX	0.02791878	0.02152284	0.01919705	0.03570803
ASH APX	0.05908629	0.06608214	0.13758096	0.2551345
SSH GPX	0.01151846	0.00956	0.00997923	0.0103138
ASH GPX	0.01767864	0.02453011	0.01353039	0.01094277

SSH: without water stress.
ASH: with water stress

3.3 Measurement and localization of Hydrogen Peroxide (H_2O_2)

The evolution of the average grade of H_2O_2 in the roots Fig. 2 showed as significant production of Hydrogen Peroxide from the 5^{th} day, which tends to increase gradually as the stress level increases. Figs. 3 and 4 confirm the presence of H_2O_2 at the roots subjected to stress, visible as fluorescent granules (few root cells are highly vacuolated). In controls Fig. 3 no trace of H_2O_2 was observed.

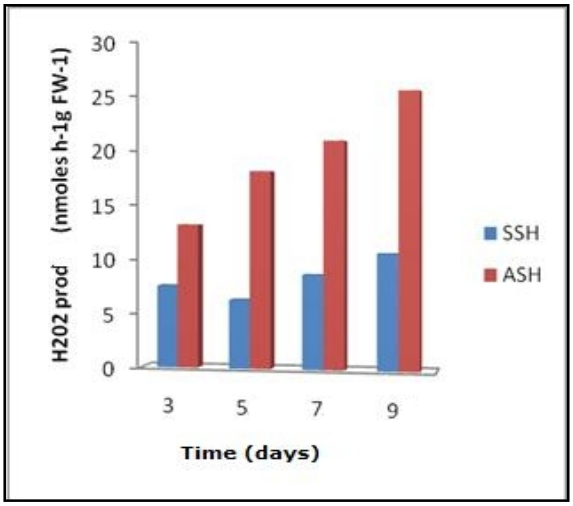

Fig. 2. Average content of hydrogen peroxide produced in the roots of durum wheat.
SSH: without water stress.

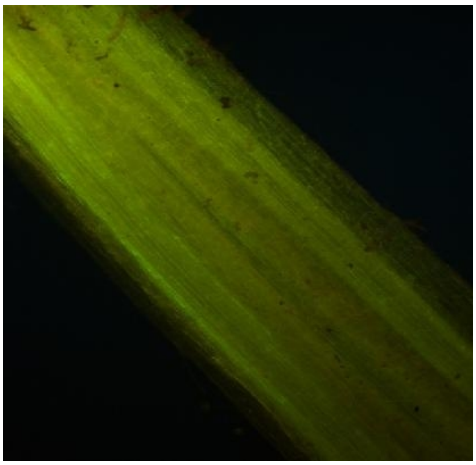

Fig. 3. H_2O_2 in controls.

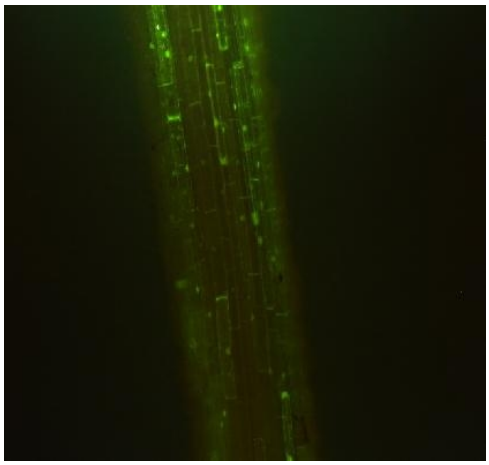

Fig. 4. Hydrogen Peroxide in stressed plants.

4. DISCUSSION

In our work, it was demonstrated that one of the consequences of water stress in roots of durum wheat, is the appearance of oxidative stress, causing he accumulation of reactive species oxygen species [18], proportional to the intensity of the induced stress.

Relative stability of H_2O_2 compared with other ROS and its ability to cross the membrane makes it suitable for signaling. It can induce the intracellular ROS scavenging system by activating the antioxidant enzymes and by modulating the expression of genes of these enzymes [19].

Faced with this attack, root cells synthesize a number of antioxidant enzymes that's cavenge these ROS. The results were demonstrated there spective increases in CAT activity, APX and GPX in stressed seeds [20].

Mitochondria are a major source of ROS in plants [21]. Nevertheless, the production of ROS from the mitochondria is variable depending on the involved organ and the environmental conditions (e.g. drought) [22].

Under normal physiological conditions, approximately 2% of the total oxygen consumed by mitochondria was converted to ROS [23-24]. The radical intermediates of ubiquin one formed by reducing the pool of ubiquin one by complex I and III of the Electron Transport Chain(ETC) are the main donors of electrons to oxygen.

ROS production close to CTE may cause membrane damage, leading to a further decrease in the efficiency of the CTE and greater loss of electrons. The result is an increase in the generation of ROS further decreasing efficiency of the CTE. When significant stress initiates the generation of ROS this can lead to deleterious production cycle. Mitochondrial Cycle of ROS generation is considered one of the main causes of cellular aging [26].

Undoubtedly, drought stress induces ROS generation as a primary response of plant and this may be mediated by hormones like ABA and ethylene as well, which may sometimes

play a downstream role too. Gross level of ROS could exacerbate the stress-induced damages to most of the cellular components unless compromised because of ROS scavenging by antioxidant system. However, depending on spatial and temporal ROS generation and scavenging (ROS management) responses can be characterized as toward conferring protection by arousing the protection system or as directly leading to injuries or death. Gradual imposition of drought stress, which is more common in nature, probably triggers ROS generation in the apoplast by plasma membrane-localized NADPH oxidase where Ca2+ plays a role as an upstream as well as downstream messenger forming a positive feedback loop. Apoplast is ideal site for initiation for signaling by ROS (localized oxidative burst) as this compartment has less redox buffering capacity, which could have otherwise diffused the signal strength. Efficiency for such an apoplast based ROS signaling system has already been appreciated in case of defense against pathogen (biotic stress). Now the fate of the cellular system subjected to water stress depends on whether intracellular ROS scavenging system, being stimulated by the ROS signal of extracellular origin, keeps the oxidative load low on the cellular components or a rapid intracellular ROS accumulation has taken over before defense system is put in place. This again depends on speed and magnitude of stress imposition as well as the plant system subject to the stress, the latter being variable genetically as regard to the chronology and propensity of ROS management toward orchestration of defense related changes under stress [27].

COMPETING INTERESTS

Authors have declared that No competing interests exist.

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