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Physiological response of wheat varieties to elevated atmospheric CO_2 and low water supply levels

S. BENCZE[+], Z. BAMBERGER, T. JANDA, K. BALLA, B. VARGA, Z. BEDŐ, and O. VEISZ

Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, H-2462 Martonvásár, Brunszvik u. 2, Hungary

Abstract

In the phytotron experiment, the effect of elevated atmospheric CO_2 (EC, 750 µmol mol^{-1}) on the drought tolerance was studied in two winter varieties (Mv Mambo, tolerant; Mv Regiment, moderately tolerant) and in one spring variety of wheat (Lona, sensitive to drought). Changes in net photosynthetic rate (P_N), stomatal conductance, transpiration, water-use efficiency, effective quantum yield of photosystem II, and activities of glutathione reductase (GR), glutathione-S-transferase (GST), guaiacol peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) were monitored during water withdrawal. Drought caused a faster decline of P_N at EC, leading to the lower assimilation rates under severe drought compared with ambient CO_2 (NC). In the sensitive variety, P_N remained high for a longer period at EC. The growth at EC resulted in a more relaxed activation level of the antioxidant enzyme system in all three varieties, with very low activities of GR, GST, APX, and POD. The similar, low values were due to decreases in the varieties which had higher ambient values. A parallel increase of CAT was, however, recorded in two varieties. As the decline in P_N was faster at EC under drought but there was no change in the rate of electron transport compared to NC values, a higher level of oxidative stress was induced. This triggered a more pronounced, general response in the antioxidant enzyme system at EC, leading to very high activities of APX, CAT, and GST in all three varieties. The results indicated that EC had generally favourable effects on the development and stress tolerance of plants, although bigger foliage made the plants more prone to the water loss. The relaxation of the defence mechanisms increased potentially the risk of damage due to the higher level of oxidative stress at EC under severe drought compared with NC.

Additional key words: antioxidant enzyme system; bread wheat; CO_2 enrichment; drought; photosynthesis; *Triticum aestivum*.

Introduction

The last 200 years of extensive human activity has resulted in the accelerating changes of global climatic processes. Weather anomalies have become more and more frequent and intense during the last century and water shortage is now a major limiting factor of crop production.

The early responses of plants to water deficit include the accumulation of physiologically active compounds, osmolytes, in the cells, helping the organs to retain water (Kameli and Lösel 1993, Sarker *et al.* 1999, Niedzwiedz-Siegien *et al.* 2004). In response to severe drought stress, a rapid decline in the chlorophyll (Chl) and protein contents may occur, accompanied by a rise in the proline concentration and the accumulation of sucrose (Baisak *et al.* 1994, Lin and Wang 2002).

Wheat plants may use different strategies to avoid the severe damage caused by water deprivation (Chaves *et al.* 2002). The adaptive mechanisms include morphological changes such as leaf rolling or changes in leaf angle, decreased stomatal conductance, osmotic adjustment, or higher leaf waxiness might also occur. However, stomata closure, preventing the water loss through transpiration,

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[+]Corresponding author; phone: +36 22569 549, fax: +36 22461 000, e-mail: bencze.szilvia@agrar.mta.hu

Abbreviations: APX – ascorbate peroxidase; CAT – catalase; *E* – transpiration rate; EC – elevated CO_2; FM – fresh mass; GR – glutathione reductase; g_s – stomatal conductance; GSH – glutathione; GST – glutathione-S-transferase; Lo – Lona; Mam – Mv Mambo; NC – normal (ambient) CO_2; P_N – net photosynthetic rate; P_{Nmax} – light-saturated net photosynthetic rate; POD – guaiacol peroxidase; PS – photosystem; Φ_{PSII} – effective quantum yield of PSII; Reg – Mv Regiment; SOD – superoxide dismutase; SVWC – soil volumetric water content; WUE – water-use efficiency.

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may result in the inhibition of CO_2 exchange, leading to reduced assimilation. The lack of equilibrium between electron transport and CO_2 fixation may lead to the electrons being transferred to O_2 molecules. Although these mechanisms protect the components of the electron transport chain from photodamage, the resulting reactive oxygen species (ROS) may react with cell compounds. Acting as the signals, ROS are able, directly or indirectly, to induce the expression of various genes involved in stress signalling (Apel and Hirt 2004, Pogány *et al.* 2006). If water deficiency develops at a slower rate, the antioxidant mechanisms are able to reduce tissue damage to a minimum, while rapid desiccation may result in a severe, possibly lethal damage (Farrant *et al.* 2004). Hardening (a lower level of stress during the development), however, may promote tolerance and adaptation (Selote and Khanna-Chopra 2006).

Research on wheat species and varieties revealed the enhancement of GR, SOD, CAT, APX, and nonspecific POD activities as a result of water deficiency, but the changes in the functioning of these enzymes were diverse (Zhang and Kirkham 1994, Sairam and Saxena 2000, Keles *et al.* 2002, Almeselmani *et al.* 2009, Takele and Farrant 2009). It was found that the tolerant genotypes tended to have higher antioxidant enzyme activities under optimal water conditions compared with those of the susceptible ones (Sairam and Saxena 2000, Sairam and Srivastava 2001, Khanna-Chopra and Selote 2007). Nevertheless, there were also some exceptions in the individual wheat genotypes (Sairam and Srivastava 2001).

As CO_2 is a limiting factor for photosynthesis in C_3 plants, a rise in the atmospheric CO_2 concentration increases the rate of assimilation and dry matter production (Kramer 1981; Lawlor and Mitchell 1991). The rate of stimulation is, however, greatly influenced by the growth type and the nutrient supplies (Poorter *et al.* 1996, Li *et al.* 2007). Atmospheric CO_2 enrichment results in a greater leaf mass, total biomass and yield, and the better tolerance of various stresses in wheat (Kendall *et al.* 1985, Harnos *et al.* 1998, Bencze *et al.* 2004, Högy *et al.* 2009). This might result partly from the fact that stomatal conductance (g_s) decreases and water-use efficiency (WUE) improves at high CO_2 concentrations, due to a considerable decline in transpiration rate (E) and a concurrent rise in P_N (Tuba *et al.* 1994, 2003). Much less is known, however, about the physiological and biochemical background of the improved stress tolerance observed at high CO_2 concentrations. The growth at elevated CO_2 was found to cause the reduced activity of certain antioxidant enzymes, *e.g.* GST (Kőmíves *et al.* 1997), CAT, SOD, and APX (McKee *et al.* 1997, Polle *et al.* 1997, Pritchard *et al.* 2000, Schwanz and Polle 2001). It had no effect on the activity of other enzymes (*e.g.* POD) or on some enzymes in certain plant species (Schwanz and Polle 2001*).* The activities of antioxidant enzymes may increase more at high than at ambient CO_2 concentrations under stress. In wheat, the SOD and CAT activities were much higher at elevated CO_2 than at the ambient concentration during the induction of water stress (Lin and Wang 2002). The SOD activity was proved to increase only in response to drought at elevated CO_2 according to other authors (Schwanz and Polle 2001).

