Antioxidant enzyme activities in *Allium* species and their cultivars under water stress

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ABSTRACT

We compared the enzymatic antioxidative defence mechanisms of some regional subspecies of *Allium* (*A. cepa* L., *A. ascalonicum* auct. hort., *A. sativum* L.) cultivated mainly in the western regions of Romania, and two modern Hungarian climate resistant F_1 hybrids. The variability in the activities of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione reductase (GR) and glutathione S-transferase (GST) and their changes under soil moisture stress were investigated. 1-week-long water stress revealed that among three *Allium* species, relative water content decreased only in *A. ascalonicum* leaves (up to 16%). Unlike root enzymes, the activities of the shoot enzymes, especially POD, GR and GST showed a stronger correlation with the water content of the leaves after one week of water withdrawal; regression coefficients (R^2) were 0.359, 0.518 and 0.279, respectively. The ancient populations with elevated (or highly inducible) antioxidant enzyme activities may be interesting for further research and for breeding of new *Allium* varieties.

Keywords: *Allium* species; superoxide dismutase; catalase; guaiacol peroxidase; glutathione reductase; glutathione S-transferase; drought stress; genetic diversity

Growth and productivity of plants depend on environmental conditions. Water deficit is a major limiting factor of crop production under continental climates and there is a continuous demand for genetically new drought-resistant crops and vegetables. One of the possibilities to improve drought resistance of cultivated crops is to find ancient regional subspecies or local races which are well adapted to local environmental conditions.

Drought is one type of oxidative stress that, at the cellular level, enhances the generation of active oxygen species (AOS), such as superoxide radicals ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\cdot}$). Plants have developed different enzymatic and non-enzymatic scavenging mechanisms to control the level of AOS. Superoxide radicals can be converted to hydrogen peroxide enzymatically by superoxide dismutases (SOD). Cellular hydrogen peroxide is removed by catalase (CAT) enzymes and other enzymatic defence systems e.g. ascorbate peroxidase (APX) and other peroxidases. The level of antioxidants and the activities of antioxidant enzymes such as H_2O_2 related SOD, CAT, APX, guaiacol peroxidase (POD), and glutathione related enzymes (glutathione reductase, GR and glutathione S-transferase, GST) are generally increased in plants under stress conditions and in several cases their activities correlate well with enhanced tolerance (Prasad et al. 1994, Foyer et al. 1997).

In our experiments, onion (*Allium cepa* L.), shallot (*Allium ascalonicum* auct. hort.) and garlic (*Allium sativum* L.) were studied. Onion and garlic plants are species of a worldwide economic importance and they have several intraspecific

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groups. Onion is propagated by seeds, bulbs or sets (small bulbs). The other plants are propagated traditionally vegetatively via sets, daughter bulbs or cloves in home gardens. In certain parts of Central Europe a temporary drought may occur in spring, which can cause stress (and decreased yields) for plants. Irrigation is necessary to avoid moisture stress during the growing period and to achieve a high onion yield (Chopade et al. 1998). Stressing plants prior to bulb formation resulted in a reduced bulb size. It was reported that soil water stress imposed at any growth stage reduces the total yield, but the greatest effect was at the 5- and 7-leaf stages or when irrigation was withheld both at the 3- and 7-leaf stages of development (Pelter et al. 2004). There are varieties adapted to bulbing in a wide range of environmental conditions, and these cultivars offer a promising source for novel genes and alleles to improve the drought resistance of cultivated crops.

We compared the antioxidative enzyme responses of some regional subspecies of *Allium* species cultivated mainly in the western part of Romania and two bred cultivars, *A. cepa* L. Makói Bronz and *A. sativum* L. Lelexir. The biological material was collected in areas where old local forms are still cultivated, the climate conditions are less favorable and the agriculture is less developed. The aim of our work was to investigate the variations present in the antioxidant response of populations and find a correlation between the activities of antioxidant enzymes and the response of different *Allium* plants to water withdrawal.

