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ORIGINAL PAPER

Response of barley seedlings to water deficit and enhanced UV-B irradiation acting alone and in combination

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Abstract Responses of barley seedlings to water deficit (WD) induced by polyethylene glycol (PEG 6000) and ultraviolet (UV-B; 280–320 nm) radiation and their interaction (UV-B + WD) were examined. A decrease in dry matter yield and water content of leaves and roots was observed following application of WD and UV-B + WD, while no changes were found after treating barley plants with UV-B. Proline content was increased in leaves under WD conditions and UV-B + WD. In contrast, UV-B treatment had no effect on the accumulation of proline in leaves of barley plants. Changes in root proline content showed a varied response: WD induced an increase in the level of this amino acid, while UV-B as well as UV-B + WD suppressed root proline content. The lipid peroxidation product malondialdehyde (MDA) was increased in leaves under WD and UV-B + WD stresses. Root MDA content increased in WD-stressed plants, but it decreased in the case of combined application of both stresses. The applied stress factors operated in a variable manner on phenylpropanoid metabolism. Phenylalanine ammonia-lyase (PAL) activity in leaves and roots was stimulated after exposure to WD and application of UV-B + WD stresses, while UV-B stress did not affect its activity. On the other hand, UV-B treatment enhanced the activity of 4:coumarate-CoA ligase (4CL) in leaves and this enhancement was positively correlated with the accumulation of anthocyanins and flavonols. However, the combined application of WD and UV-B reduced the positive effect of UV-B on the accumulation of these compounds and the activity of 4CL. Surprisingly, anthocyanins and flavonols were not detected in roots of examined barley seedlings despite increased 4CL activity. The results suggest that UV-B-induced activation of 4CL as well as accumulation of anthocyanin and flavonols in leaves is beneficial for the response to this stress factor. On the other hand, WD-induced reduction of the effect of UV-B on 4CL activity and the contents of anthocyanin and flavonol might be a cause of membrane damage in UV-B- and WD-stressed plants. In addition, conversely to what could be expected, the UV-B effect was perceived by the water-stressed roots, which exhibited reduced lipid peroxidation (MDA) and proline accumulation in WD-stressed plants exposed to UV-B.

Keywords Flavonoids · 4:Coumarate-CoA ligase · Lipid peroxidation · Proline · Phenols · Phenylalanine ammonia-lyase

Introduction

Water deficit (WD) and increased UV-B radiation in the total solar radiation reaching the Earth are important environmental factors limiting productivity of crops in many regions of the world (Caldwell et al. 2007; Tubiello et al. 2007; Farooq et al. 2009). Under natural field conditions WD and high UV-B irradiation operate separately, but frequently plants experience both stresses simultaneously. The co-occurrence of these stresses could alter the effect of the individual stress responses. Each of the stress factors may alleviate or increase the negative effect caused by other stresses (Mittler 2006). Plant performance under stress conditions depends on the balance between the

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harmful effect of stress factors and a wide variety of protective and repair processes (Bray 1997; Chaves et al. 2003; Caldwell et al. 2007). Like almost all environmental stresses, both WD and UV-B radiation enhance the production of active oxygen species (ROS), which may initiate destructive processes, e.g. lipid peroxidation and cell membrane damage (Smirnoff 1993; Mittler 2002). One of the most common metabolic responses to WD is the accumulation of free proline, which is believed to play many important functions in plant reactions to this stress factor (Ashraf and Foolad 2007; Szabados and Savouré 2010). This molecule is considered to provide protection against stress by acting as an ROS scavenger, as a protectant of the cell membrane and enzymes, and as an osmolyte favoring osmotic adjustment, which results in water retention and prevents dehydration (Smirnoff and Cumbes 1989; Bandurska 2000; Szegletes et al. 2000; Öztürk and Demir 2002; Kocsy et al. 2005). Transgenic plants of various species that accumulate increased amounts of proline appear to exhibit enhanced tolerance to water deficit (Kavi Kishor et al. 2005).

The effect of UV-B radiation on plants includes accumulation of phenylpropanoids, such as phenolic acids and flavonoids (Liu et al. 1995, Kozłowska et al. 2007, Zhang and Björn 2009). Phenylpropanoid compounds belong to a large family of plant secondary metabolites, which are involved in an array of processes, including plant reactions to both biotic and abiotic stresses (Winkel-Shirley 2001). These compounds are produced from *trans*-cinnamic acid (*trans*-CA), formed by deamination of L-phenylalanine in a reaction catalyzed by phenylalanine ammonia-lyase (PAL, Fig. 1; Hollósy 2002). Derivatives of *trans*-CA such as *p*-coumaric, ferulic and sinapic acids and their respective alcohols serve as intermediates in lignin formation (Kováčik and Klejdus 2008). Lignin deposition in cell walls increases their rigidity and contributes to both drought and UV-B resistance (Sofo et al. 2007; Yamasaki et al. 2007). Flavonoid compounds are synthesized from *trans*-CA in reactions catalyzed by cinnamate-4-hydroxylase (C4H) and 4:coumarate-CoA ligase (4CL, Fig. 1; Hollósy 2002). These compounds act as solar screens, which absorb UV-B radiation and protect plant tissue against its harmful effects (Solecka 1997; Hernández et al. 2009; Zhang and Björn 2009). Additionally, phenolic acids and flavonoids can lower generation of reactive oxygen species and prevent cellular damage (Rice-Evans et al. 1997; Gould 2004).