From the results mentioned above, it can be assumed that the elevated CO_2 concentration may affect the stress tolerance of plants *via* causing changes in the antioxidant enzyme system. To test this hypothesis, our experiment was set up with the following objectives:

(*1*) to determine the differences in the photosynthetic response of different wheat varieties to a high CO_2 in atmosphere, and how these differences contribute to responses of plants to drought stress,

(*2*) to investigate how the relationship between the functioning of photosystem (PS)II, net assimilation, and some key components of the antioxidant enzyme system are affected by drought stress and the atmospheric CO_2 concentration.

Materials and methods

Experimental design and plant material: The experiment was conducted under the controlled environmental conditions in two *PGV-36* growth chambers (*Conviron*, Winnipeg, Canada) in the phytotron of the Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Martonvásár, Hungary. The growth conditions were the same in both chambers, except for the atmospheric CO_2 concentration, which was either ambient, *i.e.* normal (NC, 390 μmol mol^{-1}) or elevated (EC, 750 μmol mol^{-1}). Three bread wheat (*Triticum aestivum* L.) varieties with a different tolerance to drought were chosen for the tests. The winter wheat varieties, Mv Regiment (Reg) and especially Mv Mambo (Mam), have better tolerance, while the spring variety, Lona (Lo), is the variety most sensitive to drought (Bencze *et al.* 2011a). Four seedlings at the one-leaf stage were planted directly (spring wheat) or after vernalization at 4°C for 42 d (winter varieties), each in 3-L pot containing a 3:1:1 mixture of garden soil, the humus rich medium Vegasca (Florasca), and sand. There were at least 8 pots of each genotype and treatment (CO_2 × water supply). The pots were placed randomly in the growth chambers and rearranged regularly.

The plants were watered daily and supplied with nutrients in tap water (0.33 g L^{-1} *Volldünger Linz* solution, Austria, 0.3 L pot^{-1}) twice weekly till the beginning of the first stress treatment. The temperature regime changed weekly, beginning with a minimum/maximum/mean of 10/12/10.7°C during the first week and increasing until it reached 20/24/22.7°C in the maturation period (Tischner *et al.* 1997). The maximum photosynthetic photon flux density also increased from 280 to

400 μmol m^{-2} s^{-1} during this period.

As the varieties had different heading dates, water was withheld during the drought-stress treatment for 7 d, starting from the 10th d after the heading of each variety (Zadoks 59 state, Tottman and Makepeace 1979). There was no significant difference in the heading dates of the plants at the two CO_2 concentrations. Reg variety, which headed the earliest, was subjected to water stress at 20ºC, while the other two varieties were stressed at the maximum daily temperature of 22ºC. The light intensity was set at maximum PPFD of 350 μmol m^{-2} s^{-1} for all the varieties during the stress treatment. The soil water content was monitored by the *ECH2O* device (*Em50* data logger and *EC-5* soil moisture sensors, *Decagon Devices*, USA). The soil volumetric water content (SVWC) of 20–30% was regarded as the control (with no physiological constraints), 15% as mild, 10% as moderate, 5–8% as severe water deficiency stress.

P_N, g_s, and E were measured with a *LI-6400* portable photosynthesis system (*Licor*, Lincoln, Nebraska, USA) on the flag leaf of each of 4 plants in the pot. Measurements were taken at 350 μmol m^{-2} s^{-1} PAR, at various recorded soil moisture levels, at the ambient temperature, and at the actual CO_2 concentration in the chamber (*i.e.* either NC or EC). The air flow rate was 500 μmol s^{-1}. Records were taken when stable values were achieved. The WUE was calculated as the ratio of P_N/E. Measurements of the effective quantum yield of PSII (Φ_{PSII}), calculated as $\Delta F/F_m' = (F_m' - F_s)/F_m'$ (where F_m' and F_s represent the maximum and steady state fluorescence levels in the light-adapted state, respectively, Genty *et al.* 1989), were carried out on the intact flag leaves, at ambient light intensities (300 μmol m^{-2} s^{-1} PAR), using a pulse amplitude modulated fluorometer (*PAM-2000*, *Walz*, Effeltrich, Germany) in the growth chambers. The saturating light was given as white light with an intensity of 8,000 μmol m^{-2} s^{-1} for 0.8 s.

Sample collection and antioxidant enzyme activities: Plant material was usually collected after the photosynthetic measurements were made and the soil moisture content was determined. In each variety, all leaf samples were collected within a 3–4 d period. The leaf samples of 0.500 g were cut from the middle region of the flag leaves (from different pots on each occasion) and kept at –80ºC until the antioxidant enzyme activity was analysed. Antioxidant enzyme activities were determined spectrophotometrically according to Janda *et al.* (2008) on five biological replicates, using the plant extracts for which the leaf samples were homogenized with sand in 2.5 mL of the ice-cold 0.5 M Tris-buffer (pH 7.5) containing 3 mM $MgCl_2$ and 1 mM EDTA, and centrifuged (10,000 × *g* for 20 min).

Glutathione reductase (GR, EC 1.8.1.7) activity was determined as the change in absorbance at 412 nm in 1 ml of 75 mM Na-phosphate buffer (pH 7.5), containing 0.15 mM diethylene triamine pentaacetic acid (DTPA), 0.75 mM dithionitrobenzoic acid (DTNB), 0.1 mM NADPH, 0.5 mM oxidized glutathione (GSH), and 50 μl of the extract (Smith *et al.* 1988). Glutathione-S-transferase (GST, EC 2.5.1.18) activity was measured at 340 nm in a final volume of 2.65 mL made up of 75 mM Na-phosphate buffer (pH 6.5), 1 mM EDTA, 1.038 mM 1-chloro-2,4-dinitro-benzole (CDNB), 3.74 mM GSH and 100 μL of the plant extract. The nonspecific guaiacol peroxidase (POD, EC 1.11.1.7) activity was determined at 470 nm in 3 mL of 0.88 mM acetate buffer (pH 5.5), 0.88 mM guaiacol, 0.0375% H_2O_2, and 50 μl of the plant extract (Ádám *et al.* 1995). Catalase (CAT, EC 1.11.1.6) activity was determined as the decrease in absorbance at 240 nm in a 3 mL reaction mixture consisting of 0.44 M Tris-HCl (pH 7.4) and 60 μL of the plant extract after the addition of 0.0375% H_2O_2 (Janda *et al.* 1999). Ascorbate peroxidase (APX, EC 1.11.1.11) activity was measured in 2.25 mL of 0.2 M Tris-HCl (pH 7.8), 5.625 mM ascorbic acid, 50 μL of the extract, and 0.042% H_2O_2 as the change in absorbance at 290 nm due to the consumption of ascorbate (Nakano and Asada, 1987). The enzyme activity was expressed in nkat g^{-1} fresh mass (FM) (Janda *et al.* 1999).

