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Glutathione status and its effect on protein content during drought and subsequent rehydration in two spring wheat cultivars *(Triticum aestivum* L.)

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ABSTRACT
Objective: Wheat crop may experience water deficit during its life cycle, which induces oxidative stress. The present study evaluated the role of oxidative stress management in the leaves of two wheat (*Triticum aestivum* L.) cultivars, Veery (drought tolerant) and Sids-1(drought susceptible) under drought stress and subsequent rehydration. The role of glutathione in the antioxidative defense system provides a rationale for its use as a stress marker.
Methodology and results: When wheat seedlings were subjected to progressing drought, the initial response was a slight oxidation of the glutathione pool, followed by increased glutathione concentrations. When the stress increased, glutathione concentrations dropped and redox state became more oxidized, which marked the degradation of the system. Proteolytic activities were assayed using azocasein in the case of vacuolar proteinases at pH 5.0. The azocaseinolytic activity was found to be confined to the vacuoles. Drought stress increased vacuolar azocaseinolytic activity at both stages of water shortage treatments, but the increase was significantly lower after rehydration and in the tolerant cultivar.
Conclusion and application of findings: The results obtained indicate that protein synthesis and degradation are affected by dehydration and it is genotype dependent. Within such response patterns the glutathione system is a valuable stress marker in ecophysiological studies.

Key words: Drought, rehydration, *Triticum aestivum*, glutathione, proteolytic enzymes, ecophysiology

INTRODUCTION

Drought is one of the major ecological factors limiting crop production and food quality globally, especially in the arid and semi-arid areas of the world. However in certain tolerant/adaptable crop plants morphological and metabolic changes occur in response to drought, which contribute towards adaptation to such unavoidable environmental constraints (Blum, 1996). Among crop plants, wheat (*Triticum aestivum*), which often experiences water-limited conditions, is an attractive study system because of the natural genetic variation in traits related to drought tolerance (Loggini *et al*., 1999). *Triticum aestivum* L. is a staple food for more than 35% of the world population and is also one of the most important grain crops in different countries, whose production status is directly related to social stability and sustainable development.

The ability of higher plants to tolerate water deficit varies considerably among different species and large differences in water loss may occur within single species and in individual organs of the same species (Bray, 1997). Among higher plants, only a small group of angiosperms (resurrection plants) can survive dehydration and can recover from complete dryness within several hours of rehydration (Bartels & Nelson, 1994). Despite significant progress in molecular biology of the drought response, the genes and/or gene products required for dehydration tolerance remain unknown (Ingram & Bartels, 1996; Bray, 1997).

Sinclair and Ludlow (1985) proposed that leaf relative water content (RWC) was a better indicator of water status than was water potential. For reorganization of plant metabolism under water deficiency, proteolysis may be an important mechanism of regulation of cellular activity. Thereafter amino acids may be released for synthesis of new proteins, aberrant proteins formed under water deficit may be degraded and certain proteins may be activated (Jones & Mullet, 1995). Protein carbonylation is an irreversible oxidative process leading to a loss of function of the modified proteins. These oxidized proteins are selectively recognized and degraded by proteolytic enzymes (Palma *et al*. 2002). Extreme environmental conditions that induce oxidative stress have been associated to increased carbonyl groups content and to an induction in protease activity. Endopeptidases, or proteases, which degrade proteins by hydrolyzing internal peptide bonds, are subsequently one of the most well-characterized cell death proteins in plants (Palma *et al*. 2002). Proteases might play a crucial role in the plant adaptation toward environmental stresses such as drought stress which may cause oxidative stress promoted by active oxygen species (AOS) such as (O_2^-, H_2O_2 and OH) (Coffeen & Wolpert, 2004). AOS are toxic to the cells and cause membrane damage by lipid peroxidation, suggesting that the tissue suffer from hydroxyl groups resulting in alterations in function of membrane (Chaves *et al*., 2003).

Plants have the ability to improve their dehydration resistance by activation of antioxidant defense system and their redox reactions play a central role in the acclimation of plants to their environment, which made glutathione a suitable candidate as a stress marker. The glutathione system is efficient provided that GSSG is rapidly reduced to GSH by glutathione reductase GR (EC 1.6.4.2). Severe drought at low relative water contents caused oxidation of the glutathione pool in wheat leaves (Loggini *et al*., 1999). The progressive increase in GSSG with increasing water deficit suggests that GSSG may mediate the water deficit induced inhibition of protein synthesis reported by several workers (Nir *et al*., 1970; Dhindsa & Bewley, 1976). It has been shown that GSSG activates a translational inhibitor, a protein kinase, which phosphorylates and thereby inactivates the initiation factor eIF-2 as discussed by Ernst *et al*. (1978, 1979).

