PHENOLIC COMPOUNDS AND PROPERTIES
OF ANTIOXIDANTS IN GRAPEVINE ROOTS (*VITIS VINIFERA* L.)
UNDER DROUGHT STRESS FOLLOWED BY RECOVERY

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ABSTRACT

Grapevine seedlings *Vitis vinifera* were grown in a greenhouse under optimum conditions (soil moisture ca 70%) and under drought stress (soil moisture ca 35%). In addition, some of the plants subjected to drought underwent subsequent regeneration under optimum conditions. Drought stress caused accumulation of total phenolic compounds in grapevine roots, which may indicate that these compounds play an important role in the adaptation of roots to growth under stress conditions. Phenolic acids found in the roots occurred in the ester-bound form only. *p*-coumaric acid was present in the highest concentrations (6.2 to 10.5 µg/g fresh matter). The content of ferulic acid was lower, ranging from 2.4 to 4.6 µg/g fresh matter. The lowest concentration in grapevine roots was achieved by caffeic acid (2.4 to 2.9 µg/g fresh matter). The levels of *p*-coumaric and ferulic acids in roots rose significantly under the drought stress, while the concentration of caffeic acid increased during the post-drought recovery period. This may suggest that some of the phenolic acids protect plants under stress conditions. All the extracts from grapevine roots had antioxidative properties, but the antiradical activity of the extracts obtained from roots subjected to drought stress was inferior to the control. The same extracts were also characterised by depressed reducing power. The results imply that tolerance of grapevine to soil drought may be associated with the value of antioxidative potential in root tissues of these plants.

KEY WORDS: antioxidant system, oxidative stress, drought stress, grapevine roots, phenolic compounds.

INTRODUCTION

Water deficit, extreme temperatures and excessive salinity may cause oxidative stress in plants. In order to avoid it, plants have developed a complex system of antioxidative defence, which can be regulated depending on the environmental conditions. Under non-stress conditions, the system itself is enough to maintain homeostasis of oxygenation and reduction reactions, which prevents oxidative damage to bio-particles. Excess of reactive oxygen species and free radicals generated by oxidative stress can be neutralized by plants in a number of ways. One possible defence mechanism involves scavenging systems, such as superoxide dismutase as well as catalyse and peroxidase. Another system employs small molecule compounds, such as glutathione, ascorbate, carotenoids, flavonoids, tocopherols, phenols, proline, etc., for neutralisation of reactive oxygen species and free radicals. Depending on the type of stress and affected organ, plants can activate different antioxidative mechanisms. It should be noted here that scavenging systems can be an important element of the processes which condition plant tolerance to stress (Qureshi et al. 2007; Weidner et al. 2007; Woźny and Przybył 2007).

Among various compounds present in plant tissues, phenolic acids and flavonoids have antioxidative properties (Sokół-Łętowska 1997; Sokół-Łętowska and Oszmiański 1998; Rosicka-Kaczmarek 2004). The extent of antioxidative effects produced by phenols largely depends on the structure of a given compound and especially on the number and distribution of hydroxyl groups (-OH) (Sokół-Łętowska and Oszmiański 1998). Antioxidative activity is much higher when a compound has two -OH groups in the ortho position, e.g. caffeic acid (Oszmiański 1995; Rosicka-Kaczmarek 2004).

Phenolic compounds relatively easily release the -OH group hydrogen atom, which means that they oxidize themselves, thus acting as antioxidants. They reduce oxides and hydroxoides, and form complexes with metals (Oszmiański 1995; Sokół-Łętowska 1997; Sokół-Łętowska and Oszmiański 1998). Having released the hydrogen atom, phenols change into chinons, which are stabilized by non-pair electrons in the benzene ring, which means they cannot react with oxygen (Rosicka-Kaczmarek 2004). It is much more difficult to notice the dependence between antioxidative activity and the number and position of substitutents in the case of falvonoids (Oszmiański 1995; Sokół-Łętowska 1997; Sokół-Łętowska and Oszmiański 1998; Rosicka-Kaczmarek 2004).