Statistical analysis: To describe the relationship between the level of the drought stress and the changes induced in various parameters, polynomial functions (2nd degree) were used, which fitted the best to the character of the relationship (*MS Excel 2007*). The significance level of differences between the CO_2 treatments at various soil water levels was ascertained using two sample *t*-test on the corresponding individual data sets (all replicates included) at NC and EC. The critical values of the correlation coefficient *R* were determined according to Fisher and Yates (1957), df = $n - 2$.

Results

Changes in gas-exchange parameters and the effective quantum yield of PSII: At the control water level, P_N was stimulated by EC in Reg and Mam (P = 0.079 and 0.074, respectively), the varieties with moderate photosynthetic rates. In Lo, which had the highest control values among the varieties, P_N was similar at both NC and EC (Fig. 1). With the decreasing water supply, P_N started to decline. The overall rate of decrease in P_N was faster at EC than at NC. At NC, P_N declined at a considerably slower rate in Mam and Reg than in Lo. In Mam and Reg, P_N exhibited similar values at both NC and EC even under mild stress (15% SVWC) while at this water supply in Lo, P_N was still maintained at higher values at EC than at NC, though it dropped rapidly

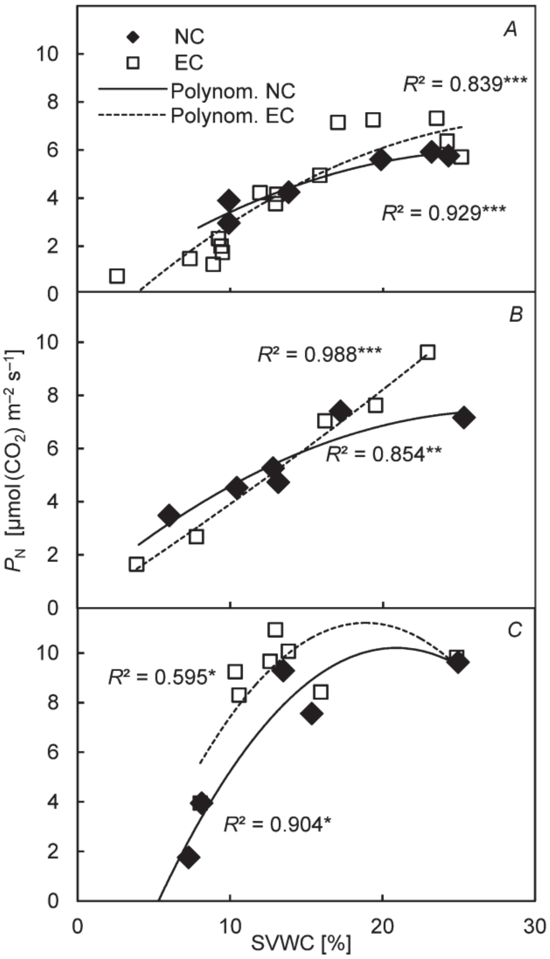

Fig. 1. Change in the net photosynthetic rate (P_N) as a function of soil volumetric water content (SVWC) during water withdrawal at ambient (normal, NC) or elevated CO_2 (EC) concentration. Winter wheat varieties: Mv Regiment (*A*), Mv Mambo (*B*), spring wheat: Lona (*C*). Each value represents the mean of four measurements on the individual plants in a pot. The R^2 values are given for polynomial functions (2nd degree). $^{+, *, **,}$ and *** – significant R^2 values at the probability level of 10, 5, 1, and 0.1%, respectively.

when drought became more severe.

There were great differences in g_s between the varieties (Fig. 2), EC resulted in either lower or similar values than those recorded at NC. No change was recorded in response to EC at the control water supply in Reg, the variety with the highest g_s under the control conditions. In Lo, which had the intermediate control g_s, a considerable decline was detected in response to EC. The value of g_s became lower also in Mam, which, however, had the lowest control values of the three varieties.

Water withdrawal usually caused a decrease in g_s at both CO_2 concentrations. At NC, a dramatic decrease was detected in Lo, while a moderate decline was found in Mam and Reg. At EC, however, there was a drop in g_s in Reg even under mild water stress, while only severe water stress induced changes in Mam. In Lo, g_s did not change significantly from the control level at EC until 10% SVWC, where the values at NC reached those at EC, while g_s started to decrease at both CO_2 concentrations under more severe drought.

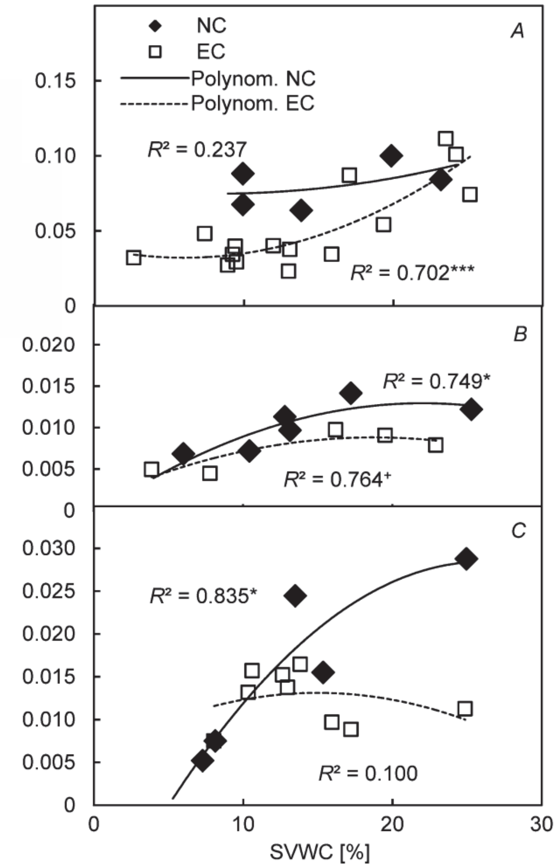

Fig. 2. Change in the stomatal conductance (g_s) as a function of soil volumetric water content (SVWC) during water withdrawal at ambient (normal, NC) or elevated CO_2 (EC) concentration. Winter wheat varieties: Mv Regiment (*A*), Mv Mambo (*B*), spring wheat: Lona (*C*). Each value represents the mean of four measurements on the individual plants in a pot. The R^2 values are given for polynomial functions (2nd degree). $^{+, *, **,}$ and *** – significant R^2 values at the probability level of 10, 5, 1, and 0.1%, respectively.