It has been suggested that sugar phosphates, NADPH, and thiol-reducing systems are required to maintain a high rate of protein synthesis probably by keeping the inhibitor in the inactive state (Jackson *et al*., 1983).This probably means that such a level of reductants (NADPPH and GSH) are needed to keep the GSSG concentration from increasing above a certain level. GSH plays a major role in maintaining the redox state of cells (Meister, 1983) and its oxidized form, glutathione disulfide (GSSG), is known to inhibit both in vivo and in vitro protein synthesis (Kosower *et al*., 1971). Mediation by GSSG of water deficit-induced damage to the protein synthesizing apparatus is suggested and discussed. Dhinds and Matowe (1981) reported that the availability of oxidants during rehydration would cause changes in the glutathione status of the tissue and GSH, as percent of total glutathione, increased significantly whereas, GSSG increased during water deficit. Lascano *et al.* (2001) found no clear differences between four different drought-tolerant wheat varieties after one month of drought in the field, but the two more resistant cultivars responded with an increase in total glutathione during the rewatering period after treatment.

Proteolytic activities in response to senescence are well documented (Noodén *et al*., 1997; Fischer *et al*., 1998) but present knowledge

of proteolytic activities under dehydration remains fragment. Zagdaska and Winiewski (1998) have indicated that the activities of proteinases degrading azocasein in wheat leaf extracts, in response to dehydration, increased about 7-fold at the acid pH optimum. Acclimation had no effect on the quantitative induction of azocaseinolytic proteolysis, but the contribution of prolonged dehydration was significantly increased in total proteolytic activity (Zagdaska & Winiewski, 1998).

The objective of the present study was to investigate mechanisms responsible for drought tolerance in two wheat cultivars, Veery (drought tolerant) and Sids-1(drought susceptible). The effect of drought was studied to elucidate the mechanisms that confer protection from oxidative stress and to analyze the recovery after rehydration. For these purposes we studied the glutathione system of the tissue and its relation to protein synthesis in both cultivars upon dehydration and rehydration.

MATERIALS AND METHODS

Two wheat (*T. aestivum* L.) cultivars, namely Veery (drought tolerant) and Sids-1(drought susceptible) was provided by Prof. M. Nageeb, Faculty of Agriculture, Alexandria University, Egypt. Seeds were grown in plastic pots (15 cm diameter x 20cm height). (40 pots per cv). Seeds were soaked in continuously aerated distilled water for 24 h in darkness to remove the dormancy of the seeds. At the end of soaking period, twelve seeds were sown in each pot containing 1700 g sterilized sandy soil(70%) and 30% vermiculite under16/8 hr light/ darkness cycle. Light intensity was 420µmol $m^{-2}s^{-1}$ at the canopy of plant supplied by a mixture of fluorescent and incandescent lamps and at controlled temperature of 28/26 ˚C and 55/60 relative humidity. The pots were irrigated by distilled water. After fifteen days from sowing, the pots were irrigated with half strength of Hoagland solution only up to twenty eight days then the pots for each cultivar were grouped into two sets In the first set, control (non-stressed) plants were maintained under these conditions throughout the experimental period. In the second set, plants were drought pretreated (drought acclimated) by cessation of watering for 8 days. After the first drought phase plants were rewatered by 150 ml distilled water for 48 h and then subjected to second drought phase extended for 12 days. To eliminate the indirect effect of drought on plant development, all measurements were carried out with the leaves at the same developmental stage (developmental control). Sampling was done around midday between 11:00 and 12:00 h from control and stressed/rewatered plants for quantifying the relative water content, carbonyl concentration level and antioxidant defense components such as glutathione status. For the antioxidant metabolites and enzyme assays, the leaves were cut into small pieces, weighed 0.2 g in replicates, frozen in liquid nitrogen and stored at −80 C. Three replicates were maintained for all the measurements.

