

Protective enzyme system and osmoprotection in durum wheat (*Triticum durum*. Desf.) leaves under water deficit

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Abstract: *Abiotic stresses, such as high temperature, and salt stress are major factors which reduce crop productivity. The effects of water deficit on physiological traits, soluble protein content and protective enzyme system of durum wheat leaves were studied . The results indicated that the stomatal resistance (SR) increased under drought condition in all studied genotypes while the chlorophyll content and relative water content (RWC) decrease in the drought condition. Soluble protein content, SDS-PAGE indicated the presence of specific bands in both control and stress condition. The peroxidase (POD) activity in durum wheat leaves was lower in control condition, and then ascending obviously under the drought condition , the highest value was obtained by the adaptive genotype Waha however the lowest activity was enregistered by the introduced genotype Colesseo. It is suggested that the soluble protein and cellular protection enzymes, such as peroxidase activity, play an important physiological functions for cellular plants protection under drought stress.*

Keywords drought, wheat, soluble protein, peroxidase, RWC, Chlorophyll, RS

Introduction

Drought, cold and salinity are the major abiotic stresses affecting wheat crops (Turner et al. 2001). As drought is the major constraint in wheat production, it is essential to develop varieties which can make use of available water resources and produce maximum yield. Drought is defined as a water stress due to lack or insufficient rainfall and/or inadequate water supply (Toker et al. 2007).

The seriousness of drought stress depends on its timing, duration and intensity (Serraj et al. 2003). Often drought is accompanied by relatively high temperatures, which promote evapotranspiration and hence could accentuate the effects of drought and there by further reduce crop yields.

Many physiological processes associated with crop growth and development is reported to be influenced by water deficits (Turner and Begg 1978 ; Bousba & al, 2009). Previous research has shown various physiological and biochemical changes in durum wheat plants (Ykhlef et al, 2011) when drought stressed, such as the regulation of stomatal aperture, osmotic pressure of cells, and protein synthesis (Lu et al., 1994; Cheng, 1995).

drought , salt, heat and oxidative stress are accompanied by the formation of ROS such as O₂, H₂O₂, and OH (Moran et al. 1994 ; Mittler 2002), which damage membranes and macromolecules. Plants have developed several antioxidation strategies to scavenge these toxic compounds. Enhancement of antioxidant defense in plants can thus increase tolerance to different stress factors. Antioxidants (ROS scavengers) include enzymes such as catalase, superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase, as well as non-enzyme molecules such as ascorbate, glutathione, carotenoids, and anthocyanins. Additional compounds, such as osmolytes, proteins can also function as ROS scavengers (Bowler et al. 1992; Noctor and Foyer 1998). The objective of the present study was to evaluate the effect of water deficit on physiological, soluble protein content and antioxidant enzymes in durum wheat by using ten cultivars comprising (drought resistant and drought sensitive cultivars) (Bousba et al, 2011)

Material and methods

Plant material and growing conditions

Ten varieties of *Durum wheat (Triticum durum*. Desf.) Local and introduced released by the technical institute Crop El Khroub Constantine 'ITGC' are the subject of this present study (**Table.1**). These cultivars are characterized by contrasting agricultural productivity.

Ten seeds are germinating in Petri dishes. After germination, seedlings are transplanted into plastic pots with mixture of (soil / sand 3:1). The pots are arranged randomly (total randomization) in green house with an average temperature of 36.75±10°C and relative humidity of 47.55±8%.

After plant emergence, pots were irrigated at field capacity and weighed every day, until flowering stage. At this stage two water regimes were imposed. They consisted of 100% (control) and 12% of field capacity (f.c), each treatment was replicated three times.

