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Has Glutathione a Key Role in the Resistance to Oxidative Stress in Durum Wheat?

By

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Summary

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Two wheat (*Triticum durum* Desf.) cultivars with different sensitivities to drought were either grown under regular irrigation or subjected to water deficit by withholding water for 35 d. Water-stressed plants were then rehydrated. Dryland conditions induced larger decreases in water potential and relative water content in the more sensitive cv. Adamello than in the more tolerant cv. Ofanto. During dehydration, the glutathione content decreased in both wheat cultivars, but only the more sensitive cv. Adamello required the induction of glutathione reductase and H_2O_2-glutathione peroxidase activities. A protective action of glutathione system against oxidation of sulfhydryl groups of soluble proteins seems to be established during the dehydration and rehydration cycle in both wheat cultivars.

Introduction

Increasing evidence suggests that water stress has its effects directly or indirectly through the formation of activated oxygen following impairment of electron transport systems. To counteract the toxicity of active oxygen species a highly efficient antioxidative defence system, composed of both non-enzymic and enzymic constituents, is present in all plant cells. In particular, glutathione is a very important soluble antioxidant, because it protects many cellular components under oxidative stress. It can directly react with free radicals. During drying it may protect, via thiol-disulfide exchange, the thiol status of proteins, so maintaining their metabolically active form and the activity of enzymes which possess exposed

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thiol groups (GILBERT & al. 1990). In addition glutathione is involved as a substrate for the glutathione peroxidases (GP, EC 1.11.1.9), which reduce H_2O_2 and organic peroxides, so protecting cell proteins and cell membranes against oxidation (NAVARI-IZZO & al. 1997).

We have previously found that in the "resurrection" plant *Boea hygroscopica* the antioxidant glutathione plays a key role in the recovery of this plant from slow dehydration (SGHERRI & al. 1994a). It has furthermore been observed that this plant, when dehydrated for 25 days until the relative water content is 24%, maintains the enzyme activities related to glutathione utilization and regeneration such as the activity of $NADP^+$-dependent glyceraldehyde-3-phosphate dehydrogenase (GA3PDH, EC 1.2.1.12), which contains essential sulfhydryl groups (-SH) (NAVARI-IZZO & al. 1997).

The aim of this work is to verify if two wheat cultivars, differently sensitive to drought, show a different utilization of glutathione and to evaluate if the glutathione system can protect -SH of soluble proteins from oxidation.

Material and Methods

Seedlings of two wheat (*Triticum durum* Desf.) cultivars, one more drought tolerant (cv. Ofanto) than the other (cv. Adamello), were grown under field irrigation and dryland conditions. Plants from both cultivars were subjected to water deficit by withholding water for 35 d, starting 30 d after sowing. Dehydrated plants were then rehydrated. Control plants (irrigated) were harvested at 30 d (initial control, C_0) and 65 d (final control, C_1) after sowing, and water stressed plants at 65 d (dried, D), 66 d (rehydrated for 1 day, R_1) and 69 d (rehydrated for 4 days, R_2) after sowing.

The relative water contents (RWC) were calculated as reported by SGHERRI & al. 1994a and the water potential (ψ_w) was measured with a pressure chamber (NAVARI-IZZO & al. 1990). Reduced (GSH) and oxidized (GSSG) glutathione contents were evaluated following the procedure of SGHERRI & al. 1994a. Glutathione reductase (GR, EC 1.6.4.2) and GP activities were determined according to SGHERRI & al. 1994b and CARMAGNOL & al. 1983, respectively. The H_2O_2 contents were evaluated as described by SGHERRI & al. 1994b. Determination of -SH and disulfide groups (-SS-) of soluble proteins was performed according to NAVARI-IZZO & al 1997. GA3PDH and stromal fructose-1,6-bisphosphatase (Fru 1,6 BPase, EC 3.1.3.11) initial and total activities were determined according to NAVARI-IZZO & al. 1997 and TAKEDA & al. 1995, respectively. Total activity of both enzymes was evaluated after incubation with 20 mM DTT for 20 min.