The aim of the study has been to investigate the quantitative and qualitative modifications of phenolic compounds and to study the antioxidational abilities and the reducing power of extracts of grapevine roots which occur under the soil drought stress and following recovery.

MATERIAL AND METHODS

Plants

The material for the study consisted of whole root systems of grapevine *Vitis vinifera*, cultivar Himrod. The seedlings from which the roots were obtained had been purchased from a plant nursery in Jabłonowo (Poland).

Plant growth conditions

The seedlings were transferred into larger pots and grown for the next 8 weeks in a greenhouse under the following conditions: soil moisture ca 70%, 27/18°C day/night cycle and 14 h photoperiod at 180-200 µm/(m²s) irradiance. During the whole period, the plants were regularly watered to maintain the optimum soil moisture (70%). The soil moisture was monitored using a VP-PRL-Nr 279462 moisture meter manufactured by Mera. When the plants reached the expected size, the tests began. The plants were divided into four groups: control 1 (C1), drought stress (S), control 2 (C2) and stress + regeneration after the stress period (S+R). The experimental conditions were as follows: C1 – plants were grown under optimum conditions for a week (soil moisture about 70%), S – plants were grown under drought stress for a week (soil moisture kept down to about 35%), C2 – plants were grown for two weeks under optimum conditions (soil moisture 70%), S+R – plants were grown for one week under drought stress (ca 35% soil moisture) and afterwards, for another week, under optimum conditions (soil moisture ca 70%).

When the experiment had terminated, the seedlings were removed from the pots and the roots were cleaned from soil by rinsing with room temperature water. Next, the whole root systems were incised. The incised roots were frozen in liquid nitrogen, lyophilized and fragmented in a mixer mill.

Extraction of phenolic compounds

Phenolic compound were extracted under nitrogen from roots three times into 80 (w/v) acetone for 15 min at 80°C, as described by Amarowicz et al (1995). After evaporating the organic solvent in a rotary evaporator at 45°C, the remaining aqueous solution was lyophilized.

Determination of total phenolics

The content of total phenolic compounds in the extracts was determined using the Folin-Ciocalteau's reagent (Naczk and Shahidi 1989) and (+)-catechin was used as a standard.

Separation and analysis of phenolic acids by HPLC

Phenolic acids (free and those liberated from soluble esters and from soluble glycosides) were isolated from the extracts according to the method previously described by Krygier et al. (1982), Zadernowski and Kozłowska (1993), and Weidner et al. (2000). An aqueous suspension of the methanolic extract (200 mg in 20 ml) was adjusted to pH 2 with 6 M HCl, and free phenolic acids were extracted five times into 20 ml diethyl ether using a separatory funnel. The ether extract was evaporated to dryness under vacuum at room temperature. The water solution was neutralized and then lyophilized. The residue was dissolved in 20 ml of 2 M NaOH and hydrolysed for 4 h under a nitrogen atmosphere at room temperature. After acidification to pH 2 using 6 M HCl, phenolic acids released from soluble esters were extracted from the hydrolysate five times into 30 ml diethyl ether. Nine ml of 6 M HCl were added to the water solution, which was then placed in nitrogen atmosphere and hydrolysed for 1 h in a boiling water bath. Phenolic acids released from soluble glycosides were separated from the hydrolysate five times into 45 ml diethyl ether. After ether evaporation, the dry residue was dissolved in 2 ml methanol and filtered through a 0.45 µm nylon filter. The sample was injected onto an HPLC column. A Shimadzu HPLC system was employed: LC – 10 ADVP pump, photodiode array detector UV-VIS SPD – M10AVP, oven CTO – 10 ASVP, Controller SCL – 10AVP. The conditions of the separations were as follows: pre-packed LUNA C_{18} column (5 µm, 4.6×250 mm; Phenomenex); mobile phase water-acetonitrile – acetic acid (88:10:2, v/v/v) (Amarowicz and Weidner 2001); flow rate of 1 ml·min⁻¹; injection volume of 20 µl; the detector was set at 280 and 320 nm; oven temperature was 20°C.