In this study we analyzed the impact of polyethylene glycol (PEG)-induced WD and UV-B irradiation, operating individually as well simultaneously, on some physiological and biochemical changes in barley leaves and roots. Plant responses were assessed by evaluating the leaf and root dry matter accumulation, hydration status, membrane damage and the level of proline, phenolic acids, anthocyanins and flavonols as well as the activity of PAL and 4CL.

Materials and methods

Plant material and growth conditions

Seeds of barley (*Hordeum vulgare* L. cv. Granal) were incubated for 7 days in a germination chamber. Seedlings of uniform size were selected and planted in pots containing Hoagland's nutrient medium, which was aerated for 3 h per day and 3 h per night. Plants were grown in a climatic chamber (65–75% relative humidity; 20/13°C day/night temperatures; 14/10 h light/dark and 180 μmol $m^{-2} s^{-1}$ photosynthetically active photon flux density (PPFD) provided by fluorescent Philips TLD 18 W/840 sun lamps). Two-week-old seedlings were divided into four batches subjected correspondingly to: (I) WD, (II) UV-B radiation, (III) a combination of WD and UV-B (WD + UV-B), and (IV) not treated (control).

WD and UV-B radiation treatments

Water deficit was imposed by immersing the root system in a nutrient solution containing 20% polyethylene glycol (PEG 6000). Water potential of the nutrient solution was decreased by −0.5 MPa. Plants were irradiated with UV-B for 8 h a day using Philips lamps (TL 20 W/01 RS) with a characteristic emission in the range of 310–320 nm. The total energy delivered by lamps at the canopy level was 0.84 W m^{-2} (measured with VLX 3 W radiometer equipment, Vilbert Lourmant, Marne La Vallée, France). Daily dosage of UV-B radiation was 24 kJ m^{-2} day^{-1}. Control plants were grown in a nutrient solution under photosynthetically active radiation. Plant material (leaves and roots) were sampled (harvested) 10 days from the beginning of stresses imposition. Three plants per treatment were used for estimation of tissue water content and biochemical parameters. Analyses were made in three replicates derived from three different plants.

Measurements

Dry matter accumulation (DM)

DM accumulation during the experimental period was measured to estimate the effect of stresses on plant growth. Ten plants from each treatment were sampled. Shoots and roots were separated. Fresh weight of shoots and roots was measured and afterwards plant material was dried at 70°C to determine dry matter. Analyses were made in ten replicates derived from ten different plants.

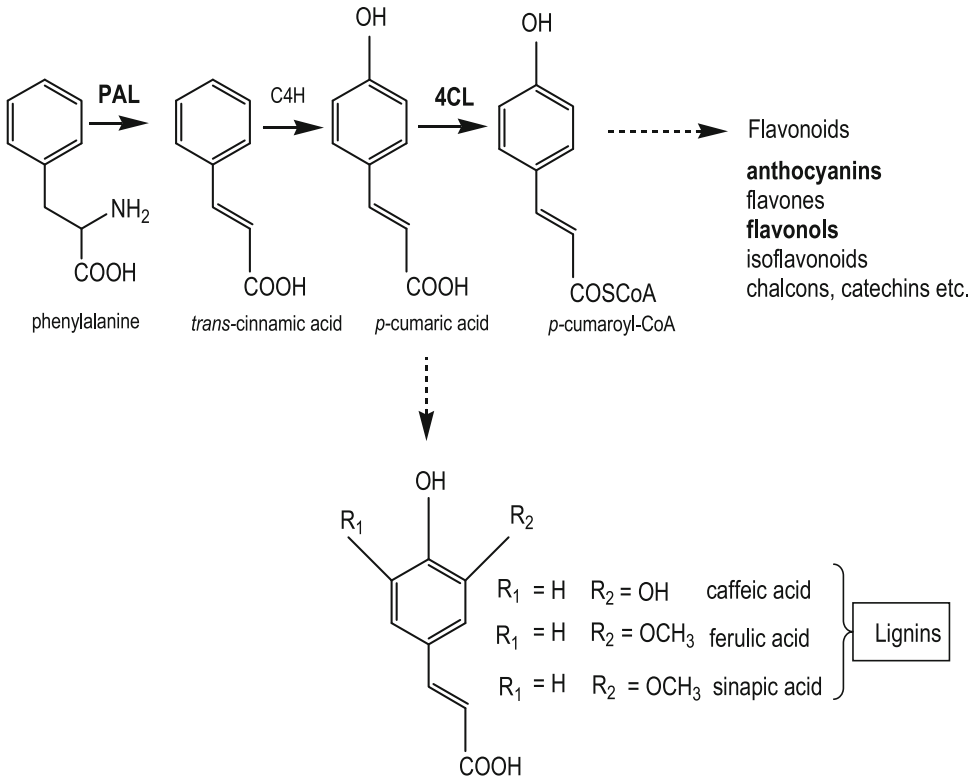

Fig. 1 Schematic diagram of biosynthetic pathway of flavonoid and lignin precursors

Water content in leaves was estimated by measuring relative water content (RWC) according to the standard method developed by Weatherly (1950) as described by Bandurska (2000) and it was calculated using the following formula:

$$\mathrm{RWC} = \frac{\text{fresh matter} - \text{dry matter}}{\text{fresh matter at full turgor} - \text{dry matter}} \times 100$$

Fresh matter of leaves was weighed immediately after harvesting. Fresh matter of full turgor was measured after placing leaf samples in distilled water for 4 h under normal room light and temperature. Samples were then oven dried at 70°C for 24 h and weighed to determine dry weight.