Relative Water Content (RWC): Relative water content (RWC) was measured using leaves after treatment. Immediately after cutting at the base of lamina, leaves were sealed within plastic bags and quickly transferred to the laboratory. Fresh weights were determined within 2 h after excision. Turgid weights were obtained after soaking leaves in distilled water in test tubes for 16 to 18 h at room temperature (about 20°C) and under the low light conditions of the laboratory. After soaking, leaves were quickly and carefully blotted dry with tissue paper in preparation for determining turgid weight. Dry weights were obtained after oven drying the leaf samples for 72 h at 70°C. RWC was calculated from the equation of Schonfeld *et al*. (1988).

Oxidative stress indices

Protein oxidation: Protein oxidation was measured as carbonyl concentration, following the protocol described by Romero-Puertas *et al*. (2002). After incubation with 10 mM 2,4dinitrophenylhydrazine (DNPH), proteins were precipitated with 20% trichloro acetic acid (TCA). To facilitate pellet re-dissolution, excess acid was washed with 10% H_2O in ethanol: ethyl acetate (1:1, v/v). The pellet was disrupted, and then incubated at 40 °C for 30 min. After incubation, the suspension was centrifuged at 12 000 *g* for 5 min, and absorbance was measured at 370 nm (carbonyl concentration) and at 280 nm (total protein concentration).

Glutathione determination: Two fresh leaves were homogenized in 1.0 mL of ice-cold 2.5 N $HClO_4$. The homogenate was filtered through three layers of cheesecloth (Miracloth) and then centrifuged at

15,000 × *g* for 5 min. The supernatant was neutralized with 5 M K_2CO_3 to pH 6.5 for glutathione determination. The levels of glutathione were measured spectrophotometrically by monitoring the reduction of 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) at 412 nm, after the method of Loggini *et al*.(1999).For measurement of total glutathione (GSH + GSSG), 150 µL of neutralized extract were added to 1.2 mL of 0.3mM NADPH, 150µL of 6 mM DTNB and 1 unit of glutathione reductase (GR). For oxidized glutathione (GSSG) determination, 150 µL of neutralized extract were incubated with 2 µL of 2-vinylpyridine for 1h at 25 °C, and then added to 1.2 mL of 0.3 mM NADPH, 100 µL of 6 mM DTNB, and 1 unit of GR. The concentration of reduced glutathione (GSH) was calculated as the difference between total glutathione and GSSG.

Antioxidant enzyme

Extraction of enzyme: The overall procedure was carried out at 0 to 4 °C. Samples (0.5g) of leaf tissue were ground and homogenized in 20 ml ice-cold extraction buffer (100 mM KH_2PO_4 / K_2HPO_4 (pH 7.8), 300mg polyvinyl pyrrolidone (PVP), 1% (v/v) Triton X-100 and 5mM ascorbate (Schwanz *et al*.,1996). The homogenate was centrifuged at 25,000×*g* (20 min, 4 °C). The extract was used for the determination of glutathione reductase(GR).

Glutathione reductase(GR) assay: GR activity was measured by following the decrease in absorbance at 340 nm due to NADPH oxidation after the method of Carlberg and Mannervik (1985) A 200 µL aliquot of enzyme extract was added to a reaction mixture containing 1.5 mL of 0.1 M potassium phosphate buffer, pH 7, 150 µL of 20 mM GSSG, 1 mL of distilled water and 150 µL of 2 mM NADPH (dissolved in Tris–HCl buffer, pH 7), in a final volume of 3.0 mL. An absorption coefficient of 6.2 mM^{-1} cm^{-1} was used for calculations. One unit of GR activity was defined as the amount of enzyme that oxidizes 1nmol of NADPH per min at 25 C. Proteins were extracted from 0.5 g ground frozen tissue with 0.5 ml of extraction buffer [0.1 M Na-phosphate buffer, 0.2% Triton X-100 (v/v), 1 mM EDTA, and 1 mM dithiotheritol (DTT at pH 7.4]. After centrifugation at 12 000 *g* for 15 min. Protein concentration was determined according to Bradford (1976); bovine serum albumin was used as standard.

Measurement of azocaseinolytic activity: It has been shown previously that the maximum azocaseinolytic activities in crude wheat leaf extract was at pH 5.0 (Zagdaska & Winiewski, 1996) and therefore changes in total azocaseinolytic activities in wheat leaves were monitored at this acid pH optimum. The reaction mixture contained in 1 ml: 0.1 ml of the enzyme extract, 0.3 ml of 0.5% azocasein and 0.6 ml of 0.25 M citrate/phosphate buffer pH 5.0. After 2 h at 37 °C the reaction was stopped by adding 2 ml of 12% trichloroacetic acid and acid-soluble products were determined spectrophotometrically at 340 nm. One unit of azocaseinolytic activity was defined as the amount of the enzyme causing a 0.01 increase in A_{340} .