Results and Discussion

After 35 d of drought plants of both wheat cultivars underwent a severe level of water stress. However dryland conditions induced greater decreases in ψ_w and RWC in the more sensitive cv. Adamello than in the more tolerant cv. Ofanto. Indeed, ψ_w and RWC of dried leaves of cv. Adamello were -2.3 MPa and 50% respectively, while dried leaves of cv. Ofanto had a ψ_w of -1.9 MPa and a RWC of 67%. Furthermore, during rehydration, the recovery of the leaf water status was more rapid in the cv. Ofanto.

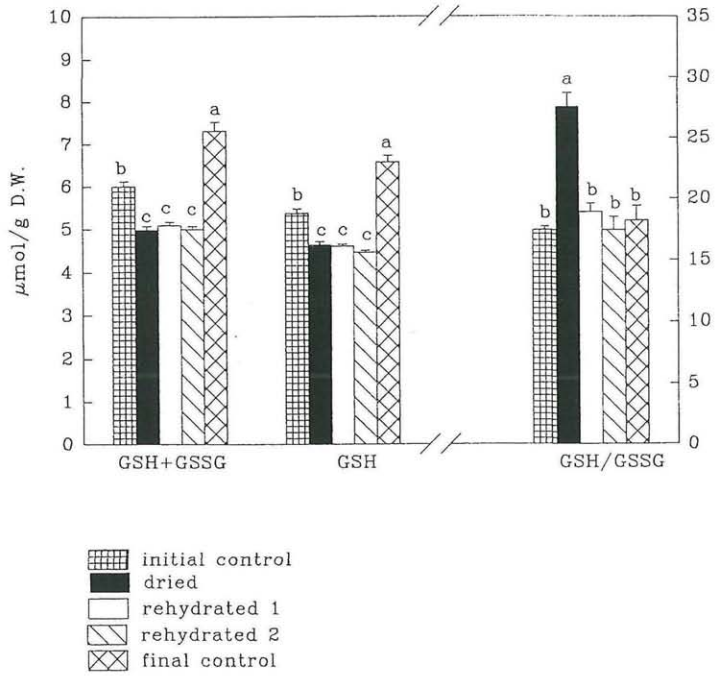

Fig. 1. Changes in the content and oxidation state of glutathione during dehydration and rehydration of cv. Adamello. The results are the means ± SE of five replicate samples. For comparisons among the means, analysis of variance was used. Histograms with different letters are significantly different at $P \leq 0.01$.

Although during dehydration the GSH+GSSG and GSH contents decreased in both cultivars of wheat (Figs. 1, 2), they were maintained at high levels. Furthermore, oxidation of GSH did not occur as it is evident from the GSH/GSSG ratio which, in comparison with C_1, remained unchanged in the cv. Ofanto (Fig. 2) and even increased in the cv. Adamello (Fig. 1) in concomitance with the increase of 36% of the GR activity. The cv. Adamello, during dehydration, required, also, the doubling of H_2O_2-GP activity, which contributed to reduce the H_2O_2 content (Fig. 3). Probably the greater ability to metabolize H_2O_2 enable the cv. Adamelloto avoid toxic effects of the oxidative stress due to an increase of superoxide radical ($O_2^{\cdot-}$) production induced by dehydration (QUARTACCI & al. 1994).

In the cv. Ofanto, on the other hand, drought did not cause changes in the H_2O_2 levels (Fig. 3), GR and H_2O_2-GP activities (data not shown). It was found that plants of cv. Ofanto subjected to two water deficit cycles did not show changes in H_2O_2 contents after each period of stress. After the second period, it was found that the $O_2^{\cdot-}$ production decreased (MENCONI & al. 1995). In maize, when drought imposition was gradual and acclimation may have occurred, GR activity.