At NC, *E* was the highest in Reg and Lo but it started to decline faster in response to decreasing water supplies than in Mam, which had already very low values (Fig. 3). EC resulted in the lower *E*, leading to a 60–200% increase of WUE in all three varieties under the control soil water level (Fig. 4). WUE was relatively high in Mam, while it was a half of this value or even less in the other two genotypes. WUE remained fairly stable at all water supply levels at NC, while at EC, it declined gradually with decreasing water supplies until it reached similar values to those at NC, either under medium (in Reg) or severe drought stress (in Mam and Lo).

Unlike the P_N, no clear differences could be detected in Φ_{PSII} in response to EC (Fig. 5), therefore no

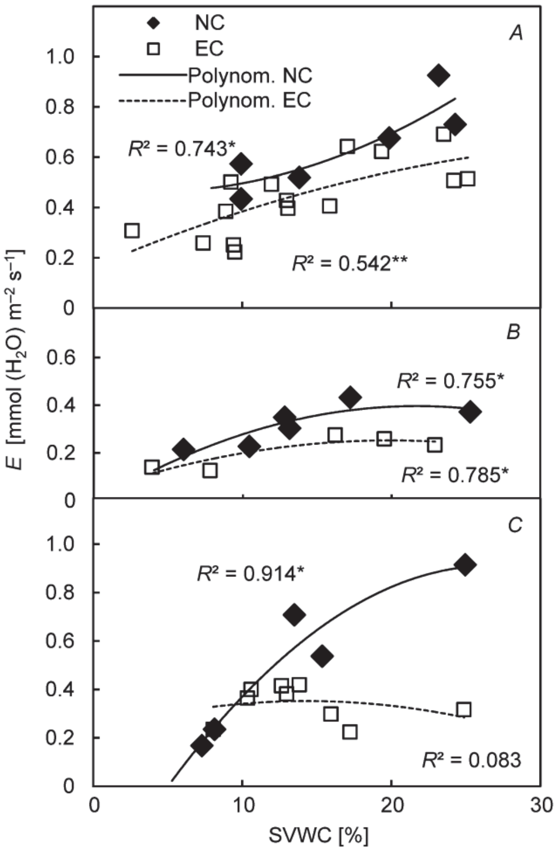

Fig. 3. Change in transpiration (E) as a function of soil volumetric water content (SVWC) during water withdrawal at ambient (normal, NC) or elevated CO_2 (EC) concentration. Winter wheat varieties: Mv Regiment (*A*), Mv Mambo (*B*), spring wheat: Lona (*C*). Each value represents the mean of four measurements on the individual plants in a pot. The R^2 values are given for polynomial functions (2nd degree). $^{+}$, *, **, and *** – significant R^2 values at the probability level of 10, 5, 1, and 0.1%, respectively.

polynomial functions were fitted to the data. In Lo, this parameter exhibited a decrease in the same range of water supply in which assimilation became close to zero (after wilting). Besides the early visual symptoms of the leaf water loss in this variety, the decrease in Φ_{PSII} also underlined the fact that Lo was the most drought-sensitive of all the varieties.

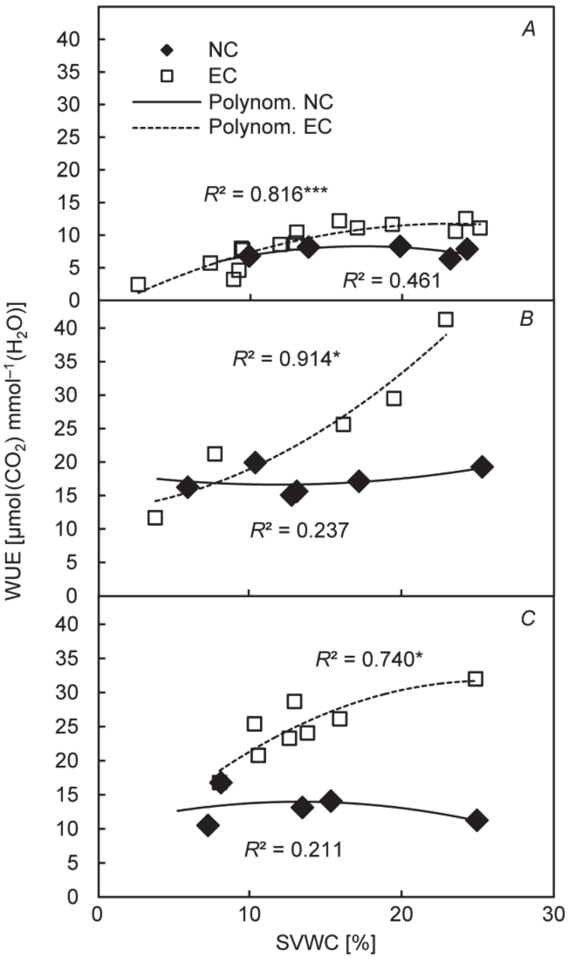

Fig. 4. Change in the water-use efficiency (WUE) as a function of soil volumetric water content (SVWC) during water withdrawal at ambient (normal, NC) or elevated CO_2 (EC) concentration. Winter wheat varieties: Mv Regiment (*A*), Mv Mambo (*B*), spring wheat: Lona (*C*). Each value represents the mean of four measurements on the individual plants in a pot. The R^2 values are given for polynomial functions (2nd degree). $^{+}$, *, **, and *** – significant R^2 values at the probability level of 10, 5, 1, and 0.1%, respectively.