Statistical analysis: The experiment was conducted in completely randomized design. Results are the mean of three measurements per treatment. The significance of difference between means values was determined by one-way analysis of variance. Duncan's multiple range test was used to compare the means of treatments at $P \leq 0.05$.

RESULTS

After completion of the first 8d drought phase, the relative water content (RWC) values were significantly decreased in wheat leaves for both cultivars and reached 45 and 56%, for *cv* Sids-1 and *cv* Veery, respectively and relative to the control (Fig. 1 A). Re-watering resulted in recovery of the decrease that occurred in the RWC and the values became as high as for the control plants, especially for *cv* Veery. Plants subjected to the second drought phase after rewatering showed a highly significant increase in RWC values that reached to 70 and 76% for *cv* Sids-1 and *cv* Veery after 8d drought relative to the control. The corresponding values after 12d drought treatment were 54 and 65%, respectively in comparison to the control (Fig. 1A).

Drought stress induced oxidative stress and increased significantly the oxidative stress parameter in both cultivars but more for *cv* Sids-1. Protein oxidation, as one of the oxidative stress indices was assessed, and a significant increase in protein carbonylation that continued steadily throughout the first drought phase, with the highest levels after 4 and 8d (Fig.1B). On the contrary, two days rehydration resulted in a marked decrease in protein oxidation and reached to values similar to corresponding control. During the second drought phase, after 2d rehydration, a steady increment

of protein carbonylation was observed, especially in sensitive cultivar (Sids-1) and reached to 162% compared to the control at the end of experimental period.

Mild drought stress resulted in a steady increase in GSSG in the fourth day of drought, thus increased from about 8 to nearly 22% of total glutathione. After the completion of the first drought phase GSSG increase further, rather sharply, to more than 46% of the total glutathione. Also imposition of severe water stress resulted in a significant decline in the total glutathione, GSH contents and GSH/GSSG ratio in leaves of both cultivars at the end of the first drought cycle (data not shown). After 2d rehydration period a sharp decline in GSSG content was observed and was almost equal to that in the control plants, reaching a level of 10% of the total glutathione. Total glutathione content after rehydration increased significantly from 115 to 158 n mol g^{-1} of original fresh weight in the case of the sensitive cultivar (Sids-1) ,this value exhibits 90% of the control. Moreover, for tolerant cultivar (Veery), the corresponding increment was from 134 to 183 n mol g^{-1} and reached 97% of the control (data not shown).Thereafter, GSSG more rapidly declined in the *cv* Veery than in the *cv* Sids-1.