Table.1 : origin and pedigrees of studied genotypes

| genotypes | Origin | pedigrees |
|------------------|---------------|--|
| Cirta | Algeria | Hedba3/GDO VZ 619 |
| Oued Znatie | Algeria | old local landrace |
| Beniswif-3 | Egypt | Corn"S"/Rufo"S" |
| Cannizzo | Italy | Advanced/improved cultivar |
| waha | ICARDA | PELICANO/RUFF//GAVIOTA/ROLETTE; |
| Beltagy3 | ICARDA | ICD97-0396-T-1AP-AP-5AP-0AP-16AP-AP |
| Djenah khotifa | Algeria | Local landrace selection from North Africa |
| Kyperounda | Cyprus | landrace |
| Colosseo | Italy | CRESO/MEXA |
| Line 3D | Egypt | / |

Measurements

stomatal resistance (Rs)

Was done with a portable leaf Porometer measurement system (AP4). The measurements are made at flowering stage on the flag leaf fully attached with three replications for each measure. stomatal resistance RS is estimated by $(m^2.s.mol^{-1})$

Chlorophyll content (SPAD Unit)

the rate of chlorophyll in the leaves is determined by using a chlorophyll meter, (model MINOLTA type SPAD). Before any measure, the device must be set (number of signal tower) and size (N = 0). In this protocol the rate of chlorophyll is estimated per unit SPAD.

Relative Water Content (RWC%)

Relative water content (RWC) was determined according to the method adopted by Turner and Kramer (1980) using the following equation:

$$\text{RWC} = [(\text{FW} - \text{DW}) / (\text{TW} - \text{DW})] \times 100.$$

With **FW**: fresh weight, **DW**: dry weight and **TW**: fresh weight at full turgidity.

Estimation of soluble protein content

Total soluble protein was determined by following the method of Bradford (1976). Fresh plant material of 0.5g was homogenized in 5ml of extraction buffer (50mM Tris-HCl; pH-8, 1mM PMSF, 10% (v/v) glycerol) and centrifuged. Aliquot of the extract was used for determining protein concentration using bovine serum albumin (BSA) as a standard.

SDS-PAGE

Was conducted on 12.5 % acrylamide gels according to the description of Laemmli, 1970. An electrophoresis calibration kit was used to determine the molecular weight of proteins and the gels were stained with 0.03% Coomassie Brilliant Blue.

Peroxidase (POD) activity

The peroxidase (POD) activity was determined according to Fielding & al. (1978). The POD reaction system consisted of the following components: 2.91 ml phosphate buffer (10 mmol L⁻¹, pH 7), 0.05 ml guaiacol (20 mmol/L), 40 mmol/L H₂O₂, and 0.02 mL enzyme solution. The POD activity was determined by spectrophotometer (HITACHI) at 470 nm colorimetric wavelength, and the units represented are $\mu\text{Kat}/\text{mn}$.

Data analysis

The experiments were performed in completely randomized design with three replicates. Differences among the treatment as well as between the cultivars were tested using the STATISTICA software program (Version7.0). Hence analysis of variances of all the parameters was performed and differences at $p < 0.05$ were considered significant.

Results and Discussion

In this study, stomatal resistance developed by the ten varieties studied is also significantly affected by the water deficit ($p < 0.01$) (**Table. 2**). The differences were observed since the installation of water stress and stomatal resistance increases as the stress becomes more severe (12% f.c). Oued znatie was the genotype showing the highest value ($54.3 \text{ m}^2 \cdot \text{s} \cdot \text{mol}^{-1}$) compared to other cultivars, followed by the introduced variety Collesseo, enregistreng an smaller increase of this trait (**Fig. 1**). In this concern (Sarda et al, 1993), reported that *durum wheat* under conditions of drought condition, seems to favor the first strategy is to enable the closure of stomata and limit the intensity of transpiration by plants. The maintaining of satisfactory photosynthetic activity by the local variety (Oued znatie) against the applied water stress in our study, despite the decrease in internal CO_2 concentration due to the closure of stomata, can result by the establishment of the non stomatic mechanisms as a means of adaptation to limit the water loss (Ykhlef and Djekoune, 2000 ; Shao, 2005).