Determination of tannins with the vanillin method

The content of tannins in the extract was determined using the modified Vanillin Assay (Price et al. 1978). Results were expressed as absorbance units at 500 nm per mg of extract (A_{500}/mg). Briefly, to 0.5 ml of extract, 2.5 ml of solution B (obtained by dissolving 0.5 g of vanillin in 100 ml of solution A) was added. Solution A was made by adjusting 4 ml of concentrated HCl to 100 ml with methanol. The samples were left in the dark at room temperature for 20 min, and then absorbance was measured at 500 nm.

Determination of tannins using the protein precipitation method

The content of tannins in the extract was determined using the method described by Hagerman and Butler (1978). Briefly, 2 ml of bovine serum albumin (BSA) solution (concentration 1 mg·ml⁻¹ in 0.2 M acetate buffer at pH 5.5 containing 0.17 M NaCl) was added to 1 ml of the extract. The sample was left for 15 min at room temperature and then centrifuged (5000 × g). After 15 min of centrifugation, the supernatant was removed and the pellet was washed with 1 mL acetate buffer and centrifuged again (5000 × g/15 min). The sediment was dissolved in 4 mL of

solution containing: 1% SDS and 5% TEA, and 1 ml of 0.01 M $FeCl_3$ in 0.01 M HCl was added. The samples were left for 30 min at room temperature and the absorbance was measured at 510 nm. The results were expressed as absorbance units at 510 nm per 1 mg of extract (A_{510}/mg).

Antiradical activity of roots extracts

The antiradical activity was analyzed with the method described by Yen and Chien (1995). Briefly, 0.1-0.5 mg of an extract dissolved in 0.1 ml of methanol was added to 2 ml of methanol; then, 0.25 ml of a DPPH (2,2-diphenyl-1--picrylhydrazyl) solution in the concentration of 1 mg·ml^{-1} methanol was added. The mixture was left in the dark at room temperature for 20 min. After that time, absorbance was read at a wavelength of 517 mm. The analysis was performed in three replications.

Reducing power of extracts

Reducing power of phenolics present in the extracts was determined using the method described by Yen and Chen (1995). Briefly, 1 ml of water containing 0.1-0.5 mg of an extract was pipetted into a tube. Next, 2.5 ml of 0.2 M phosphate buffer, pH 6.6, and 2.5 ml of 1% solution of potassium ferrocyanide were added. The mixture was incubated in a water bath at 50°C for 20 min. Afterwards, 2.5 ml of 10% TCA (trichloroacetic acid) solution was added. To 2.5 ml of this sample, 2.5 ml of 0.1% $FeCl_3$ in distilled water were added. After 10 min absorbance was measured at a wavelength of 700 nm. The analysis was performed in three replications, and means for the values thus obtained were derived.

Total Antioxidant Activity (TAA)

The Total Antioxidant Activity of the extracts was determined according to the Trolox equivalent antioxidant activity (TEAC) assay described by Re et al. (1999). TAA was expressed as mmol Trolox equivalent/g of extract.

Statistical evaluation

All experiments were repeated four times, with three replications for each sample. The presented values are the means of twelve determinations ± SD. Statistically significant differences in the mean values were tested by Student's t-test.

RESULTS

Dry matter content

The highest dry matter content was determined in roots of grapevine plants which had grown under strong drought stress (S sample), where it reached 22.7%, being significantly higher than in the control sample C1 (14.9%) (Table 1). In the roots of plants which had undergone drought stress followed by recovery (S+R sample), the dry matter content (17.7%) was lower than in the S sample but similar to that found in the C2 sample (16.4%).