MATERIAL AND METHODS

Plant material

Onion (*Allium cepa* L.), shallot (*Allium ascalonicum* auct. hort.) and garlic (*Allium sativum* L.) cultivars – four of each – hereafter referred to as "populations", were estimated (Table 1). The *A. cepa* populations were numbered Ac 1–4, the *A. ascalonicum* were called Aa 1–4, and the *A. sativum* lines were numbered As 1–4. The old regional varieties were gathered from the western part of Romania and maintained by the group of Dr. E. Madoşă at the Banat's University of Agricultural Sciences in Timisoara. Small bulbs

Table 1. Origin of *Allium* populations used to study the variation in antioxidant enzyme activities

Population number	Botanical names of the crop group	Other names in the literature	Origin/Source	English name
Ac 1*	*Allium cepa* L., common onion group	*Allium cepa* var. *cepa* cv. Makói Bronz	Makó, Hungary	onion
Ac 2	*Allium cepa* L., common onion group	*Allium cepa* var. *cepa*	Deszk, Hungary	onion
Ac 3	*Allium cepa* L., common onion group	*Allium cepa* var. *cepa*	Valcani, Romania	onion
Ac 4	*Allium cepa* L., common onion group	*Allium cepa* var. *cepa*	Gelu, Romania	onion
Aa 1	*Allium cepa* L., aggregatum group, *Allium ascalonicum* auct. hort.	*Allium cepa* var. *ascalonicum*	Buteni, Romania	shallot
Aa 2	*Allium cepa* L., aggregatum group, *Allium ascalonicum* auct. hort.	*Allium cepa* var. *ascalonicum*	Ohaba Lungă, Romania	shallot
Aa 3	*Allium cepa* L., aggregatum group, *Allium ascalonicum* auct. hort.	*Allium cepa* var. *ascalonicum*	Buzad, Romania	shallot
Aa 4	*Allium cepa* L., aggregatum group, *Allium ascalonicum* auct. hort.	*Allium cepa* var. *ascalonicum*	Tela, Romania	shallot
As 1	*Allium sativum* L., common garlic group	*Allium sativum* var. *sativum*	Chesânţ, Romania	garlic
As 2	*Allium sativum* L., common garlic group	*Allium sativum* var. *sativum*	Nădab, Romania	garlic
As 3	*Allium sativum* L., common garlic group	*Allium sativum* var. *sativum*	Bârsa, Romania	garlic
As 4*	*Allium sativum* L., common garlic group	*Allium sativum* var. *sativum* cv. Lelexir	Makó, Hungary	garlic

Ac 1–4 = *A. cepa* populations; Aa 1–4 = *A. ascalonicum* populations; As 1–4 = *A. sativum* populations
* kindly provided by the Onion Breeding Research Station, Makó, Hungary

(sets) and cloves were stored at 4°C during winter and were planted in April in 15 l containers, filled with a mixture of "Bioland B" plant soil: sand:perlite = 4:1:1. The experiments were carried out in two seasons; at least 10 plantlets of each population were grown in one container. Plants were kept in a greenhouse in a 14/10-h day/night period, with 300 μmol/m^2/s light intensity at 22/25°C night/day temperature. Metal halogen, 400 W Philips 40 HP1-Tplus type lamps and Philips TL D58/840NG type strip lights were used as light sources. Drought stress was carried out by suspending watering of the 6-week-old sprouts and watered sprouts were used as control. At this age the plantlets were at the 3- to 5-leaf stages. Watering ensured approximately 40% water content of the soil mixture, which decreased to 22% after 7 days. The analysis was performed in three replicates on three plants.

Determination of water content

After exposing plants to drought, the fresh weight (fw) of leaves was measured for control and stressed plants. The leaves were then imbibed in distilled water for 24 h and the turgid weight (tw) was recorded. The plant material was dried for 24 h (80°C) and the dry weight was measured (dw). The relative water content (RWC) was calculated according to the following formula:

$$RWC\ (\%) = 100 \times (fw - dw)/(tw - dw)$$

Activity measurements of antioxidant enzymes

Enzyme activities were determined one week after water withdrawal. 1 g of plant tissue from control and treated plants was homogenized on ice in 4 ml extraction buffer (50mM phosphate buffer pH 7.0, containing 1mM EDTA, 1mM phenylmethylsulfonyl fluoride and 1% polyvinylpolypirrolidone). The homogenate was centrifuged for 25 min at 15 000 × g and 4°C. The supernatant was used for enzyme activity assays. The means ± SD were calculated from the data of at least 3 independent measurements.

