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Impact of UV-B on drought- or cadmium-induced changes in the fatty acid composition of membrane lipid fractions in wheat

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Abstract

UV-B radiation may have either a positive or negative impact under the same conditions in wheat, depending on the type of secondary abiotic stressor: Cd or drought. Supplemental UV-B prevented the wilting and leaf rolling induced by PEG treatment. In contrast, combined UV-B and Cd treatment resulted in pronounced oxidative stress. The opposite effect of UV-B radiation in the case of drought or cadmium stress may be related to the alteration induced in the fatty acid composition. UV-B caused changes in the unsaturation of leaf phosphatidylglycerol fractions, and the accumulation of flavonoid in the leaves may prevent the stress induced by subsequent drought treatment. However it resulted in pronounced injury despite the increased flavonoid content in roots exposed to Cd. This was manifested in a drastic decrease in the unsaturation of the leaf monogalactosyldiacylglycerol and the root phosphatidylglycerol and digalactosyldiacylglycerol fractions. Data on the flavonoid content and fatty acid composition showed that oxidative stress was induced by drought in the leaves, by Cd in the roots, and interestingly, by UV-B radiation in both the leaves and roots. The additive effect of the combined stresses was also detected in the roots. The results presented here suggest a relationship between the capacity of the plant to remodel the fatty acid composition and its resistance to various stress factors.

Abbreviations: DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; *t*16:1, *trans*-Δ^3-hexadecanoic acid; 16:0, palmitic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid.acid

Keywords: Cadmium; Drought; Fatty acid composition; Flavonoids; UV-B radiation; Wheat

1 Introduction

Several reports have demonstrated the direct and indirect effects of UV-B radiation, an integral component of sunlight, on the plant growth and metabolism of various plant species, including a range of morphological, physiological and biochemical changes (Zlatev et al., 2012). However, as well as being a damaging agent UV-B also has an important role as a regulatory signal. The perception of and responses to UV-B by plants stimulate protective mechanisms, including the accumulation of UV-absorbing phenolic compounds and the modification of the biochemical composition (Jenkins, 2008). Under natural conditions plants are often subjected to multiple stress factors, so the impact of any particular stress may be aggravated or attenuated by the simultaneous action of another stressor. In a previous study it was demonstrated that UV-B radiation may have either a positive or negative impact under the same conditions in wheat, depending on the type of secondary abiotic stress factor: Cd or drought (Kovács et al., 2014). Supplemental UV-B light prevented the wilting and leaf rolling induced by PEG treatment. In contrast, combined UV-B and Cd treatment resulted in pronounced oxidative stress, which was manifested in the further enhancement of the leaf MDA content, root antioxidant enzyme activities and also root polyamine content compared to the effect of single stress factors, Cd or UV-B radiation (Kovács et al., 2014).

The ability to adjust membrane lipid fluidity by changing the level of unsaturated fatty acids is a feature of stress-tolerant plants, resulting in an environment suitable for the function of critical integral proteins. Changes have been described in the total lipid content, in the saturation of the total lipids, in the ratio of the membrane lipid fractions, and in the fatty acid composition of individual membrane fractions during single abiotic stresses, such as drought (Zhong et al., 2011; Filek et al., 2012) or heavy metal (Pál et al., 2007; Bernat et al., 2014). Nevertheless, no reports have yet been published on the combined effect of either drought or Cd treatment and UV-B radiation on the fatty acid composition of the individual membrane fractions in wheat plants.

As a continuation of previous work, the aim of the present study was to reveal the possible biochemical changes behind the opposing effects of UV-B radiation on drought and cadmium stress. Changes were investigated in the fatty acid composition of the different membrane fractions, together with the levels of certain flavonoids, phenolic compounds with antioxidant characteristic. Acquiring better knowledge of the regulation of fatty acid unsaturation may help us to understand plant adaptation to changing environmental conditions.