Phenolic compounds

The total concentration of phenolic compounds in extracts from grapevine roots was determined using a reaction with Folin-Ciocalteu's reagent. The results are illustrated in Table 2. The highest level of phenolic compo-

TABLE 1. The content of dry weight in grapevine roots after drought stress and recovery. Symbols of samples, C1, C2, S and S+R, are explained explained in Material and methods. Asterisks denote differences assessed by t-Student's test as significant at $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$ (***).

Sample	Dry weight content (%)
C1	14.89±0.31
S	22.67±0.56 (*)
C2	16.41±0.31
S+R	17.71±0.47

TABLE 2. Comparison of the total phenolic content in grapevine roots after drought and recovery. Symbols of samples, C1, S, C2 and S+R, are explained in Material and methods. Asterisks denote differences assessed by t-Student's test as significant at $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$ (***).

Sample	mg/g FW
C1	9.57±0.06
S	15.72±0.09 (***)
C2	13.31±0.15
S+R	13.49±0.19

unds, 15.7 mg/g fresh weight, occurred in roots of grapevine plants which were subjected to drought stress (S). They contained much more phenolic compounds than roots of the control sample (C1) – 9.6 mg/g FW. The content of phenolic compounds in roots of the sample left to regenerate after a period of drought stress (S+R) was lower than in the S sample (13.5 mg/g FW). The content of phenolic compounds in the sample S+R was similar to that found in the sample C2 (13.3 mg/g FW).

Content of tannins

The content of tannins in roots of grapevine plants was analyzed with the Vanillin Assay and the BSA Precipitation Method (precipitation of tannins with protein). The vanillin assay is used to detect the presence of catechins and condensed tannins. The roots of grapevine plants which had been affected by drought stress (S), significantly more condensed tannins were found (0.65 A_{500}/mg extract) versus the control sample (C1; 0.56 A_{500}/mg extract). Also the S+R sample (drought stress followed by regeneration) contained much more condensed tannins (0.75 A_{500}/mg extract) than the sample which had only undergone drought stress (S). The results are demonstrated in Table 3. The concentration of tannins in samples as determined by the BSA precipitation method can be seen in Table 4. This method is used to determine the presence of both condensed and hydrolyzing tannins. Samples S and C1 contained

TABLE 3. Comparison of the content of tannins in extracts of grapevine roots after drought stress and recovery according to the Vanillin Assay. Symbols of samples, C1, C2, S and S+R, are explained in Material and methods. Asterisks denote differences assessed by t-Student's test as significant at $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$ (***).

Sample	A_{500}/mg extract
C1	0.56±0.02
S	0.65±0.02 (**)
C2	0.66±0.03
S+R	0.75±0.02 (**)

TABLE 4. Comparison of the content of tannins in extracts of grapevine roots after drought stress and recovery according to the BSA precipitation method. Symbols of samples, C1, C2, S and S+R, are explained in Material and methods. Asterisks denote differences assessed by *t*-Student's test as significant at $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$ (***).

Sample	A_{510}/mg extract
C1	0.294±0.005
S	0.291±0.003
C2	0.441±0.006
S+R	0.387±0.011 (*)

TABLE 5. Content of ester-bound *p*-coumaric acid in extracts of grapevine roots after drought stress and recovery. Symbols of samples, C1, C2, S and S+R, are explained in Material and methods. Asterisks denote differences assessed by *t*-Student's test as significant at $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$ (***).

Sample	µg/ g FW
C1	6.22±0.11
S	10.53±0.22 (***)
C2	9.94±0.25
S+R	9.23±0.21 (*)

TABLE 6. Content of ester-bound ferulic acid in extracts of grapevine roots after drought stress and recovery. Symbols of samples, C1, C2, S and S+R, are explained in Material and methods. Asterisks denote differences assessed by *t*-Student's test as significant at $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$ (***).