Changes in antioxidant enzyme activity: At NC, the antioxidant enzyme activities of the plants exposed to water shortage depended very much on the genotype (*e.g.* GR, GST, APX, and CAT), while the POD activity remained fairly constant in all the varieties (Figs. 6–8). In Mam, no significant differences were detected for GR, POD, CAT, or APX, while GST decreased due to drought stress. In Reg, the activities of GR, GST, APX, and CAT increased under mild water stress and remained at similar levels under medium drought, but it started to decrease under severe water shortage. Increases in the activity of the same enzymes in Lo were gradual, but fairly moderate; only GST and APX gave a more pronounced response.

Growing the plants at EC did not result in any general pattern of changes in antioxidant enzyme activity (Figs. 6–8). The APX activity was unchanged in all the varieties, while the activity of GR and GST changed only in Mam and that of POD in Reg in response to EC, in all cases exhibiting the decrease. The CAT activity increased, however, in Mam and Lo. In other words, in Reg, the only enzyme exhibiting any change in the activity was POD, in Mam, GR and GST decreased while CAT increased, and in Lo, only a rise in the CAT activity was observed. It could be seen that, compared to the variation among the varieties observed at NC, the levels of enzyme activity were much the same in all three varieties at EC, with values of around 30 nkat g^{-1}(FM) for

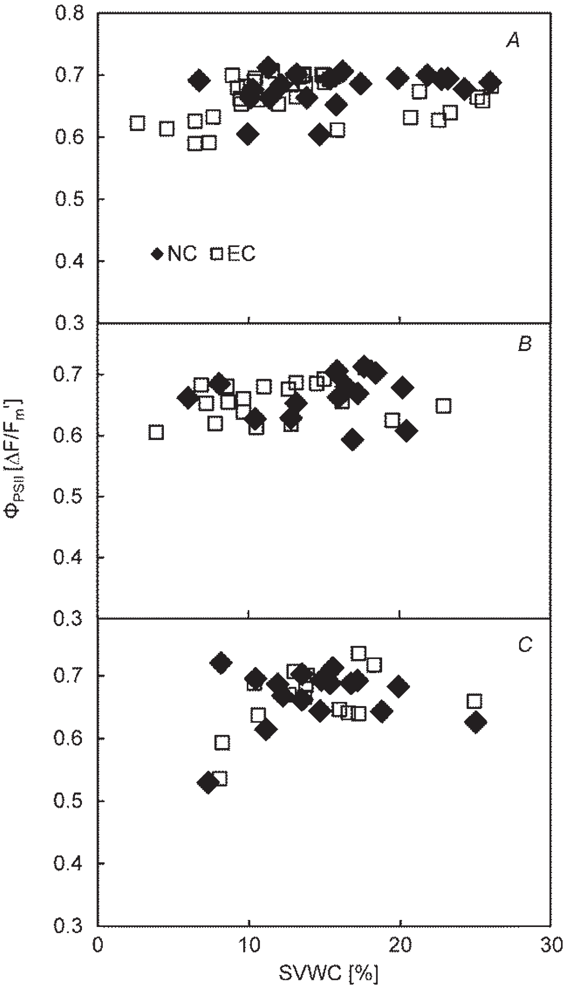

Fig. 5. Effective quantum yield of PSII (Φ_{PSII}) [ΔF/F'$_m$] at various levels of soil volumetric water content (SVWC) and atmospheric CO_2. Winter wheat varieties: Mv Regiment (*A*), Mv Mambo (*B*), spring wheat: Lona (*C*). Each data point is the average value of four plants in the same pot. NC – normal CO_2; EC – elevated CO_2.

GST, 43–50 for GR, and 100 for APX. POD had similar values in Reg and Mam (540 and 650 nkat g^{-1}, respectively), but it showed a higher activity in Lo [(900 nkat g^{-1}(FM)]. The CAT activity was only slightly lower [9,000 nkat g^{-1}(FM)] in Reg than that recorded in Mam and Lo at EC [10–11,000 nkat g^{-1}(FM)].

Although the plants tended to respond more intensely to drought at EC, the difference between the CO_2 concentrations was not always evident (Figs. 6–8). The GR activity values induced by drought were within the same range at both EC and NC. GST had very similar values at NC and EC in Reg and Lo, but in Mam, compared to the decline observed at NC, the GST activity increased at EC in response to drought, reaching significantly higher values than those recorded at NC (P=0.0079). The POD activity increased considerably at EC in Reg under the mild water stress (P=0.0009). The APX activity did not differ with the CO_2 concentration in Mam, but it remained high longer at EC in Reg (P=0.0031). Although the APX activity induced by severe drought in Lo was similar at EC and NC, under mild water stress (15% SVWC) it was considerably lower at EC than at NC (P=0.0028). In response to drought stress, CAT exhibited slightly or substantially higher activity at EC than that at NC in all the varieties (P=0.0054–0.0612).

The maximal values of activity induced by drought and EC were very similar in all the varieties for GST (40–50), APX (130), and CAT [12,000 nkat g^{-1}(FM)]. POD had values of around 1,000 nkat g^{-1} in Reg and Lo and 700–800 nkat g^{-1} in Mam. GR had different levels of activity in all three varieties [70, 60, and 50 nkat g^{-1}(FM) in Reg, Mam, and Lo, respectively].

Differences between the varieties: The variance observed in the response of the antioxidant system either to EC or drought can be attributed to differences between the genotypes. There was no or very little change in the enzyme activity of Mam in response to drought at NC, but at EC, the activity of most enzymes showed a slightly increasing trend, resulting in significantly higher values of GST, POD, and CAT (Figs. 6*B*, 8*B*). A pronounced increase in activity was detected due to drought stress at EC in Reg (Figs. 6*A*, 8*A*), where the activity of all the enzymes was greatly enhanced even under mild water deficiency. In this variety, the overall changes exhibited a fairly similar range at both CO_2 concentrations except for POD, which had much higher values under mild drought at EC than at NC. The higher activity of APX and CAT was maintained significantly longer under EC than under NC. In Lo in general, the rise in the enzyme activity in response to water shortage was similar at both CO_2 concentrations (Figs. 6*C*, 8*C*). At the control water supply level, the activity of most enzymes was the same at both NC and EC; CAT, however, had higher activity at EC, and the values induced by drought were also slightly, though significantly, higher at EC. The APX activity in Lo, which began to rise under the mild water deficiency at NC, started to increase under stronger drought stress at EC.