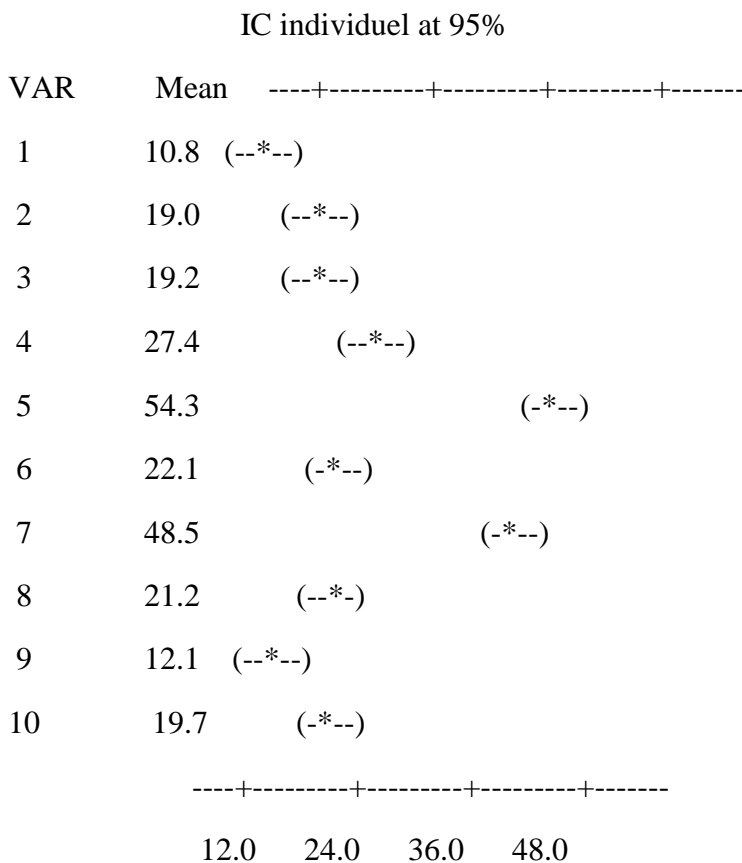


Figure. 1 means of stomatal resistance ($\text{m}^2/\text{s}/\text{mol}$) for studied genotypes

The variation of the rate of chlorophyll, under water stress provides information about the behavior of varieties towards the water deficiency. The chlorophyll rate of the local varieties Cirta and Oued znatie decreased under severe water regime (12% f.c), in contrast, the introduced varieties Bensouif and Beltagy were characterized by the highest rates under the same condition. The result of ANOVA for this trait showed that chlorophyll content changed significantly under drought stress (Table 2). The difference for this trait was also observed among genotypes. The interaction between drought treatment and genotypes was found significantly. The decrease in chlorophyll under stress condition can be attributed to the sensitivity of this pigment to increasing environmental stresses, especially to salinity and drought, which has been reported by several researchers (Mekliche et al., 2003; Younis et al., 2000); many studies indicated that stay-green is associated with improved yield and transpiration efficiency under water-limited conditions in sorghum, maize and wheat (Borrell et al. 2000; Verma et al. 2004). This is in good agreement with the result of Rong-hual et al, 2006, that chlorophyll content and photosynthetic traits were significantly different between drought tolerance and sensitive genotypes under water deficit condition, and values of these photosynthetic traits under drought stress were highly associated with grain yield.

In well condition of water supply, Relative water content is significantly higher ($p < 0.05$) compared to drought conditions (**Table. 2**), it also notes that the effects of interaction (genotypes x water regime), were highly significant, it means that the different studied varieties varied in their behavior against the drought. This result confirms a previous finding on durum wheat (El Jaafari. 2000), that showed the effect of water stress on RWC in wheat plants (Atefeh et al, 2011). For protein soluble content a total of 29 bands were detected with different molecular weights ranging from 9 to 112 KDa. Among such bands, protein bands were clearly observed in stress condition (13 bands), while the other 15 bands were varied in some distinctive genotypes under well watered condition. The hierarchical classification based on eucliden distance shows six distinguished groups (**Fig. 2**), where as some newly induced soluble protein bands were expressed under water drought stress such as

(112, 97, 57, 48 and 15) KDa in Colesseo, Line3D, Kyperounda, Beniswif and Cirta varieties.