Sample	µg/ g FW
C1	2.44±0.05
S	4.63±0.09 (***)
C2	3.42±0.07
S+R	3.84±0.13 (**)

TABLE 7. Content of ester-bound caffeic acid in extracts of grapevine roots after drought stress and recovery. Symbols of samples, C1, C2, S and S+R, are explained in Material and methods. Asterisks denote differences assessed by *t*-Student's test as significant at $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$ (***).

Sample	µg/g FW
C1	2.41±0.05
S	2.54±0.06
C2	2.71±0.07
S+R	2.92±0.06 (*)

comparable levels of these two types of compounds (0.291 and 0.294 A_{510}/mg extract, respectively). During the regeneration period, tannins were observed to have increased in number, reaching the value of 0.39 A_{510}/mg extract. The highest concentration of these compounds was found in the roots from the C2 sample (0.44 A_{510}/mg extract). It should be noted here that the amount of condensed tannins in the S+R sample (0.39 A_{510}/mg extract) was much lower than in the C2 sample.

Content of phenolic acids

The qualitative assays of the grapevine root extracts enabled us to determine that they contained *p*-coumaric, ferulic and caffeic acids. All these phenolic acids occurred in the roots in the ester-bound form; no free and glycoside-bound phenolic acids were discovered in the root extracts. The content of *p*-coumaric acid in the sample subjected to drought stress (S) was significantly higher than in the control (C1) (Table 5). The concentration of ester-bound *p*-coumaric acid in the S sample was 10.5 and in the C1 sample – 6.2 µg/g FW. During the recovery period following the drought, the amount of this acid in grapevine roots declined. The samples contained the following amounts of ester-bound *p*-cumaric acid: 9.2 in the S+R sample and 9.9 µg/g FW in the C2 sample.

The content of ester-bound ferulic acid in grapevine roots was lower than that of *p*-coumaric acid, ranging from 2.4 to 4.6 µg/g FW. The highest content of this acid was found in the roots of plants subjected to drought stress (S) (Table 6). In this sample, 4.6 µg ferulic acid/g FW was determined versus 2.4 µg/g FW in the control sample (C1). While the plants regenerated after the stress, the amount of ester-bound ferulic acid in roots decreased. In the S+R sample, its content went down to 3.8 µg/g FW, which was still significantly more than in the control sample C2 (3.4 µg/g FW). The content of caffeic acid in grapevine roots was the lowest among all the three phenolic acids. It varied from 2.4 to 2.9 µg/g FW. The content of ester-bound caffeic acid in the control sample C1 was 2.4 µg/g FW. It

tended to increase slightly in the samples under drought stress (2.5 µg/g FW) but no significant differences were found between these two samples (Table 7). The amount of ester-bound caffeic acid was also observed to have risen in the roots of grapevine plants during the recovery period after drought stress (sample S+R; 2.9 µg/g FW).

DPPH• free radical scavenging capability of extracts

Aqueous solution of a DPPH• (2,2-diphenyl-1-picrylhydrazyl) free radical is deep red in colour. Under the effect of antioxidants found in extracts, this radical becomes paler so that eventually a colourless product is obtained. As a result of this reaction the absorbance of the solution decreases. The higher the content of antioxidants in a solution, the paler the solution. A DPPH• solution shows the maximum absorbance at a wavelength of 517 nm. The results of our assays are presented in Figure 1. All the analysed extracts proved to be capable of scavenging a DPPH• free radical. The highest scavenging capacity was demonstrated by the most concentrated solutions. The sample subjected to drought stress (S) was less capable of scavenging a DPPH• free radical than the control sample C1. The absorbance measured at a wavelength of 517 nm for the S sample, at the extract concentration of 0.04 mg per sample, was 0.42, whereas an analogous measurement for the C1 sample was 0.34. This seems to suggest that under drought stress the quantities of antioxidants in roots tend to decline. In the S+R sample, the absorbance was identical to that in the C1 sample (0.34), while the lowest absorbance was detected in the C2 sample (0.27).