Discussion

Response of plants to drought: A decline in both P_N and g_s was recorded in response to drought. Great differences were found between the varieties both at the control water supply and in the rate of decrease in response to water deficit. P_N decreased dramatically in the sensitive variety due to drought, while it declined gradually in the more tolerant varieties. Similar results were reported under drought conditions by other authors (Inoue *et al.* 2004a,b), but the findings differed under the control water conditions: in the present work, the lower values of P_N were found in the more tolerant varieties, while the highest rate was observed in the sensitive variety. Φ_{PSII} also decreased in the sensitive variety in response to severe drought when P_N was already close to zero. As P_N usually decreased faster than Φ_{PSII} in response to drought, this referred to a lack of equilibrium between the electron transport and CO_2 fixation.

A considerable variation between the varieties was also found for *E* and WUE. Mam had much higher WUE than the other two varieties. In all varieties, however, WUE had relatively stable values at all water levels at NC. This was similar to the results reported by Robredo *et al*. (2007) for water-stressed plants, but contrasted with the findings of others (Martin and Ruiz-Torres 1992) where an initial increase in WUE followed by a subsequent decline was reported.

The antioxidant enzyme activity of the varieties tested here gave diverse responses to drought stress. The most tolerant variety, Mam, had the lower GST activity in response to water deficiency, while no change was observed for the other enzymes. Lo exhibited a slight or moderate, though gradual increase in the activities of GR, GST, APX, and CAT. Reg, the moderately tolerant variety, responded even to mild drought stress with the higher activity of the above four enzymes, while a decline was observed under severe drought. This corresponded to the results reported for most wheat genotypes in the case of CAT and APX (Hameed *et al*. 2011), while other genotypes exhibited increases or remained unchanged under severe stress, as it was also found here for Mam and Lo. Some authors also reported increases in the activity of APX, CAT, GR, and POD, but there are also some discrepancies in the response to drought depending on the genotypes and/or the level of drought stress (Zhang and Kirkham 1994, Schwanz *et al*. 1996, Sairam and Saxena 2000, Keleş *et al*. 2002, Lascano *et al*. 2001, Lin and Wang 2002, Almeselmani *et al*. 2009, Takele and Farrant 2009, Salazar-Parra *et al*. 2012). In the present work, the activity of POD was found to remain stable even under severe drought in all the varieties,

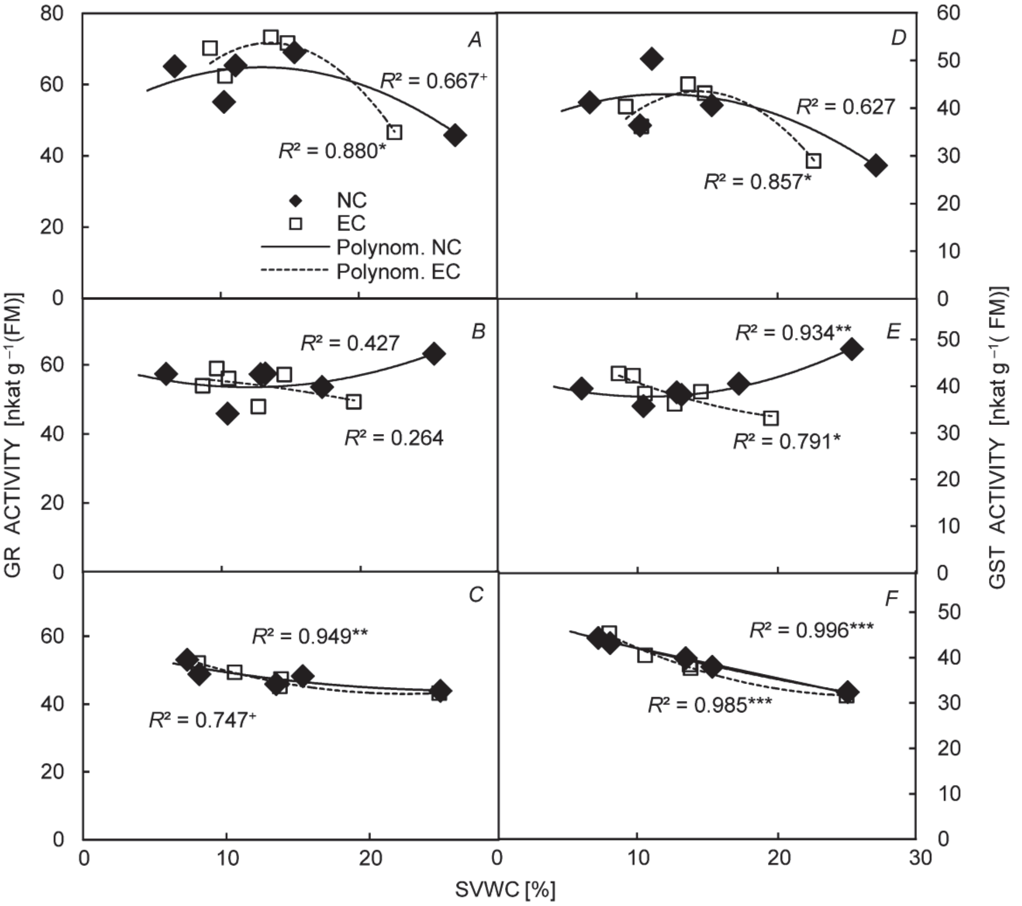

Fig. 6. Change in the activity of glutathione reductase (GR) and glutathione-S-transferase (GST) in response to elevated CO_2 concentration and drought. Winter wheat varieties: Mv Regiment (*A,D*), Mv Mambo (*B,E*), spring wheat: Lona (*C,F*). Each value represents the average of five leaf samples from the same pot. NC – normal CO_2, EC – elevated CO_2, SVWC – soil volumetric water content. The R^2 values are given for polynomial functions (2nd degree). $^{+, *, **,}$ and *** – significant R^2 values at the probability level of 10, 5, 1, and 0.1%, respectively.