Water content in roots (WC) was estimated by measuring fresh weight and dry weight following oven drying of fresh root samples at 70°C. WC (%) was calculated using the following formula:

$$\mathrm{WC} = \frac{\text{fresh matter} - \text{dry matter}}{\text{fresh matter}} \times 100$$

Lipid peroxidation (MDA)

Leaf and root samples (0.3 g) were homogenized in a chilled mortar with 3 cm^3 of 0.1 M potassium phosphate buffer (pH 7.0) with 30 mg of Polyclar AT added and centrifuged at 16,000*g* for 30 min at 4°C. The level of lipid peroxidation in the supernatant was measured as the amount of malondialdehyde (MDA) determined as thiobarbituric acid (TBA) reactive substances according to Dhindsa and Matowe (1981). The volume of 2 cm^3 of 20% trichloroacetic acid (TCA) containing 0.5% TBA was added to 0.5 cm^3 of the supernatant. The mixture was incubated at 95°C for 30 min, quickly cooled in an ice bath to room temperature and then centrifuged at 10,000*g* for 10 min. Absorbance of the supernatant was determined at 520 and 600 nm. The value for nonspecific absorption at 600 nm was subtracted from the value at 532 nm. The concentration of MDA was calculated using absorption coefficient of 155 mM^{-1} cm^{-1} (Heath and Packer 1968). The level of MDA was expressed in mmol g^{-1} of dry matter (DM).

Proline content (PC)

Leaf and root samples (0.3 g) were homogenized with 5% TCA. The homogenate was centrifuged at 5,000*g* for 15 min and the supernatant was used for proline determination by measuring the quantity of the colored reaction product of proline with ninhydric acid according to Bates et al. (1973). Absorbance was read at 520 nm. The PC value in the sample was calculated from the proline standard curve and expressed in mg g^{-1} DM.

Phenolic acid content (PAC)

Leaf and root samples (0.2 g) were cut into pieces and homogenized with 5 cm^3 of 80% methanol. The homogenate

was centrifuged at 18,000*g* for 30 min. The Folin-Denis method was used to estimate PAC contents in the supernatant according to the method described by Swain and Hillis (1959). The level of PAC was calculated from the standard curve, using coumaric acid as a standard and expressed in μg g^{-1} DM.

Flavonol content (FC)

Leaf and root samples (0.5 g) were cut into pieces and homogenized with 5 cm^3 of methanol, HCl and distilled H_2O (90:1:1, v/v/v). The solution was stirred and heated (60°C) for 10 min, cooled at room temperature for 15 min and centrifuged at 23,000*g* for 30 min (Day 1993). The levels of flavonols were estimated by measuring absorbance of the supernatant at 254 nm with a UV/visible spectrophotometer (Jasco V-530 UV–VIS Spectrophotometer). FC was calculated using the calibration curves of quercetin (Stefova et al. 2001) and was expressed in μg g^{-1} DM.

Anthocyanin content (AC)

Plant material was homogenized with 0.5 N HCl, and centrifuged at 23,000*g* for 30 min. Absorbance of the supernatant was measured at 530 nm using a spectrophotometer. AC was calculated using a calibration curve of cyaninchlorid according to Arakawa (1991) and it was expressed in μg g^{-1} DM.

Enzyme activity

Phenylalanine ammonia-lyase (PAL) activity was determined using the method of Cahill and McComb (1992) according to Politycka (1999). Samples (0.5 g) were ground in a mortar at 4°C with 0.1 M Tris–HCl buffer at pH 8.9, containing 10 mmol of 2-mercaptoethanol and 50 mg of Polyclar AT. The homogenate was centrifuged at 12,000*g* for 20 min at 4°C. The reaction mixture contained 80 mmol of borate buffer, pH 8.9, 30 mmol of L-phenylalanine and 0.5 cm^3 of enzyme extract (supernatant) in a 2.5 cm^3 volume. The reaction proceeded for 24 h at 30°C and was interrupted by the addition of an equal volume of 2 M HCl to the incubation mixture. The product of the reaction catalyzed by PAL—*trans*-cinnamic acid—was determined at 290 nm (Jasco-V-530 UV/VIS spectrophotometer). Enzyme activity was expressed as nkat mg^{-1} protein.