Table.2 Analysis of variance for studied variables Chlorophyll (U.SPAD), RWC% & Stomatal resistance m²s/mol

| Source of variation | Chlorophyll (U.SPAD) | | | | | RWC% | | | | Stomatal resistance m ² s/mol | | | |
|---------------------|----------------------|--------|-------|--------|---------|--------|--------|-------|---------|--|--------|--------|---------|
| | DF | SS | MS | Fcal | P | SS | MS | Fcal | P | SS | MS | Fcal | P |
| Genotypes | 9 | 1380.8 | 153.4 | 12.17 | 0.00*** | 976.4 | 108.5 | 2.570 | 0.02* | 11433.4 | 1270.4 | 81.37 | 0.00*** |
| treatments | 1 | 2427 | 2427 | 192.49 | 0.00*** | 4737.3 | 4737.3 | 112.1 | 0.00*** | 4896.1 | 4896.1 | 313.61 | 0.00*** |
| Interaction | 9 | 461.8 | 51.3 | 4.07 | 0.00*** | 897.4 | 99.7 | 2.360 | 0.03* | 3853.5 | 428.2 | 27.43 | 0.00*** |
| Error | 40 | 504.3 | 12.6 | | | 1690.4 | 42.3 | | | 624.5 | 15.6 | | |
| Total | 59 | 4774 | | | | 8301.5 | | | | 20807.4 | | | |

*, ** & *** significant at 5% , 1% et 0.1% respectively

Chen and Wang, 2003; Zhu and Zhang, 2003; Xie et al., 2005) founded that the synthesis of some original proteins (namely stress-induced proteins) may be induced or upregulated to adjust osmotic potential of cells in order to keep a certain turgor and thus to ensure the normal proceeding of physiological processes such as cell growth, stomatal opening and photosynthesis it can concluded that to cope with environmental stress, plants activate a large set of genes leading to the accumulation of specific stress-associated proteins (Vierling 1991; Ingram and Bartels 1996; Bohnert and Sheveleva 1998; Thomashow 1999; Hoekstra et al. 2001). Heat-shock proteins (Hsps) and late embryogenesis abundant (LEA)-type proteins are two major types of stress-induced proteins that accumulate upon water, salinity, and extreme temperature stress. They have been shown to play a role in cellular protection during the stress (Bakalova et al. 2008 ; Thomashow 1998).