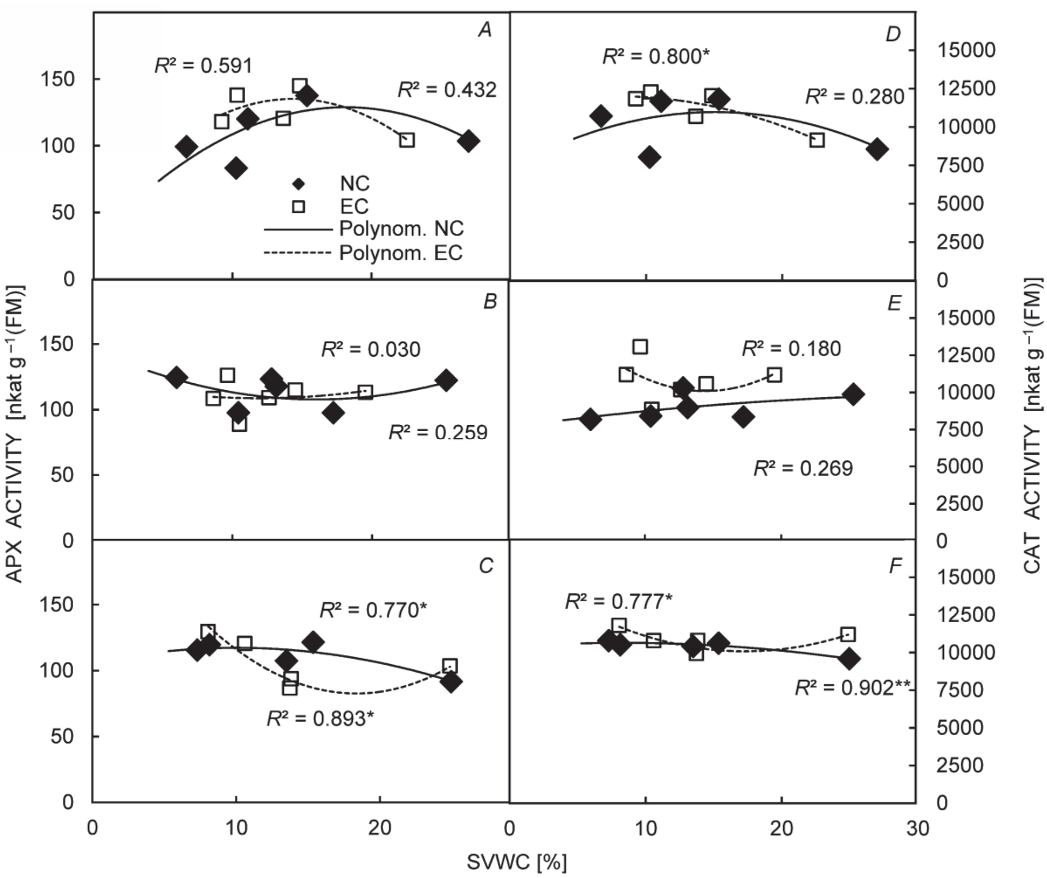

Fig. 7. Change in the activity of ascorbate peroxidase (APX) and catalase (CAT) in response to elevated CO_2 concentration and drought. Winter wheat varieties: Mv Regiment (*A,D*), Mv Mambo (*B,E*), spring wheat: Lona (*C,F*). Each value represents the average of five leaf samples from the same pot. NC – normal CO_2, EC – elevated CO_2, SVWC – soil volumetric water content. The R^2 values are given for polynomial functions (2nd degree). $^{+}$, *, **, and *** – significant R^2 values at the probability level of 10, 5, 1, and 0.1%, respectively.

as reported earlier by Lin and Wang (2002), but in contrast with others (Sairam and Saxena 2000, Hameed *et al.* 2011).

Response of plants to elevated CO_2 concentration: EC increased P_N at the control water level in the varieties that had lower basic values, but not in Lo which had the highest ambient rate of P_N. The g_s showed an adaptation to EC in two varieties, as it had much lower values in plants grown at EC than at NC, similarly to what was found in grass species (LeCain *et al.* 2003) and wheat (Garcia *et al.* 1998). In all the varieties, *E* was lower and WUE of plants was higher at EC than at NC, in accordance with previous data (Chaudhuri *et al.* 1990, Tyree and Alexander 1993, Tuba *et al.* 1994, 2003). As EC decreases transpiration in both C_3 and C_4 plants, this reduction in *E*, combined with increased P_N, can contribute to the higher WUE (Prior *et al.* 2011). Besides enhancing leaf WUE, EC can, however, stimulate photosynthesis even more greatly in the lower layers of the foliage (up to more than 100%), than at the top of the plants (Bencze *et al.* 2011b).

In the plants grown at EC, a decrease was observed in the activity of GR and GST in Mam and for POD in Reg, but there was an increase in CAT in Mam and Lo compared with the NC values. As the enzyme activity only declined due to EC in the varieties that had higher NC values, this led to a fairly relaxed state of the antioxidant enzyme system; the activities of GR, GST and APX had the same low values in all the varieties. The higher rate of electron consumption in photosynthetic carbon fixation could be a possible explanation for the low activation level of the antioxidant enzyme system (Tausz *et al.* 2013, Salazar-Parra *et al.* 2012). These findings, highlighting the differences in the basic characteristics of the varieties, might also explain the discrepancy in the literature regarding the reaction of plants to EC with some authors reporting no significant change for GR, APX, CAT, or POD (Schwanz *et al.* 1996, Lin and Wang 2002, Mishra *et al.* 2008), while others found a decrease or no change for CAT and APX, depending on the species (Schwanz and Polle 2001, Schwanz *et al.* 1996) or a decline in GST, GR, CAT, and APX (Kőmíves *et al.* 1997, McKee *et al.* 1997, Polle *et* *al*. 1997, Pritchard *et al*. 2000, Gillespie *et al*. 2011) in response to EC.

Response of plants to EC and drought: To certain levels of drought stress, the plants grown at EC had higher photosynthetic rates than those at NC, as it was also reported in the case of P_{Nsat} by Tausz-Posch *et al*. (2013). EC enhanced drought tolerance in the sensitive variety more than in the more tolerant genotypes; Lo plants had higher assimilation rate at EC than at NC under medium drought stress, while the more tolerant genotypes had similar values at both CO_2 concentrations even under mild stress. These results were in accordance with previous findings (Lin and Wang 2002), where a drought-sensitive variety was reported to benefit more from growing at a high CO_2 concentration, based on the leaf Chl content.

Despite the fact that photosynthesis was stimulated by EC at the control level, severe drought resulted in even lower photosynthetic rates at EC than at NC. This suggests that though the plants could deal with the increasing level of stress better at EC than at NC, severe drought resulting in the drop in the leaf water content might lead to a more dramatic collapse of homeostasis in plants with bigger foliage at EC. This was supported by the data of the leaf absolute water contents (not included here) which showed that the leaves of all the varieties had higher water content at about 10% SVWC at EC than at NC, but started to lose water much faster at EC (in Mam and Lo; no data were available for Reg below 10% SVWC). The value of Φ_{PSII} also demonstrated the limitation of photosynthesis under drought in Lo, which had the poorest tolerance of drought. The decline in the photosynthetic rate in response to drought was caused mainly by the stomata closure but also by the damage to PSII (Petridis *et al*. 2012). This was probably also the case for Lo, which exhibited a dramatic decrease in Φ_{PSII} in response to drought while in the other two varieties only P_N declined.