SOD activity was determined spectrophotometrically by measuring the ability of the enzyme to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) in the presence of riboflavin in light (Dhindsa et al. 1981). One unit (U) of SOD was the amount that causes a 50% inhibition of NBT reduction in light. The enzyme activity was expressed in terms of specific activity (U/mg protein). CAT activity was determined by the decomposition of H_2O_2 which, in turn, was measured by the decrease in absorbance at 240 nm (Upadhyaya et al. 1985). One U equals the amount of H_2O_2 (in μmol) decomposed in 1 min. POD activity was determined by monitoring the increase in absorbance at 470 nm during the oxidation of guaiacol (Upadhyaya et al. 1985). The amount of enzyme producing 1 μmol/min of oxidized guaiacol was defined as 1 U. GR activity was determined by measuring the absorbance increment at 412 nm when 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) was reduced by GSH, generated from glutathione disulfide (GSSG) (Smith et al. 1988). The specific activity was calculated as the amount of reduced DTNB, in μmol/min protein mg, $\varepsilon_{420} = 13.6 mM^{-1}\ cm^{-1}$. GST activity was determined spectrophotometrically by using an artificial substrate, 1-chloro-2,4-dinitrobenzene (CDNB), according to Habig et al. (1974). One U is the amount of enzyme producing 1 μmol conjugated product in 1 min, $\varepsilon_{340} = 9.6 mM^{-1}cm^{-1}$. The protein contents of the extracts were determined by the method of Bradford (1976).

RESULTS AND DISCUSSION

6-week-old *Allium cepa* and *Allium sativum* plants from all varieties maintain the water content in their leaves for 7 days without watering (Figure 1A). During this 7-day period, the soil water content declined by more than 40%, which caused a mild drought stress. However, the RWC values in leaves decreased in three *A. ascalonicum* lines (marked as Aa 2, Aa 3 and Aa 4; see Table 1) confirming that shallot is the most sensitive to water withdrawal. Some of the local onion and garlic populations even showed a slight increase in water content of their leaves (Figure 1A). Caldwell et al. (2003) suggested that onion plants appear to tolerate drought through increasing their osmotic adjustment. Barrowclough and Peterson (1994) reported that the vitality of the onion root epidermis is inversely related to the ambient moisture level. Since these cells provide the major site for ion uptake in roots with a mature exodermis, their death may reduce the efficiency of ion uptake and influence several physiological processes.

The antioxidant enzyme activities were determined in leaves and roots of *Allium* species. The basic activity of the investigated enzymes was generally similar in shoots of different varieties