Reducing power of the extracts

Under the influence of the reducing power of the extracts, Fe^{3+} ions were reduced to Fe^{2+} ions, and the colour of the reaction mixture changed from yellow to various tints of blue and green. The content of the reduced Fe^{2+} ions was measured by analysing the amount of Prussian blue produced in the solution, using absorbance measurements at a 700 nm wavelength. The results are shown in
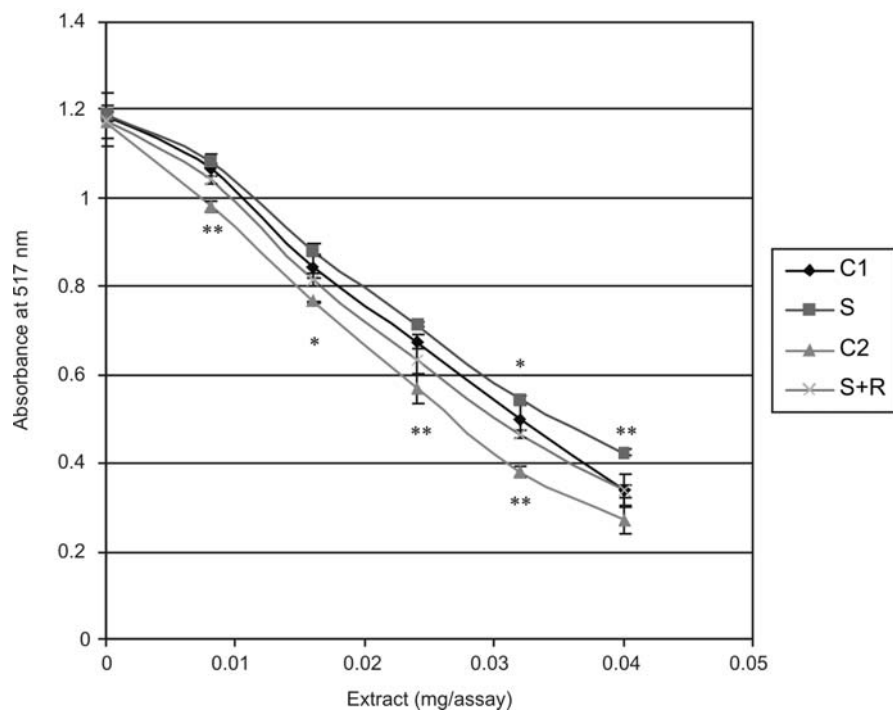
Fig. 1. Scavenging effects of grapevine roots after drought stress and recovery on DPPH[·]. Symbols of samples, C1, C2, S and S+R, are explained in Material and methods. Vertical lines denote standard deviation. Asterisks denote differences assessed by t-Student's test as significant at $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$ (***).

Figure 2. All the samples revealed reducing properties. The sample exposed to drought stress (S) was characterized by a much weaker reducing power than the C1 sample. Regarding the S and C1 samples, significant differences occurred at three extract concentrations: 0.4, 0.6 and 0.8 mg/sample. Stronger reducing power than in the above samples was determined for the S+R sample, while the C2 sample proved to possess the strongest reducing properties. At the extract concentration equal 0.8 mg/sample, the absorbance for the samples C1, S, C2 and S+R was, respectively: 0.97, 0.92, 1.21 and 1.11.