No clear differences were detected in Φ_{PSII} between the CO_2 concentrations at any soil moisture levels in any of the varieties. This was in accordance with the findings of some researchers (McKee *et al*. 1997).

The decrease in g_s due to drought was greater at EC than at NC in one variety, as it was also found in *Arabidopsis* in response to osmotic stress (Leymarie *et al*. 1999). In the other two varieties, however, the decrease was either smaller or g_s was maintained at a similar level longer at EC than at NC.

Even under more severe soil water levels, E was lower and WUE was higher at EC than at NC in all the varieties. Although WUE remained at similar levels at NC, it decreased gradually at EC with decreasing water supply levels, until it reached values similar to those at NC. This suggests that EC successfully improved WUE even under the medium drought conditions. The above results indicated that the elevated WUE at EC could be attributed not only to the increase of P_N even under moderate water deficiency, but also to the permanently low values of E. This is in accordance with previous findings (Chaudhuri *et al*. 1990, Tuba *et al*. 1994, 2003), which suggested that WUE improves at high CO_2 concentration due to the considerable decline in E and a concurrent rise in P_N. In another study, the reduction in E and the enhancement of P_N due to EC increased both the instantaneous and the whole-plant WUE also under irrigation and drought-stress conditions (Robredo *et al*. 2007), leading to a much slower depletion of soil moisture at EC.

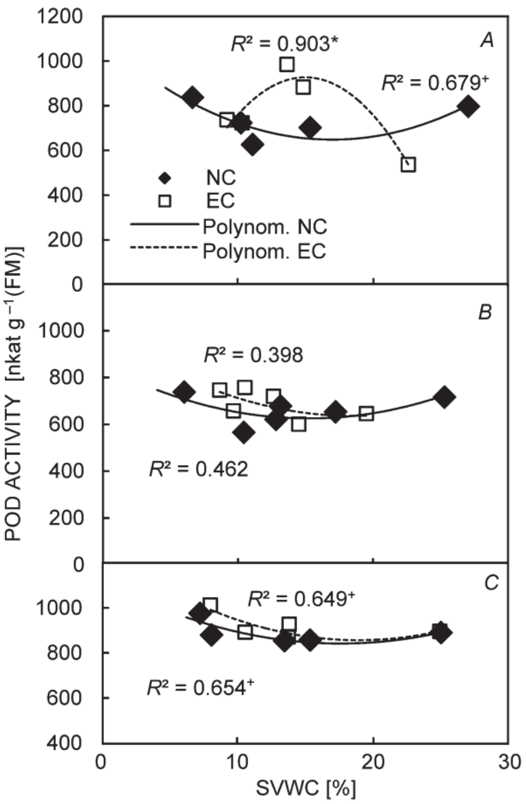

Fig. 8. Change in the guaiacol peroxidase (POD) activity in response to elevated CO_2 concentration and drought. Winter wheat varieties: Mv Regiment (*A*), Mv Mambo (*B*), spring wheat: Lona (*C*). Each value represents the average of five leaf samples from the same pot. NC – normal CO_2, EC – elevated CO_2, SVWC – soil volumetric water content. The R^2 values are given for polynomial functions (2nd degree). $^{+, *, **,}$ and *** – significant R^2 values at the probability level of 10, 5, 1, and 0.1%, respectively.

EC resulted in generally more pronounced antioxidant enzyme responses to drought. Compared with the NC values, most varieties showed increases in most enzyme activities at least at certain levels of drought. These findings were in accordance with those reported for the effect of drought and oxidative stress (O_3) on CAT and APX activity in plants grown at EC (Lin and Wang 2002, Gillespie *et al*. 2011). The stimulation of the antioxidant enzyme system reported here, caused by EC and drought, led to maximum activity values of APX, CAT, and GST very similar in all the varieties. The enhanced increase in the antioxidant enzyme activity at EC suggests a higher level of oxidative stress in the plants adapted to EC but subjected to water deficiency. This idea was confirmed by the fact that P_N declined faster at EC than at NC, while the Φ_{PSII} value was similar to that recorded at NC.

Differences between the varieties: The varieties represented different types of ecological adaptation. The most successful variety, best adapted to drought conditions, was Mam, which had moderate P_N, low g_s, and high WUE. In the earlier work, this variety was found to have the greatest antioxidant enzyme capacity even under control water supply (Bencze *et al.* 2011a), and no further increase was detected in the present work in response to drought. The other winter wheat variety, Reg, which had record grain yield potential and headed very early, had a similarly moderate rate of P_N, but very high g_s and low WUE. Despite the fact that the antioxidant enzyme activities were lower at control water level in Reg than in Mam, Reg responded very quickly, even to mild oxidative stress. Lo, being a spring variety with the relatively short vegetation period, had high P_N, and moderate g_s and WUE, but it had very low antioxidant enzyme potential, which gave little response to stress, probably making this variety very prone to drought. In addition, the antioxidant enzyme system of Lo exhibited the least reaction to EC with very little difference between the CO_2 concentrations in response to drought. In fact, Lo had lower APX values under mild drought stress at EC.

Unlike the sensitive variety, Reg responded quickly to drought even at EC and the antioxidant enzyme activity was maintained at a high level longer at EC while it declined at NC. These results are in good accordance with those reported by other authors for the activity of the same enzymes; APX, CAT, and POD in both sensitive and tolerant varieties (Lin and Wang 2002). The fact that no decline was observed in the enzyme activity of the other two varieties studied here could be due to the shorter period of drought applied here compared to the previous work.

The above findings suggest that there was a considerable variation between the different varieties in their response to EC, as could be seen from the changes in P_N, g_s, E, and WUE, as well as in the antioxidant enzyme system. This resulted in different responses to drought compared with those observed at NC. In all the varieties, the changes induced by EC led, however, to a relaxed state of the antioxidant enzyme system with the low activities of GR, GST, POD, and APX, while drought at EC resulted in the pronounced stimulation of the system with high APX, CAT, and GST activities. This could be explained by the higher level of oxidative stress at EC when drought became more severe; the assimilation was reduced by a greater extent at EC than at NC, while the electron transport was maintained at similar levels at both CO_2 concentrations.

These results indicated that although EC had generally very favourable effects on the development and stress tolerance of plants; certain general effects, such as the bigger foliage, might make the plants more prone to water loss and the relaxation of the defence mechanisms might also increase the risk of damage caused by the higher level of oxidative stress at EC than at NC.

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