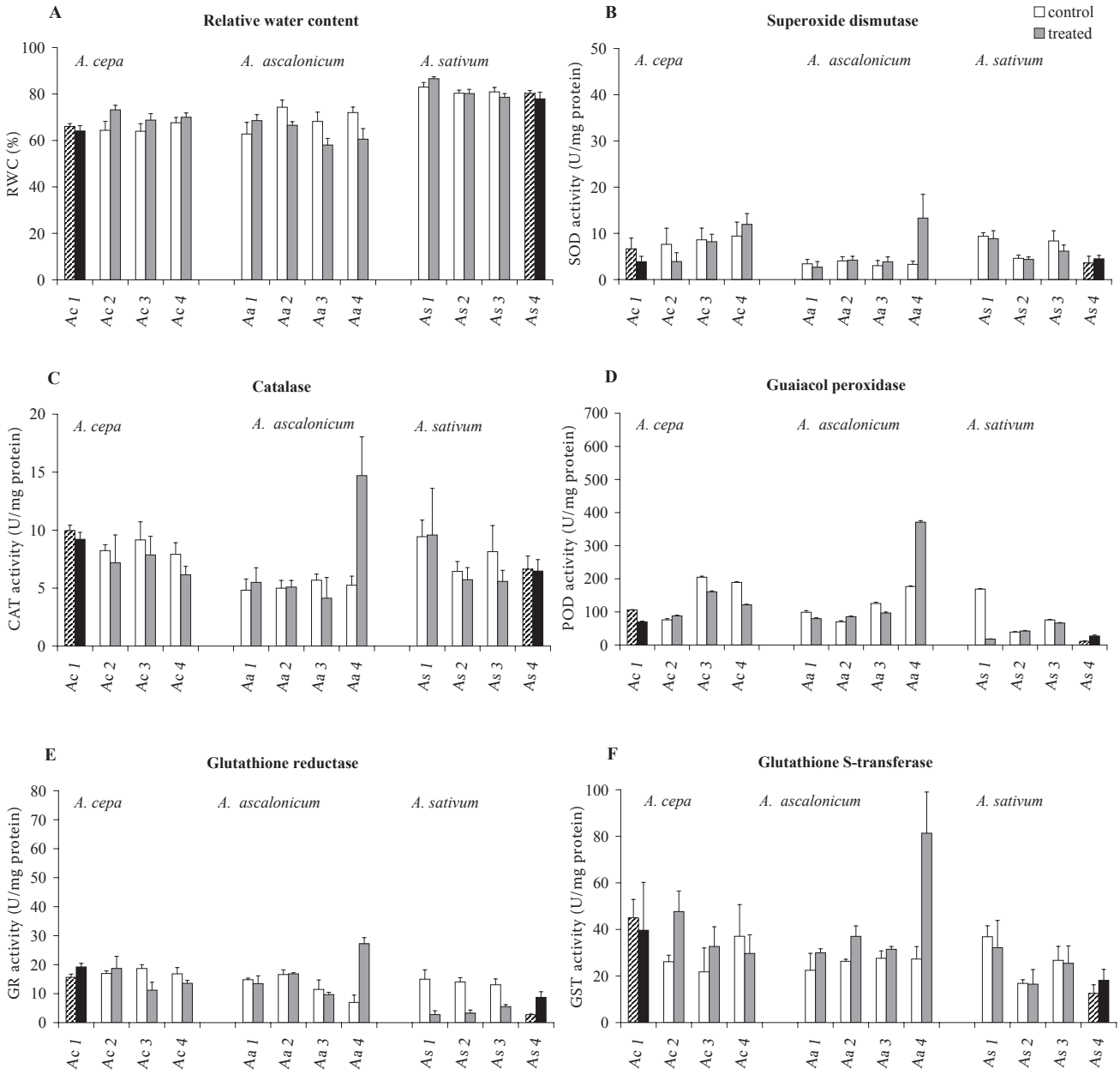

Figure 1. Relative water content (RWC %) of shoots (A) and changes in superoxide dismutase (B), catalase (C), guaiacol peroxidase (D), glutathione reductase (E) and glutathione S-transferase (F) activities of 6-week-old *Allium* populations after 1-week drought stress. Ac 1 and As 4 are bred cultivars. Abbreviations for populations are indicated in Table 1. Data are means ± SD of three samples

of the three *Allium* species, however we detected an approximately twofold enhancement of the measured enzyme activities in the Aa 4 genotype of *A. ascalonicum*, a species exhibiting a decrease in shoot RWC after one week of water withholding (Figure 1B–F). Comparison of the antioxidant enzyme activities in shoots of *A. cepa* lines also revealed that the populations named Ac 3 and Ac 4 had higher SOD and POD activities than the cultivated F_1 hybrid Makói Bronz (named Ac 1) (Figure 1B–F). Comparing the antioxidant enzyme activities of selected *A. sativum* lines to the bred garlic As 4 control cultivar, As 1 stands out with higher shoot antioxidant enzyme activities, especially in the case of POD, SOD, CAT and GST enzymes.