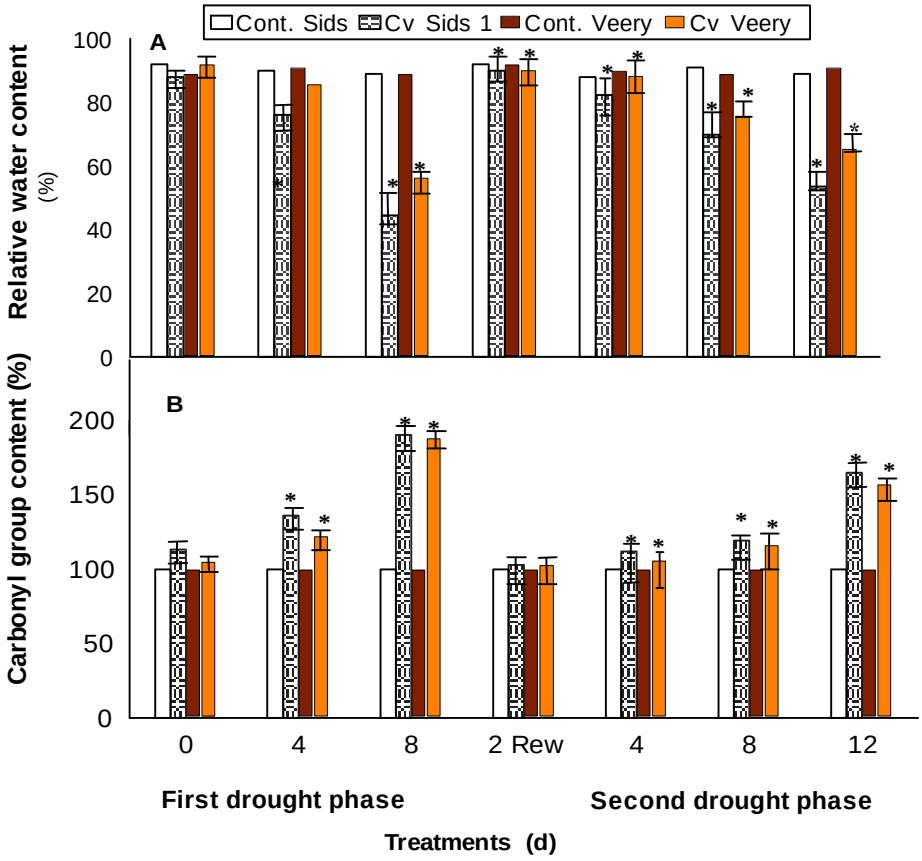

Figure 1: relative water content (%) (A) and Carbonyl group content (B) of well watered and drought stressed wheat cultivars *cv* Sids-1 (sensitive) and *cv* Veery (tolerant) subjected to two drought phases interrupted by 2d rewatering. Each value represents the mean ±SE of five replicates. Cont. Sids-1; Control of sensitive cultivar, *cv* Sids-1; drought stressed sensitive cultivar, Cont. Veery; Control of tolerant cultivar, *cv* Veery; drought stressed tolerant cultivar, 2 Rew; 2 days rewatering. Significant differences ($P<0.05$) between treatments according to LSD test are shown by an asterisk.

Glutathione reductase activity had a similar tendency in the two wheat cultivars tested. Statistically increased activity (1.8 fold) was found after 2 d drought as compared to the control (Fig. 2). Prolonged exposure to

drought significantly decreased the GR activity after 8d drought. GR activity for *cv* Sids-1 and *cv* Veery dropped to 54 and 71% ($P<0.05$), respectively and relative to the 2d drought stressed wheat plants, but was still higher than in the corresponding control (Fig. 2). The activity of GR increased in both cultivars after rewatering period, the increase was more significantly in *cv* Sids-1 than in *cv* Veery, reaching values similar to those found in the corresponding control (Fig. 2). However, the GR activity of wheat plants exposed to the second drought phase after rehydration, had a tendency to increase even after 8d drought in both cultivars. Thereafter, at the end of the second drought phase a more significant decrement in GR activity was observed clearly in the sensitive cultivar (Sids-1) more than in tolerant cultivar (Veery). The decrement at the end of the second 12d drought phase were nearly similar to the values obtained after the completion of the first 8d drought phase in non acclimated plants. No significant changes in the activity of GR enzyme in control were observed during the experimental period.