The assay of 4:coumarate-CoA ligase (4CL) activity was performed according to Knobloch and Hahlbrock (1977). Samples (0.5 g) were ground with liquid nitrogen. Frozen powder was mixed with 100 mM Tris–HCl buffer at pH 7.8 containing 5 mM 2-mercaptoethanol and 5% glycerol. Next 0.1 g cm^{-3} Dowex AG 1-X2 was added and stirred for 15 min at 4°C. Extract was centrifuged at 23,000*g* for 30 min. The reaction mixture (0.2 cm^3) contained 100 mM Tris–HCl (pH 7.8), 0.1 mM coumaric acid, 0.5 mM ATP, 0.3 mM CoA, 5 mM $MgCl_2$ and 0.1 cm^3 of supernatant. The activity of 4CL was determined spectrophotometrically at room temperature. The formation of CoA esters of coumarate acid derivatives was measured as the increase in absorbance at wavelengths of 333 nm according to the absorption maxima for 4-coumaroyl-CoA (Stöckigt and Zenk 1975). The molar absorption coefficient values for 4-coumaroyl-CoA (21 mM^{-1} cm^{-1}) were used to calculate enzyme activities. The specific enzyme activity is expressed as picokatals per milligram of total extractable protein.

Protein was determined according to the method applied by Bradford (1976), using bovine serine albumin as a standard.

Statistical analysis

ANOVA was performed to determine the significance of differences between means by Tukey's test at $P < 0.05$. Statistical analyses were performed using Statistica 8.0 software.

Results

The assayed parameters of the test barley plants are influenced by the investigated stress factors. Application of WD individually and in conjunction with UV-B decreased leaf and root dry matter accumulation (Fig. 2A, B) as well as RWC in leaves and WC in roots of the test plants (Fig. 2C, D). There is no effect of UV-B treatment alone on dry matter accumulation or water content in leaves and roots of barley plants.

Membrane damage, expressed as the MDA content, was increased as compared with controls by approximately 20% in leaves of water-stressed plants and by 40% in double-stress applications (Fig. 3A). In contrast, in roots of WD-stressed plants MDA content was increased by 20% above the controls, but for the combination of WD and UV-B it decreased by 40% below the controls (Fig. 3B).

The increase in free proline content of leaves was quite pronounced for WD, amounting to about 18% above the control value, while UV-B had almost no effect (Fig. 3C). The combination of WD and UV-B profoundly stimulated the accumulation of proline in leaves of the test plants as compared to the controls (40% higher than controls). In the case of roots, it was observed that WD activated the accumulation of free proline (20% higher than controls), but for combined WD and UV-B the free proline was

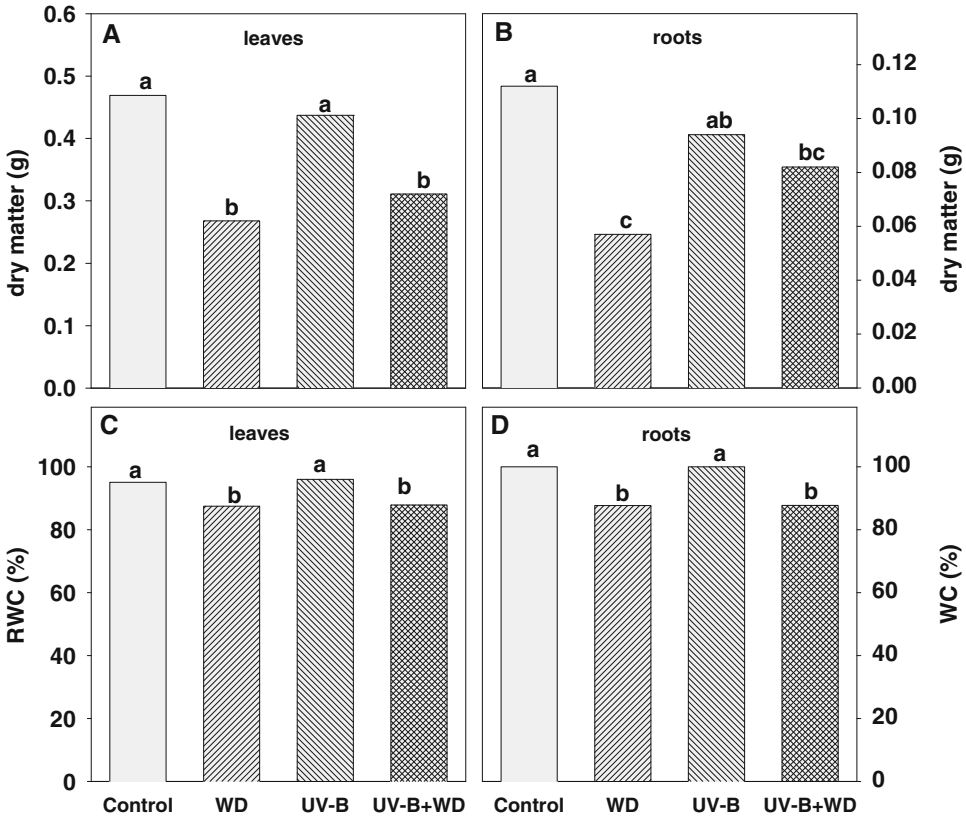

Fig. 2 Dry matter accumulation (**A**, **B**) and water content in leaves and roots (**C**, **D**) of barley seedlings treated with WD induced by PEG and UV-B radiation, acting individually and in combination. Different letters indicate significant differences between means of 10 replicates ($P < 0.05$)