In roots, there was a higher variability in the antioxidant enzyme activities both within and among species and elevated activities were detected in several *Allium* populations after the water stress (Figure 2A–E). SOD activity was rather high in roots of Ac 4 and the stress treatment induced

it outstandingly in roots of Ac 3. In contrast to this, the hydrogen peroxide scavenging CAT and POD activities in the roots of these plants were low. Glutathione related enzymes, such as GR and GST, which have an important role for example in detoxification processes, were also investigated. Very high GST enzyme activities were measured in the roots of bred cultivars (Ac 1 and As 4) and in *A. ascalonicum* populations. *A. ascalonicum* populations can be characterized with relatively high SOD, POD and GST activities under control circumstances (Figure 2A–E). Root GR activities showed a different pattern: the bred garlic As 4 had a very high GR activity already under non-stressed conditions, while this enzyme was strongly induced in other *A. sativum* populations and in Ac 3 roots (Figure 2D). The drought treatment enhanced the GR enzyme activity in roots of several *Allium* plants and in shoots only in Aa 4 (Figures 1 and 2).

Changes in the antioxidant enzyme activities were compared with plant water content after drought stress. This comparison revealed no correlation for the shoot SOD and CAT activities, while the POD, GR and GST activities increased with the decreasing water content. The regression coefficients (R^2) were between 0.279 and 0.518 (Figure 3). It seems that the inducible antioxidative enzymes in *Allium* shoots are advantageous in stress situations. In roots, only GR and GST activities changed in relation to the water content, but these data showed even lower correlation (data not shown).