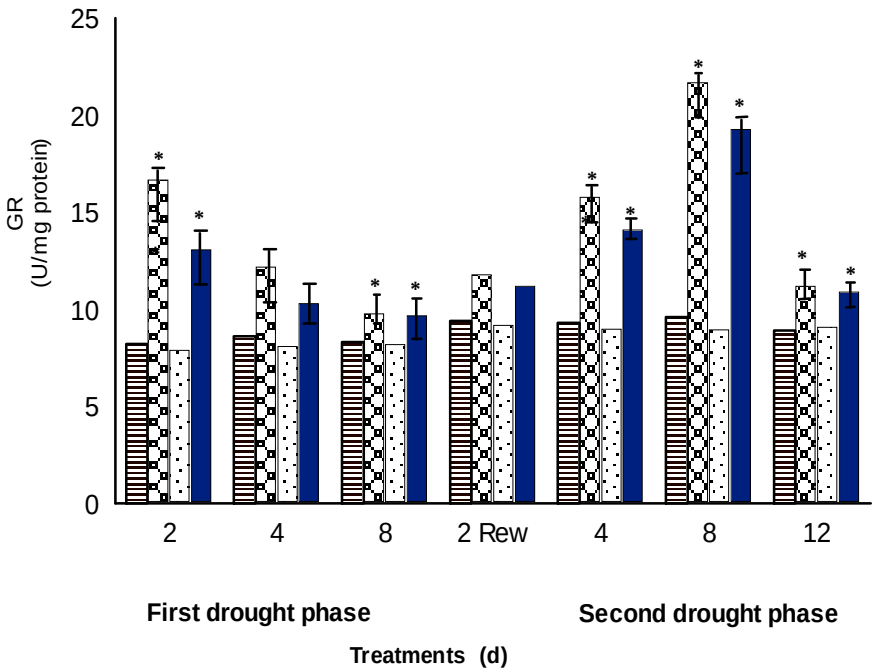

Figure 2 : Glutathione reductase(GR) activity of well watered and drought stressed wheat cultivars $_{cv}$Sids-1 and $_{cv}$ Veery subjected to two drought phases interrupted by 2d rewatering. Each value represents the mean ±SE of five replicates. Cont. Sids-1; Control of sensitive cultivar, *cv* Sids-1; drought stressed sensitive cultivar, Cont. Veery; Control of tolerant cultivar, $_{cv}$Veery; drought stressed tolerant cultivar, 2 Rew; 2 days rewatring. Significant differences ($P<0.05$) between treatments according to LSD test are shown by an asterisk.

To explore the relationship, if any, between glutathione status and protein synthesis, it was considered worthwhile to compare the protein content to those of GSSG changes during the two drought phases interrupted by rehydration period. The results of protein contents are shown in Figure 3 A. A significant decrease in the protein content was detected during the first drought phase in the two cultivars studied. Completion of the first drought phase resulted in a significant decrease in the protein content in drought sensitive cultivar (Sids-1) reaching 43% of the control (Fig. 3A). The corresponding values for the tolerant cultivar (Veery) was 52% of the control with well watered leaves. In contrast, protein content in rehydrated wheat leaves was higher in the two cultivars studied and reached to values similar to the control plants. The protein content was still high in the acclimatized wheat leaves exposed to the second drought phase after rehydration(Fig. 2A). At the end of the second drought phase the protein content for *cv* Sids-1 and *cv* Veery were 66 and 69%, respectively compared to the controls.

Total azocaseinolytic activities in the control, fully-turgid wheat leaves, were found to be slightly but insignificantly higher in *cv* Veery leaves (2.97–4.96 units mg^{-1} protein h^{-1}) than in the leaves of *cv* Sids-1 (2.20–3.78 units mg^{-1} protein h^{-1}) (Fig. 3). The azocaseinolytic activities increased significantly throughout the first drought cycle and increased from 1.5–3-fold (Fig. 3B). After 2d rehydration the activities of the enzymes were reduced to values near to the corresponding control. In drought acclimated wheat leaves, azocaseinolytic activity increased in the second drought period to a still lesser extent about 2-fold from 3.11 to 6.72 units mg^{-1} protein h^{-1} in control leaves as compared to 7.17 units mg^{-1} protein h^{-1} in drought tolerant wheat cultivar. The corresponding values for drought sensitive wheat cultivar were 6.03units mg^{-1} protein h^{-1}.