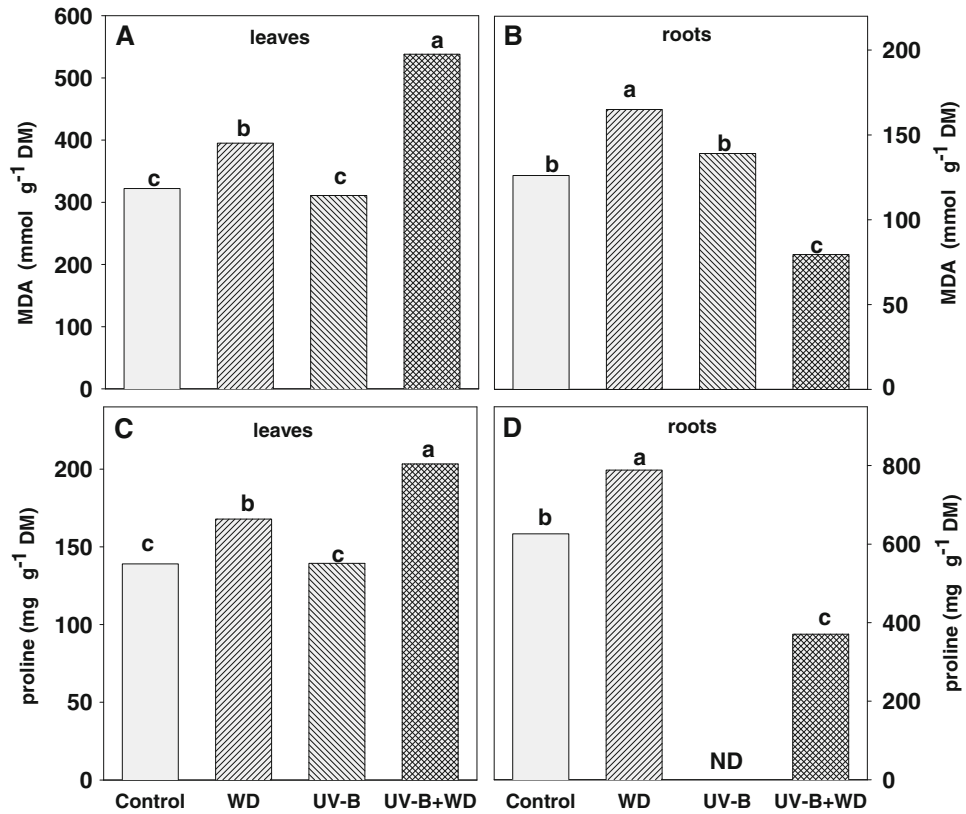

Fig. 3 Membrane damage (**A**, **B**) and proline content (**C**, **D**) in leaves and roots of barley seedlings treated with WD induced by PEG and UV-B radiation, acting individually and in combination. Different letters indicate significant differences between means of three replicates ($P < 0.05$)

sharply reduced (50% below controls) (Fig. 3D), while after UV-B treatment no detectable amount of proline was manifested.

The effectiveness of the applied stress factors on phenylpropanoid metabolism varied in the different organs of the test plants. In leaves of plants subjected to WD the activity of PAL was stimulated but 4CL did not change (Fig. 4A, C). UV-B radiation did not influence PAL activity, whereas it highly enhanced 4CL activity in leaves. When stresses were applied together, leaf PAL activity increased to the same extent as under the influence of WD alone. However, leaf 4CL activity did not change. In roots of plants subjected to WD the activity of PAL increased by about 24% over the controls, while it did not change in UV-B treated plants (Fig. 4B). Application of both stresses caused 59% increase of root PAL activity. On the other hand, neither WD nor combined application of both stresses led to any considerable changes of 4CL activity, whereas UV-B significantly stimulated the activity of this enzyme in roots (Fig. 4D). The amounts of phenolic acids were significantly decreased in roots of test plants under WD stress. However, there were no changes in these metabolites recorded in the application of UV-B and both stresses in roots as well as in the application of all stresses in leaves (Fig. 5A, B). The level of anthocyanins was increased in leaves of WD and UV-B treated plants by about 2.5- to 4-fold that of controls, respectively (Fig. 5C). In contrast, flavonol content was increased in leaves of UV-B treated plants by about 38% over the control value (Fig. 5D). There were no changes in leaf flavonol content of WD and WD + UV-B treated plants. Surprisingly, anthocyanins and flavonols were not detected in roots of examined barley seedlings.