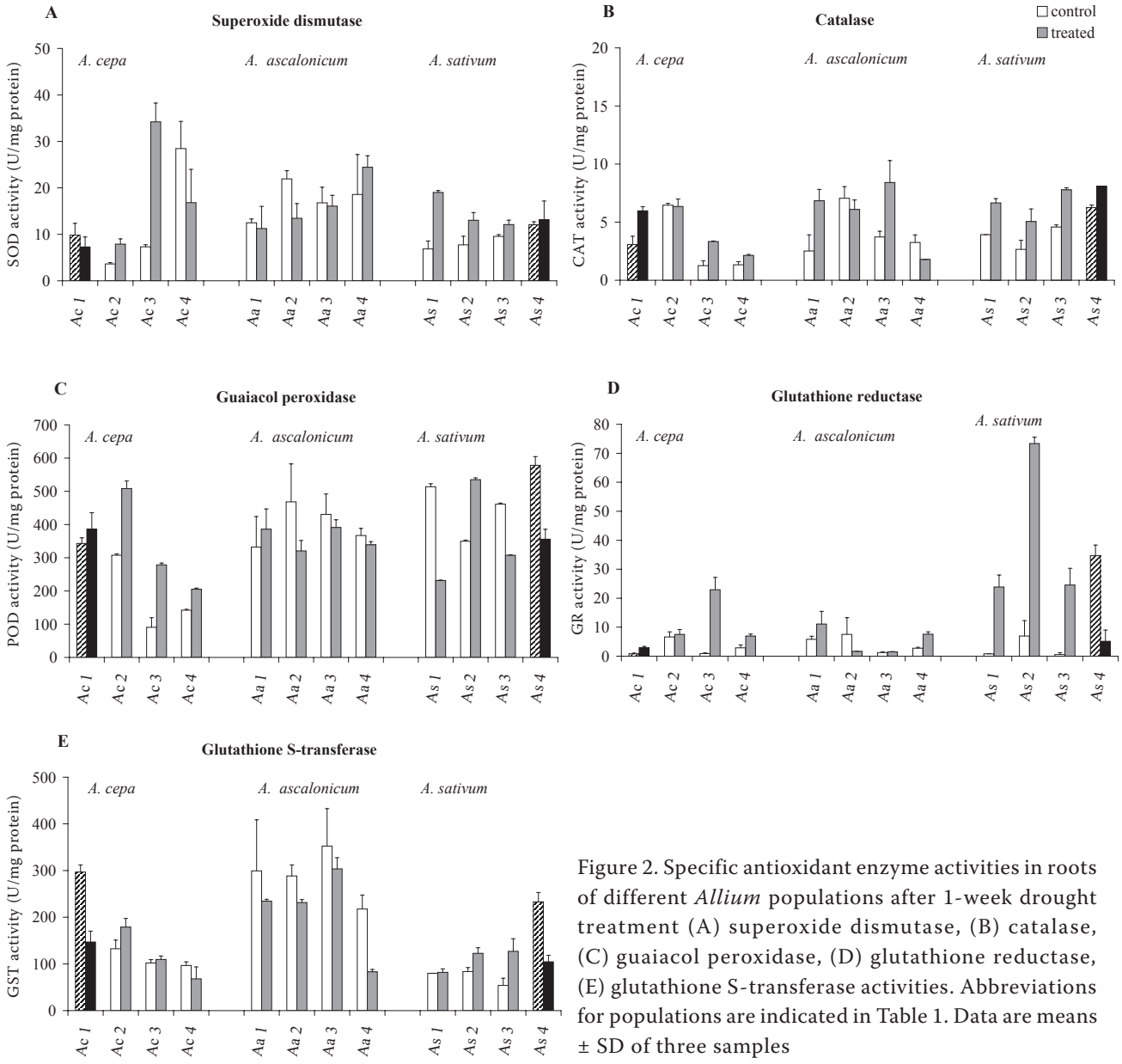

Figure 2. Specific antioxidant enzyme activities in roots of different *Allium* populations after 1-week drought treatment (A) superoxide dismutase, (B) catalase, (C) guaiacol peroxidase, (D) glutathione reductase, (E) glutathione S-transferase activities. Abbreviations for populations are indicated in Table 1. Data are means ± SD of three samples