Total antioxidant activity

The ability to scavenge an ABTS[+·] – 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) free cation radical is presented in Table 8. The results which define the antioxidative capacity of the extracts are expressed in Trolox equivalents. A colour extract of an aqueous ABTS[+·] cation radical becomes colourless when a solution of the extract is added to it. The phenolic compounds from the extract have anti-radical properties, which means that they scavenge the cation radical ABTS[+·]. The higher the scavenging capacity, and consequently the higher the concentration of antioxidants in the solution, the lighter the colour of the sample and the lower its absorbance measured at a 734 nm wavelength. All the analyzed samples proved that they were able to scavenge the cation radical ABTS[+·]. The results can be seen in Table 8. Similarly to the previous research, the sample exposed to drought stress was significantly less capable of scavenging the radical (1.08) versus the control
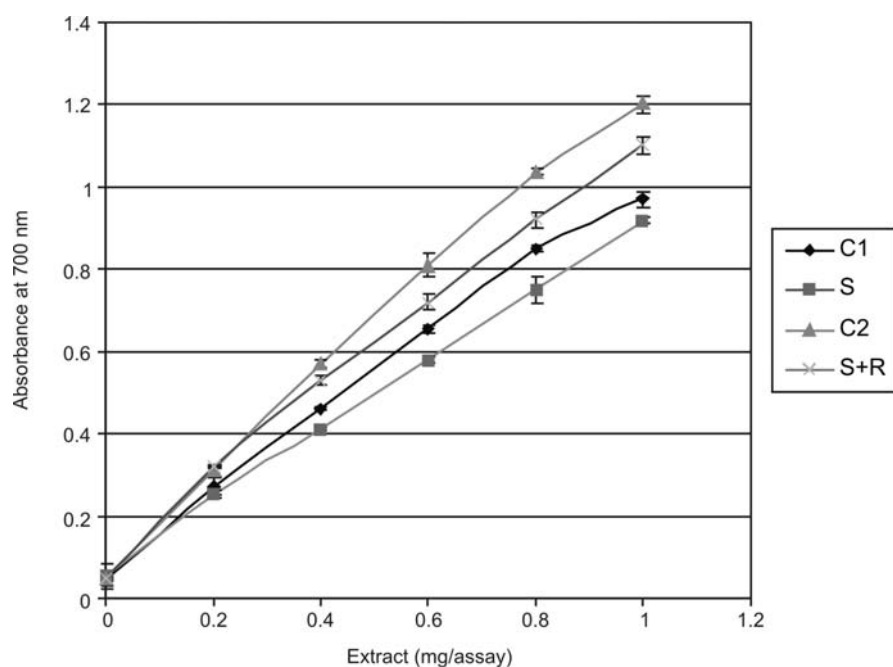

Fig. 2. Reducing power of extracts of grapevine roots after drought stress and recovery. Symbols of samples, C1, C2, S and S+R, are explained in Material and methods. Vertical lines denote standard deviation. Asterisks denote differences assessed by t-Student's test as significant at $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$ (***).

TABLE 8. Scavenging effects of extracts of grapevine roots after drought stress and recovery on ABTS$^{+\bullet}$. Symbols of samples, C1, C2, S and S+R, are explained in Material and methods. Asterisks denote differences assessed by t-Student's test as significant at $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$ (***).

Sample	mmol of Trolox/ g of extract
C1	1.47±0.02
S	1.08±0.05 (**)
C2	1.30±0.04
S+R	1.26±0.02 (*)

sample C1 (1.47 nmol Trolox/g extract). The S+R sample was more effective in scavenging the radical (1.26 nmol Trolox/g extract) than the S sample. The scavenging capability of the C2 sample in terms of the ABTS$^{+\bullet}$ radical was 1.30 nmol Trolox/g extract.

DISCUSSION

Plant root systems are studied in many laboratories worldwide. Sowiński (2000), who examined maize, found out that growth of maize roots is strongly correlated with the temperature of the substratum. Richter et al. (1998) proved a relationship between the size of a root system in maize and accumulation of dry matter in its shoots. In turn, Hashimoto et al. (2004) showed that roots not only play an important role in the uptake of water and mineral salts from soil but also participate in plants' tolerance to many stresses, such as drought, high salinity and chill. Noteworthy is the fact that Xiong et al. (2006) developed new approaches to studying plant drought tolerance and identified the inhibition of lateral root development by drought stress as an adaptive response to the stress.