Discussion

The negative effects of WD (PEG −0.5 MPa) on dry matter accumulation as well as tissue water content and the absence of changes in these parameters in UV-B (24 kJ m^{-2} day^{-1}) treated plants indicate that the reaction of barley seedlings to the applied stress factors was heterogeneous. In the experiment on black mustard *(Brassica nigra)* and turnip *(Brassica rapa)* both moderate soil WD (drought) and UV-B radiation at the levels of 6 and 17 kJ m^{-2} day^{-1} had detrimental effects on plant growth indices, but the effect of WD was much stronger than that of UV-B (Conner and Zangori 1998). A stronger effect of severe WD (PEG −2.0 MPa) than that of UV-B (13.6 kJ m^{-2} day^{-1}) on dry matter of aboveground parts was found in soy (*Glycine max)* (Sullivan and Teramura 1990). Conversely, a higher dose of UV-B (49 kJ m^{-2} day^{-1}) caused a stronger effect than moderate WD (PEG −0.5 MPa) on pea and wheat dry matter accumulation

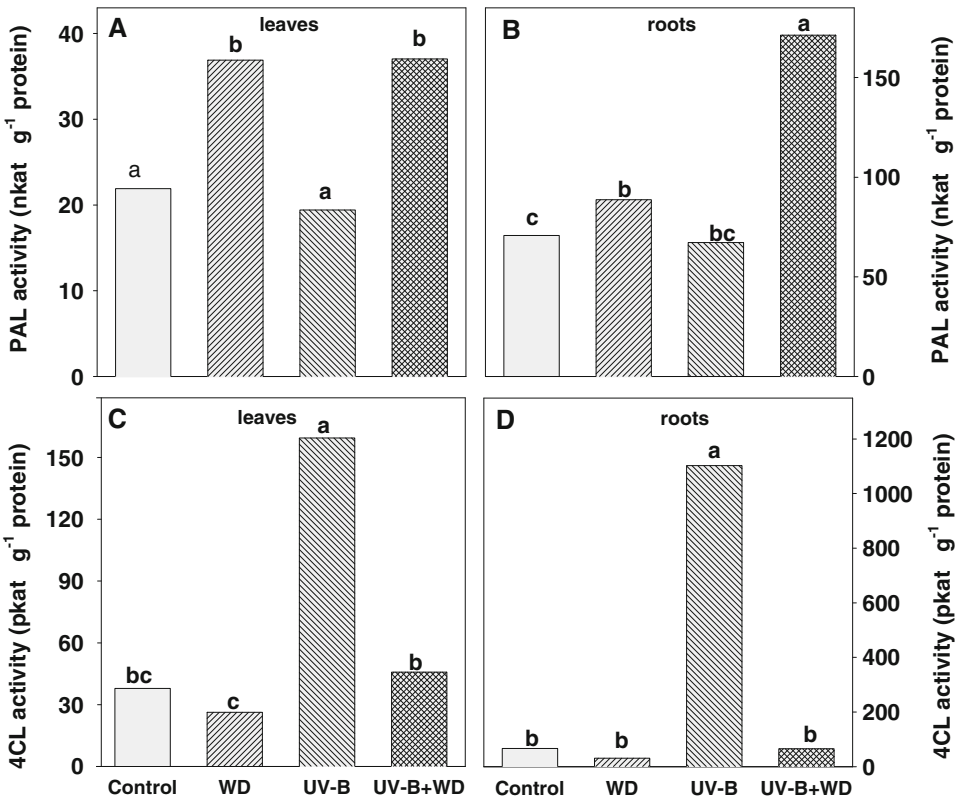

Fig. 4 Activity of phenylalanine ammonia-lyase (**A**, **B**) and 4:coumarate-CoA ligase (**C**, **D**) in leaves and roots of barley seedlings treated with WD induced by PEG and UV-B radiation, acting individually and in combination. Different letters indicate significant differences between means of three replicates ($P < 0.05$)