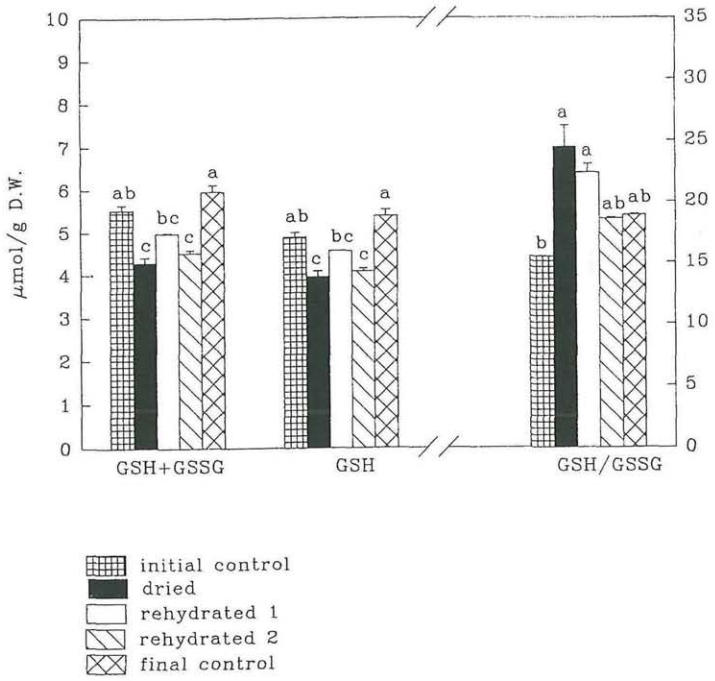

Fig. 2. Changes in the content and oxidation state of glutathione during dehydration and rehydration of cv. Ofanto. The results are the means ± SE of five replicate samples. The significance of the letters is the same as in Fig. 1.

and H_2O_2 levels were unaffected by dehydration (BROWN & al. 1995). It has been suggested that when plants are allowed to acclimate to drought, the $O_2^{\cdot-}$ level may not rise (SGHERRI & al. 1993). In our experiment plants of both cultivars of wheat required 35 days to suffer a severe water deficit stress. As this is an extended period, it is possible that plants of more tolerant cv. Ofanto have had time to respond to drought by avoiding oxidative stress. Indeed, defence processes involved in maintenance, when they include enzyme induction or antioxidant synthesis, have an energetic cost which has an effect on plant productivity (MENCONI & al. 1995).

Furthermore, the fact that cv. Adamello suffers water stress more than cv. Ofanto is also confirmed by the induction of xanthophyll cycle shown only by the first cultivar (LOGGINI & al. unpublished data).

The maintaining of low concentrations of H_2O_2 is important in plant cells, because H_2O_2 at low concentration (10-100 µM) inhibits chloroplast -SH-enzymes (TANAKA & al. 1982). Afterwards, a low H_2O_2 content and a high GSH/GSSG ratio enable both wheat cultivars to maintain, during dehydration and rehydration, the -SH of soluble proteins in the reduced state (about 90% of -SH). Similar effects appeared in the activation state of two important enzymes of photosynthesis, Fru

1,6 BPase and GA3PDH, which remained at control levels (50% for both enzymes in both cultivars). Generally, these enzymes decreased their activities during drought (STEWART & LEE 1972, HARTEN & EICKMEIER 1986). Also a previous study on the "resurrection" plant *Boea hygroscopica* revealed that, when the plants were dehydrated for 25 days to reach a RWC of 24%, the GSH/GSSG ratio as well as the GA3PDH activity remained high (NAVARI-IZZO & et al. 1997).

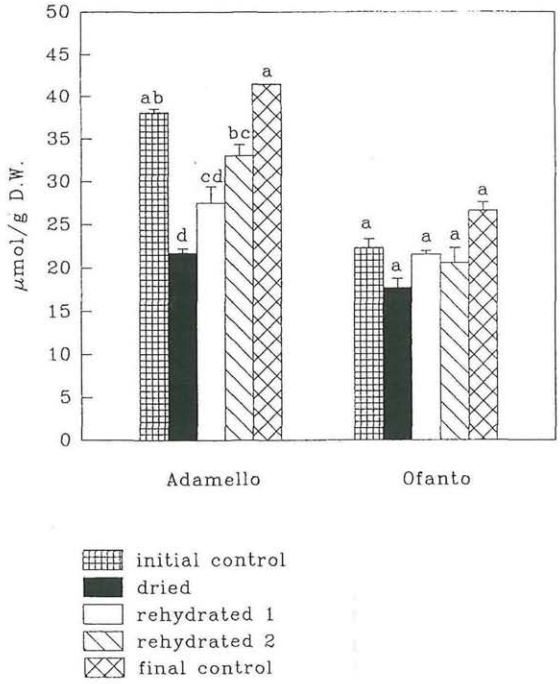

Fig. 3. Changes in the levels of H_2O_2 during dehydration and rehydration of cv. Adamello and cv. Ofanto. The results are the means ± SE of five replicate samples. The significance of the letters is the same as in Fig. 1.

Acknowledgements

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