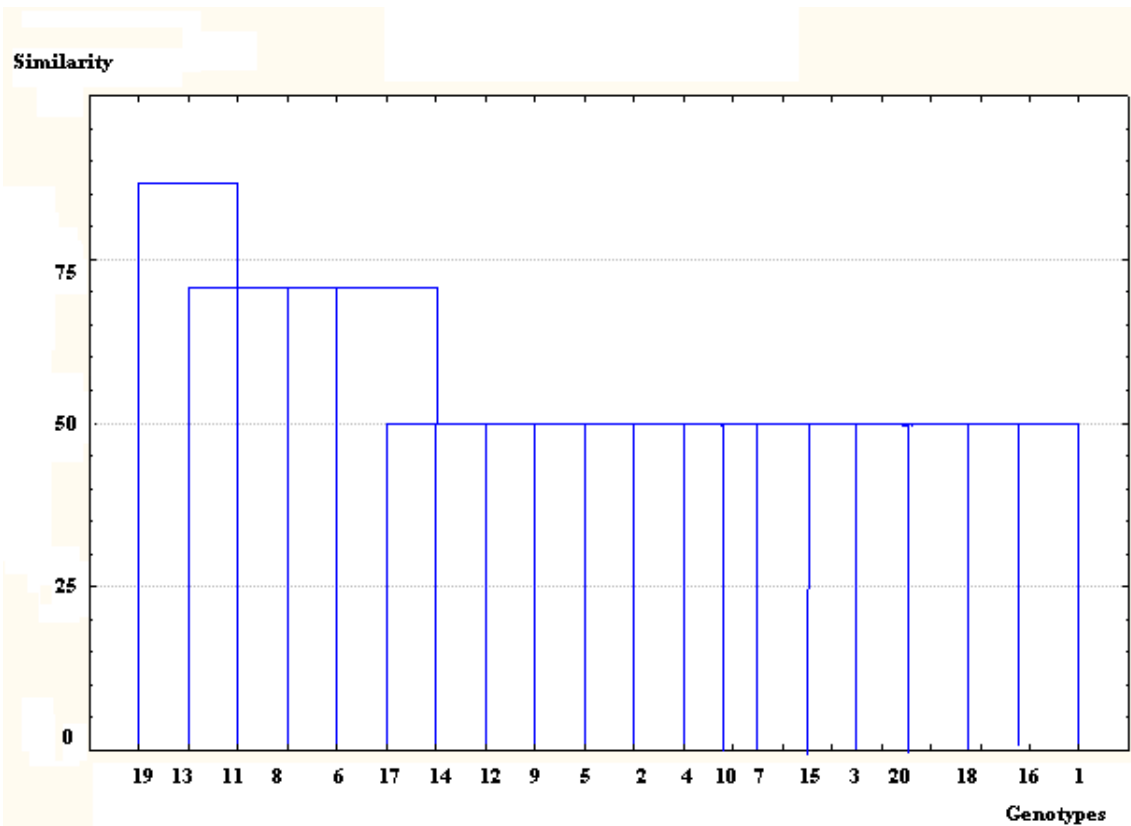


Figure. 2 protein profiles of studied genotypes under water deficit

1 Cirta, 2 Cirta s, 3 Oued Znatie, 4 Oued Znaties, 5 Bensouif, 6 Bensouifs, 7 Cannizo, 8 Cannizo s, 9 waha, 10 waha s, 11 Beltagy, 12 Beltagy s, 13 Djenah khotifa, 14 Djenah khotifa s, 15 Kyperounda, 16 Kyperounda s, 17 Colosseo, 18 Colosseo s, 19 Line3D & 20 Line3D s

Figure 3 shows, that the POD activity in all genotypes gradually increased with the drought treatment. So the highest activity of peroxidase was obtained in the adapted genotype (waha) (0.16 μ Kat/mn) followed by Cirta, Oued znatie Beniswif and Beltagy in stress condition, however the sensitive genotype Collesseo shows the lowest value in the same condition (0.06 μ Kat/mn) (**Fig. 3**). Increased activity of POX has been suggested as an adaptive mechanism to reduce the H₂O₂ and offer protection against oxidative damage (Agarwal and Pandey, 2004; Nagesh & al, 2008). It was reported that a positive correlation was observed between the activity of protective enzyme system and the antioxidation protection under drought (Wang et al., 2008; Dongxiao & al., 2010).

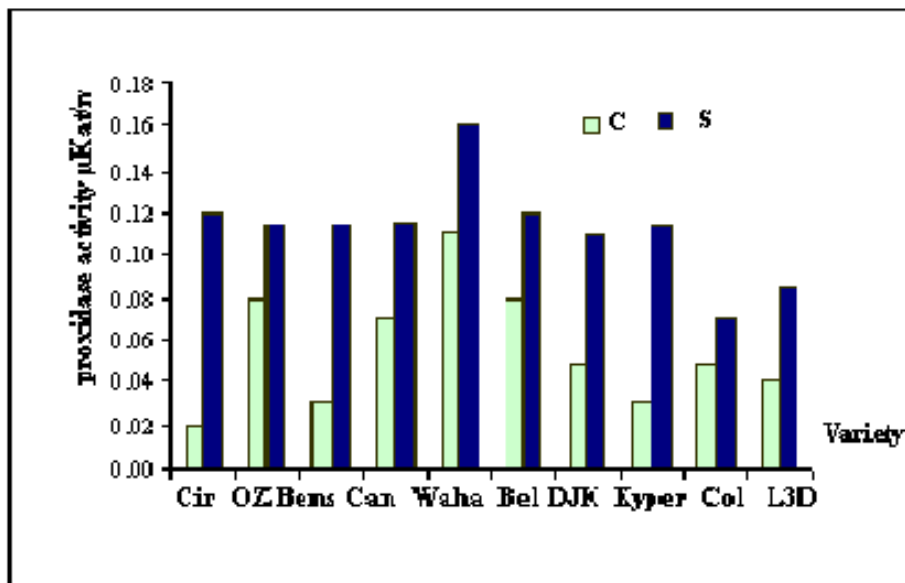


Figure. 3 the effect of drought stress on peroxidase (POD) activity in *Durum wheat* leaves.

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