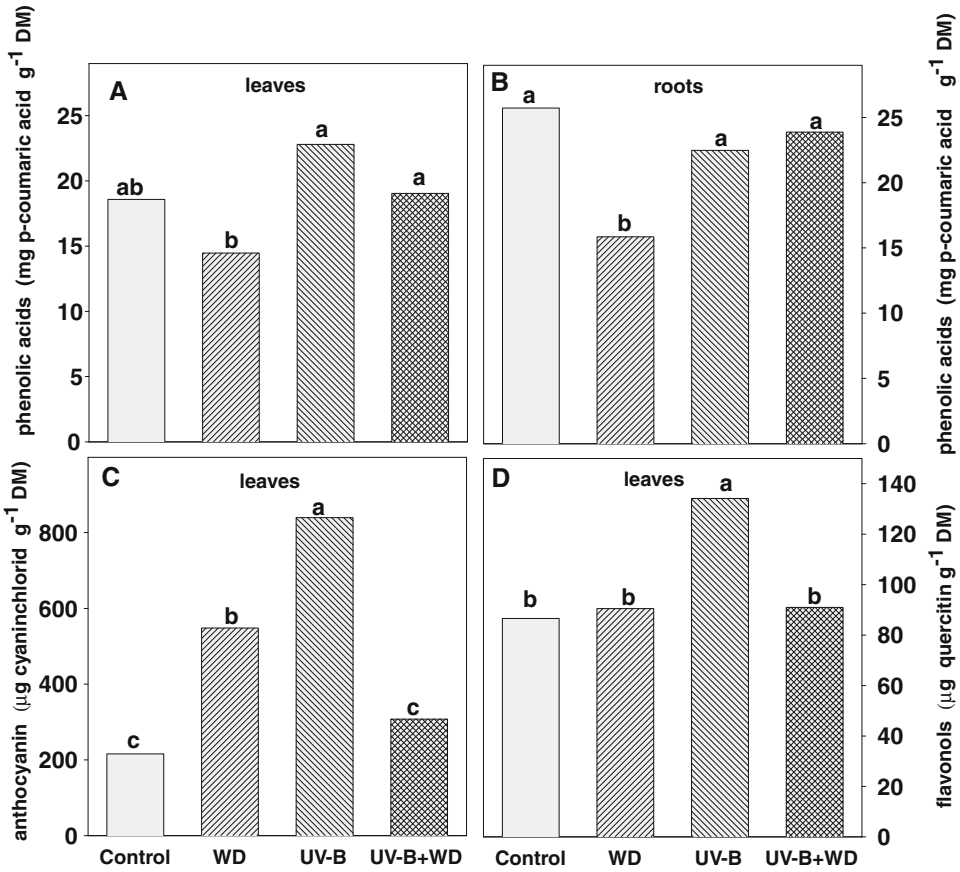

Fig. 5 Soluble phenol levels in leaves and roots (**A**, **B**), anthocyanin (**C**) and flavonol (**D**) levels in leaves of barley seedlings treated with WD induced by PEG and UV-B radiation, acting individually and in combination. Different letters indicate significant differences between means of three replicates ($P < 0.05$)

(Alexieva et al. 2001). The effect of combined action of WD and UV-B radiation on growth parameters in the test barley plants was generally similar to the effect caused by WD only. However, in wheat the combination of WD (PEG −0.5 MPa) with UV-B (3.5 kJ m^{-2} day^{-1}) caused more severe reduction of fresh matter of aboveground parts than stress factors applied separately (Tian and Lei 2007).

Inhibition of plant growth under water deficit conditions is a consequence of reduced cell turgor (Spollen et al. 1993). The results of this study indicate that a moderate WD contributed only slightly to changes in leaf hydration, while causing rather high growth inhibition in these organs. A significant factor in the mechanism of cell enlargement is cell wall extensibility (Cosgrove 1993; Passioura 1994). Under water deficit conditions cell wall extensibility may be reduced due to the formation of cross-linking between polymers forming them as well as deposition of lignins (Bacon et al. 1997; Lee et al. 2007; Moura et al. 2010). The biosynthesis of lignin and cross-linking formation of primary cell walls occur through a sequence of reactions, in which phenolic acids are involved (Fry 1986). The first stage in the formation of phenolic acids is deamination of phenylalanine catalyzed by PAL (Whetten et al. 1998). In the present study the action of WD and combined action of both factors contributed to increased PAL activity in leaves and in roots, which was accompanied by growth inhibition in these organs. In turn, an increase in PAL activity together with a lack of changes or a reduction of phenolic acid levels, which was observed in this study, indirectly indicates their consumption in metabolic processes responsible for lignification of cell walls under WD conditions. In our earlier studies we showed that WD contributed to lignification of cell walls in both leaves and roots of barley (Bandurska et al. 2005). Recently Fan et al. (2006) shown that WD induced by PEG initiated alterations in accumulation and metabolism of wall-linked phenolic substances, which are involved in inhibition of wall extensibility and root growth.

A slight reduction of water content in barley leaves under the influence of WD deficit may indicate a rapid closure of stomata. A significant role in this respect is played by abscisic acid (Chaves et al. 2003). This is confirmed by the results of our previous investigations, in which we observed a rapid increase of ABA level under the influence of WD in roots and next in leaves of barley, which was correlated with a high RWC level in leaves (Bandurska and Stroiński 2003). However, closure of stomata may lead to a reduced intensity of photosynthesis and, as a consequence, growth inhibition (Farooq et al. 2009). Another effect of stomatal closure, causing a reduction of

carbon dioxide assimilation at an unchanged level of absorption of light energy by the photosynthesizing organ, is an increase in the level of reactive oxygen species (ROS) in cells and damage to cell structures through lipid peroxidation (Syros et al. 2004; Miller et al. 2010). Several studies also showed that enhanced UV-B radiation caused generation of ROS and an increase of lipid peroxidation in many plants (Fedina et al. 2003; Feng et al. 2007; Tian and Lei 2007). In the present study only WD caused an increase in MDA level in leaves and roots of barley. Similarly, in leaves of beans, mulberry and wheat, as well as in leaves and roots of cucumber, the action of WD resulted in an increase in lipid peroxidation (Sairam et al. 1997; Reddy et al. 2004; Türkan et al. 2005, Arasimowicz-Jelonek et al. 2009a, b). Tian and Lei (2007) reported that the individual action of WD and UV-B radiation induced an increase in leaf MDA level in wheat. In turn, the simultaneous action of both factors resulted in their synergistic effect on the increase in MDA level. A similar reaction was also observed in our study in barley leaves, where the simultaneous application of WD and UV-B had a stronger effect on increased MDA level than single action. However, in barley roots the simultaneous action of both factors was antagonistic in character.