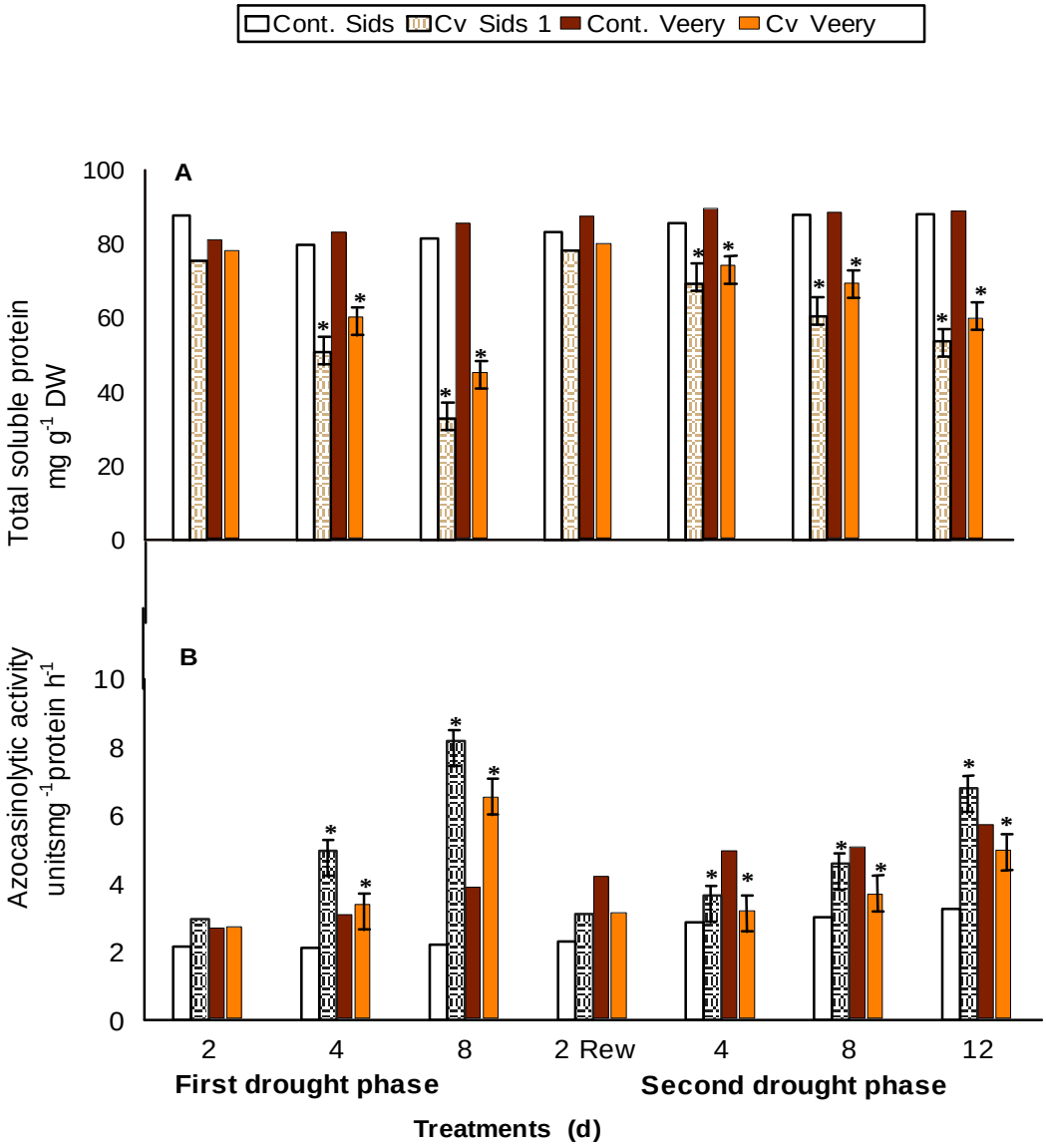

Figure 3: Total protein content (A) and Azocaseinolytic activity (B) of well watered and drought stressed wheat cultivars *cv* sids (sensitive) and *cv* Veery (tolerant) subjected to two drought phases interrupted by 2d rewatering. Each value represents the mean ±SE of five replicates. Cont. Sids-1; Control of sensitive cultivar, *cv* Sids-1; drought stressed sensitive cultivar, Cont. Veery; Control of tolerant cultivar, *cv* Veery; drought stressed tolerant cultivar, 2 Rew; 2 days rewatering. Significant differences ($P<0.05$) between treatments according to LSD test are shown by an asterisk.

DISCUSSION

The relative water content in wheat leaves of the examined cultivars varied significantly in response to drought treatments before and after rehydration, suggesting that rewatering after the release of stress enabled full recovery of plant vigor (Fig. 1A). By the time plants were subjected to the second drought treatment, the effects of water stress imposed at the preceding drought treatment had diminished. This phenomenon is in agreement with similar results reported by Techawongstin *et al*. (1993) in water-stressed hot pepper.

Protein carbonylation is an irreversible oxidative process leading to a loss of function of the modified proteins. The present experiment showed a significant increase of carbonyl concentration in response to drought stress in both cultivars tested and after prolonged drought stress, indicating the presence of oxidized protein which are selectively recognized and degraded by proteolytic enzymes (Fig.1B). It was similarly observed that extreme environmental conditions that induce oxidative stress have been associated with increased carbonyl groups content and to an induction in protease activity(Palma *et al.* 2002).

Drought stress caused marked decreases in GSH content in sensitive cultivar and to a lesser extent in the tolerant cultivar (Fig.2 A and B). GSSG levels in the tolerant cultivar (Veery) showed a slight increase during the progressive water shortage followed by a highly significant increase in the last dates of the first drought phase, while in the sensitive cultivar the rise was more intense (Fig.2). The ratio of GSH to GSSG was directly related to the level of drought stress, however, after 2d rewatering, the acclimated wheat leaves for both cultivars, showed higher values at the end of the second drought phase compared to results obtained after the completion of the first drought phase (Fig. 2C). The higher GSH/GSSG ratio could be the result of higher GR activity observed after rehydration period and these results are consistent with earlier studies (Bartoli *et al*., 1999; Loggini *et al*., 1999) and are supported by the observations of Herbinger *et al.* (2002). As stated before, GSH is important antioxidant (May *et al*., 1998a), which is necessary for coping with several environmental stresses. Depletion of GSH/GSSG, as observed under drought treatment (Fig.2C), led to the appearance of oxidative stress in wheat leaves to a high extent, in agreement with May and Leaver (1993). Also the decline of total glutathione concentration in both cultivars after rehydration was moderate and indicative of sufficient defense capacities.