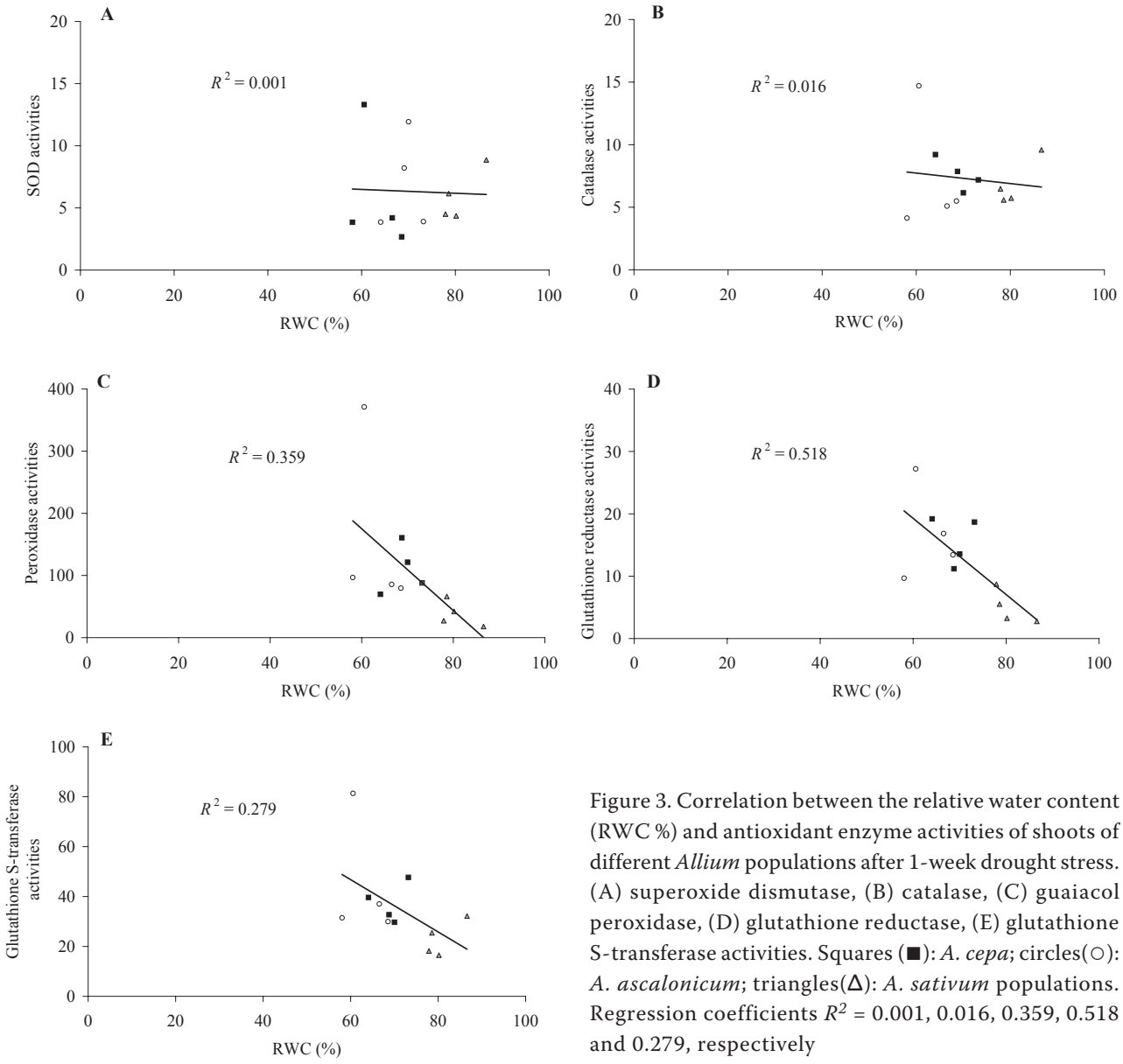

Figure 3. Correlation between the relative water content (RWC %) and antioxidant enzyme activities of shoots of different *Allium* populations after 1-week drought stress. (A) superoxide dismutase, (B) catalase, (C) guaiacol peroxidase, (D) glutathione reductase, (E) glutathione S-transferase activities. Squares (■): *A. cepa*; circles(○): *A. ascalonicum*; triangles(Δ): *A. sativum* populations. Regression coefficients R^2 = 0.001, 0.016, 0.359, 0.518 and 0.279, respectively

Having compared the antioxidant enzymes in *Allium cepa* L. and *Allium fistulosum* L. Štajner et al. (1998) reported that *A. fistulosum* has higher activities of antioxidant enzymes (SOD, CAT, glutathione peroxidase) than *A. cepa* and has a higher resistance to oxidative stress. Investigating the effect of salinity stress on antioxidant enzymes of some onion cultivars revealed that salt stress induces the activities of CAT, SOD and POD enzymes significantly and this is accompanied by increased glutathione levels. The most salt tolerant cv. had the highest antioxidant enzyme activities and GSH levels at different salt concentrations (El-baky et al. 2003). The ability of the fast, effective antioxidant response in stress situations can improve the tolerance. The processes connected with the enhancement of ROS or oxidising the glutathione pool could induce the protective mechanisms in plants (Foyer et al. 1997). In our previous investigations, higher POD, GST and glutathione peroxidase activities were also connected with elevated abiotic stress resistance (Csiszár et al. 2004).

Compared to the other *Allium* species, *A. ascalonicum* plants appear to be the most sensitive populations to moisture stress. Several antioxidant enzymes worked at a relatively high level in roots of shallot plants (SOD, GST and in some cases even CAT, POD), but this was not the case in the shoot. We experienced strong induction of these enzymes following the 1-week water stress only in the Aa 4 shallot populations. This *A. ascalonicum* has extremely inducible shoot SOD, CAT, POD, GR and GST activities. The increase in the activity

of scavenging enzymes could be a sign either of the severe oxidative stress or an efficient stress response mechanism (Zlatev et al. 2006).

In conclusion, the present study demonstrated that cultivated *Allium* varieties could be characterized with different antioxidant enzyme activity patterns. Changes in the glutathione related enzymes (GR, GST) and POD activities in shoots after 1-week water stress were associated with the relative water content of leaves. Our results suggest an important role of inducible antioxidant enzymes in the drought stress response. Ancient populations with elevated (or highly inducible) antioxidant enzyme activities present new opportunities for breeding and for further investigations of the drought tolerance mechanisms of different *Allium* plants.

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