Accumulation of free proline is a characteristic and well-documented reaction of plants to the action of water deficit (Verbrugen and Hermans 2008). Under the influence of UV-B a slight increase in the level of this amino acid was found in leaves of pea, wheat and white clover (*Trifolium repens*) populations, while a lack of changes was observed in leaves of wheat (Alexieva et al. 2001; Hoffmann et al. 2003; Tian and Lei 2007). Moreover, in wheat seedlings combined application of both stresses had a stronger (synergistic) effect on proline accumulation than stress factors applied separately (Tian and Lei 2007). In the present study a mild WD contributed to an increase in the level of this amino acid both in leaves and roots, which is consistent with our earlier investigations (Bandurska et al. 2005). In contrast, we observed a lack of effect of UV-B on the accumulation of free proline in barley leaves. However, simultaneous action of WD and UV-B had a synergistic effect on leaf proline accumulation. The negative and antagonistic effect of UV-B radiation on proline level in roots—in contrast to WD—came as a surprise.

There is some evidence that plants exposed to UV-B accumulate proline, which can protect plants against lipid peroxidation induced by UV-B light (Saradhi et al. 1995; Alia et al. 1997). The results of this study do not confirm such an effect of UV-B radiation on the accumulation of proline and lipid peroxidation. Under WD conditions and the simultaneous action of both stressors, leaf proline accumulation was accompanied by an increase in the level of lipid peroxidation. However, in our opinion this may not be evidence for the protective role of proline. This is because the increase in the level of lipid peroxidation is a consequence of damage to cell membranes. It was demonstrated by other authors that an exogenous application of proline did not have a protective effect against UV-B-induced increase in lipid peroxidation (Fedina et al. 2003).

Several studies have suggested that flavonoid pigments exert a protective function against UV-B-induced damage because of their antioxidant capacity (Middleton and Teramura 1993; Bharti and Khurana 1997; Hernández et al. 2008). In our study this is indicated by a considerable increase in leaf anthocyanin and flavonol levels under the influence of UV-B radiation and a lack of peroxidation damage in cell membranes, as well as a lack of a negative effect of this factor on biomass accumulation. PAL activity is a key factor responsible for increased accumulation of flavonoids and other phenolic compounds in barley leaves under UV-B radiation (Liu et al. 1995). It has been reported that PAL activity positively correlated with anthocyanin synthesis in fruit of grapes, strawberries and apple (Horbowicz et al. 2008). Other authors have demonstrated that when apples ripen, anthocyanin accumulation decreases, even though PAL activity is relatively high (Wang et al. 2000). Our results show that UV-B radiation enhanced the production of anthocyanins and flavonols. However, no effect of UV-B radiation on PAL activity was found. It seems that the enhanced production of anthocyanins and flavonols induced by UV-B was due to increased activity of 4CL. This enzyme catalyzes activation reactions of derivatives of cinnamic acid, which are transformed into respective thiol esters of coenzyme A, constituting precursors for the synthesis of different secondary metabolites (Dixon and Palva 1995). The UV-B induced de novo synthesis of 4CL ligase and the accumulation of flavonoids were demonstrated in cell suspension cultures of parsley (Kuhn et al. 1984). In leaves of *Arabidopsis thaliana* a 30% increase was observed in the expression of 4CL genes under the influence of UV-B radiation (Kimura et al. 2003). *Arabidopsis* mutants with a defect of synthesis of flavonoid compounds were sensitive to UV-B radiation and, on the other hand, mutants with elevated accumulation of these compounds were tolerant to this stress factor (Li et al. 1993; Bieza and Lois 2001). Similarly, UV-B screening capacity and a lower degree of damage in barley primary leaves were mainly due to UV-B induced flavonoid accumulation (Schmitz-Hoerner and Weissenböck 2003). Moreover, Middleton and Teramura (1993) demonstrated the photoprotective function of flavonoids in UV-B treated soybean plants. A pronounced increase in the level of UV-B absorbing compounds and a greater tolerance to UV-B as well as to drought were observed in poplar species originating from high altitudes than those originating from lower altitudes (Ren et al. 2007).

At the lack of coenzyme A, 4:coumarate-CoA ligase may catalyze the synthesis of nucleotides such as adenosine-5′-tetraphosphate and diadenosine tetraphosphate, whose functions in plants have not been clarified to date (Pietrowska-Borek et al. 2003). It could have occurred in the roots of barley plants treated with UV-B radiation in this study, where a considerable increase was observed in the activity of 4CL and a lack of that of anthocyanins and flavonols.

In leaves of pea plants both WD and UV-B increased anthocyanin contents when imposed alone and had a synergistic effect when imposed together (Nogués et al. 1998). Our results also showed that WD treatment caused a considerable increase in the level of leaf anthocyanins, which correlated with PAL activity. In turn, under the influence of the combined action of both stress factors, despite increased PAL activity, the content of anthocyanins did not change. Moreover, a lack of effect was found for WD and the simultaneous action of WD and UV-B radiation on 4CL activity.

From our results it appears that the activation of 4CL as well as accumulation of anthocyanin and flavonols in leaves provoked by UV-B seems to be beneficial for the response to this stress factor. On the other hand, WD-induced reduction in the effect of UV-B on 4CL activity as well as anthocyanin and flavonol contents seems to be a cause of membrane damage in UV-B- and WD-stressed plants. However, conversely to what could be expected, the UV-B effect was perceived by the water-stressed roots. It was exhibited by reduced lipid peroxidation (MDA) and reduced proline accumulation in roots of WD-stressed plants exposed to UV-B.

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