Numerous studies have revealed that environmental stresses often raise the accumulation of phenylopropanoids, which are believed to play a regulatory role in some metabolic processes (Dixon and Paiva 1995; Solecka 1997; Janas et al. 2000; Wróbel et al. 2005). Also the research presented in this paper has demonstrated that the total content of phenols strongly increases in grapevine roots under drought stress.

Using AIP (2-aminoindan-2-phosphonic amid), the strongest inhibitor of PAL (phenylalamine ammonia-lyase), Szafrańska et al. 2002 made it evident that PAL (E.C.4.3.1.5) is the main enzyme in the synthesis pathway of phenolic compounds. They proved that application of AIP to soybean roots causes a decline in the content of some derivatives of cinnamic and benzoic acids, as a result of which plants are less tolerant to chill stress. Their results have been confirmed by Janas et al. (1999), who additionally showed that AIP has some influence on other physiological processes which affect plants' tolerance to stress conditions.

The present study has revealed large changes in the composition of phenolic acids in grapevine roots drought stress. The levels of ester-bound p-coumaric and ferulic acids in roots rose significantly under the drought stress. Also some previous experiments have showed that the chilling stimulated increased accumulation of some phenolic compounds (free gallic acid and catechins) in germinating *Vitis riparia* seeds (Wróbel et al. 2005). Janas et al. (2000)

demonstrated the presence of six phenolic acids in soybean roots: p-hydroxybenzoic, vanillic, syringic, anisic, p-coumaric and ferulic and during 24 h of low temperature exposure; also, an increase in the relative level of ester-bound-soluble phenolic acids occurred. It should be stressed that in plants subjected to cold and then to freezing treatments, the levels of p-coumaric, sinapic and ferulic acids increased about 3-, 4- and 5-fold, respectively (Solecka et al. 1999). Acclimation of plants in cold and the frost-thaw treatment resulted in the promotion of phenolic estrification. It can be added that hydroxycinnamoyl esters, present in cell vacuoles, may be used as substrate for vacuolar peroxidases in a peroxidase /phenolic/ ascorbate scavenging system which protect plant cells from the stress-induced oxidative damage due to increased production of active oxygen species (Solecka et al. 1999).

Anioxidant and antiradical activity of grape extracts has been investigated using several methods such as liposome system, a β-carotene-linoleate model system, lipid peroxidation, human LDL oxidation, serum total antioxidant activity, scavenging of DPPH$^{\bullet}$ radical, the FRAP assay and the ORAC assay (Amarowicz and Weidner 2009). Significant correlation between the content of total phenolics and antioxidant activity was found (Alonso et al. 2002, Weidner et al. 2007). Heo et al. (2007) found that there was no synergistic effect in a model system among the phenolic studied. Only an additive effect of antioxidant capacity was observed.

Among plant organs, root tissue is severely affected by drought due to its direct contact with drying soil. Among the most common responses to water stress are electron leakage through tylakoid membranes, damage of chlorophylls, decrease in antioxidant systems, increase in production of H_2O_2, O^-_2, lipid peroxidation and decrease in photosynthesis (Qureshi et al. 2007).

The above results suggest that all the extracts from grapevine roots had antioxidative properties, but the antiradical activity of the extracts obtained from roots subjected to drought stress was lower to the control. The same extracts were also characterized by depressed reducing power. Similar results had been obtained in our earlier studies on antioxidant properties of *Vitis amurensis* seeds under osmotic stress (Weidner et al. 2007). The extracts from seeds germinated under optimum conditions exhibited strong antiradical properties against the DPPH$^{\bullet}$ radical as well as reducing power. As regards the extracts from grape seeds germinated under osmotic stress, this capability was much weaker. The research demonstrated that antioxidants could interfere with the oxidation process induced by various stresses by acting as oxygen scavengers, therefore the tolerance to drought stress might be correlated with an increase in the antioxidant potential.

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