In contrast with our results Loggini *et al.*, 1999 compared two wheat cultivars with different drought tolerance and found that both cultivars responded with a decline in total glutathione concentration and showed a higher (more reduced) GSH/GSSG ratio after drought stress. Lascano *et al.* (2001) found no clear differences in total glutathione between four different drought-tolerant wheat varieties after one month of drought exposure in the field, but the two more-resistant cultivars responded with an increase in total glutathione content during the re-watering period after treatment. The increase of glutathione pool during water shortage confirms the important role of GSH in plant protection against oxidative stress and could be necessary to regulate the levels of ascorbic acid (AsA) and dehydroascorbate (DHA) (Noctor & Foyer, 1998) and (Foyer *et al*., 2001).

The high activity of GR, especially during the first drought phase, maintained glutathione pool in its reduced status. This is confirmed by the different behavior of cultivars, in which a relative low GR activity, also at high degree of drought, was associated with low GSH and high GSSG levels (Fig. 2 A, B and Fig. 3). On the other hand, in particular, after 2d rehydration period and throughout the second drought phase, GR activity increased significantly in both cultivars and consequently a significant increase in GSH content was determined (Fig.2D). This highlights the regulative action of GR in the homeostasis of glutathione pool in wheat plants subjected to water deficit conditions.

The decrease of protein content in drought stressed wheat leaves appeared to be due to change of the relative water content of stressed plants and was more significant in sensitive cultivar (Sids-1) than the tolerant cultivar (Veery). It also coincides in time with the decreased rate of protein synthesis: as GSSG start to decline, the rate of protein synthesis increased. The possibility of a causal relationship between increased GSSG and decreased protein synthesis is strongly supported by the observation that GSSG inhibits in vivo and in vitro protein synthesis (Ernst *et al*., 1978, 1979; Jackson *et al*., 1983.

Drought has been associated with decreased protein levels, increased proteolytic activity, and the up-regulation of cysteine protease genes (reviewed in Beers *et al*., 2004). At the end of the first drought phase, the higher azocaseinolytic activity in dehydrated leaves was observed in both cultivars and parallel with the significant increment of GSSG content, reached to 3.5 and 2 fold increase, respectively relative to control.

A similar mutual compensation of the proteolytic activities was observed in dehydrated non-acclimated flag leaves (Zagdaska, 1995).

A significant negative correlation was found between the soluble protein content and drought severity in both varieties, but to a lesser extent in the tolerant cultivar (Veery). Lower azocaseinolytic activity was referred to cultivar type in order of increasing drought stress and associated with a distinct reduction of soluble protein content in the wheat leaves. These findings indicate that the reduction in soluble protein content of leaves upon exposure to water deficit is associated with the cultivar type of plant rather than drought effect. This is consistent with the finding by Brouquisse *et al*.(1992) who reported that the decrease in protein content in response to drought differed according to the maize types. Thus, it may be proposed that in *cv* Sids-1 protein hydrolysates are exported to sinks such as the youngest leaves. In contrast, in *cv* Veery amino acids for the synthesis of new, stress-induced proteins more suited for survival under water deficiency are needed (Ingram & Bartels, 1996; Bray, 1997).

CONCLUSION

The recent literature confirms a central role of glutathione metabolism in plant responses to environmental stress. The results of this study showed that antioxidant protection in both cultivars is mainly due to the enzymes and molecules involved in glutathione metabolism and rewatering regulates the timing of drought effect in part by delaying the increased proteolytic activity and subsequent protein degradation. These finding might imply that, with drought, alteration of the GSH/GSSG pool is not the only explanation of the induction of drought stress, and other mechanisms of injury might be involved. In this respect, it is known that plants subjected to environmental stresses augment their capability to draw back AOS (May & Leaver, 1993), so that they can survive, but irreversible damage and cell death occurs when that capability is overridden.

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