2 Materials and methods

2.1 Plant material and growth conditions

A winter wheat (*Triticum aestivum* L.) variety Mv Emese from the Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences (MTA ATK), Martonvásár, Hungary was used for the experiments. The seeds were germinated on filter paper for three days at 22 °C and grown on modified Hoagland solution (Pál et al., 2005) for two weeks at 20/18 °C with 16/8-h light/dark periodicity in a Conviron G-48 plant growth chamber (Controlled Environments Ltd., Winnipeg, Canada) at a photosynthetic photon flux density (PPFD) of 250 µmol m^{-2} s^{-1} provided by F96T12/CW/VHO 215W cool white fluorescent lamps (Sylvania, Canada). Half the plants were grown under control light (CL) and the others under control light supplemented with UV-B radiation (UV), achieved using 7 UV-B Narrowband TL 100W/01 lamps from Philips (with maximal radiation at 311 nm) during the 16 h light photoperiod. The dose of UV-B radiation was 430 µW cm^{-2}. After two weeks the seedlings of each treatment (CL and UV) were divided into three groups. The first group was the control (C or UV C), which received no secondary stress, the second group was treated with 50 µM $Cd(NO_3)_2$ for 7 days (Cd or UV+Cd) and the third group was treated with fifteen percent polyethylene glycol (PEG-6000) for 5 days (PEG or UV+PEG). Each treatment consisted of seven glass beakers, each containing twelve seedlings. The second nd and third rd leaves and roots of the control, UV-B, Cd-treated and PEG-treated plants were sampled at the end of the experiment.

2.2 Lipid extraction and fatty acid analysis

The lipids were extracted according to the method of Bligh and Dyer (1959) using 1 g plant tissue. The various lipid classes were separated by TLC on silica gel plates, after which the fatty acids were transmethylated (Pham-Quoc et al., 1994).

The GC–FID analysis of fatty acid methyl esters (FAME) was carried out using a Shimadzu Model GC–FID2010 system (Shimadzu Co., Kyoto, Japan) fitted with an SP-2380 capillary column (30 m×0.25 mm I.D., d_f=0.20 µm) (Supelco/Sigma-Aldrich Co.; St. Louis, MO, USA). In this analysis, the injector and initial oven temperatures were 200 and 175 °C, respectively. After 8 min, the oven temperature was increased to 240 °C at a rate of 50 °C/min and kept at this level for 5 min. Samples were injected in split mode with a 1/5 split ratio. The He carrier flow-rate was 37.8 cm/s. Heptadecanoic acid was used as internal standard for the quantification. Integration was performed using GC solution software 2.10 (Shimadzu Co., Kyoto, Japan).

The double bond index (DBI) was calculated from the mol% values using the following formula: DBI=1×(%18:1)+2×(%18:2)+3×(%18:3). DBI=1×(percent 18:1)+2×(percent 18:2)+3×(percent 18:3). The percentage of unsaturation was calculated using the following formula: % unsat=(18:1+18:2+18:3)/ (16:0+16:1+18:0+18:1+18:2+18:3).%unsat=(18:1+18:2+18:3)/(16:0+16:1+18:0+18:1+18:2+18:3).

2.3 Flavonoid extraction and analytical procedure

The quantity of flavonoids, namely rutin, myricetin and quercetin, were measured both in the free and methanol-soluble bound forms according to Meuwly and Métraux (1993) using 1 g of plant tissue. After the HPLC separation (Alliance 2690 system, Waters, Milford, MA, USA), carried out on a reverse phase column (ABZ+, 150×4.5 mm, 5µm.5 µm, Supelco, Bellefonte, USA), the flavonoids were measured using a W996 photodiode array detector (Waters, USA) in the 230–300 nm range.

2.4 Statistical analysis

The results are the means of five repetitions for HPLC and three repetitions for the GC analysis for each treatment. Changes in these parameters were compared to the same day control. The data were statistically evaluated using the standard deviation and *t*-test methods.

3 Results

3.1 Fatty acid composition in different membrane fractions

The following lipid fractions were examined: monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), phosphatidylethanolamine (PE) and phosphatidylglycerol (PG). In the leaf, the MGDG and DGDG fractions were characterized by a high proportion of linolenic acid (18:3), while in the PE and PG fractions palmitic acid (16:0) and 18:3 was the most abundant. In contrast, in the root the 16:0 and linoleic acid (18:2) were dominant in all the investigated membrane fractions.

3.1.1 Changes in leaf membrane fractions

In the galactolipid fractions (MGDG and DGDG) the initial proportion of polyunsaturated 18:3 fatty acid was so high (94.84% (94.84 percent in the MGDG and 85.02% 85.02 percent in the DGDG) that under the present conditions the single stress factors

applied did not influence it (Table 1). Nevertheless, the combined UV+Cd and UV+PEG treatments cause a significant decrease in the percentage of unsaturation and in the DBI of the MGDG fraction, especially due to the higher level of 16:0 and the lower proportion of 18:3 (Table 1).

Table 1 Effect of cadmium or drought stress with or without UV-B treatment on the fatty acid composition (mol%), double bond index (DBI) and percentage of unsaturation (%unsat) of various lipid classes obtained from wheat leaves. Data represent mean values±SD (*n*=3); different letters denote significant differences from control samples taken on the same day at $p<0.05$.

Fraction	Treatment	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	%Unsat	DBI
MGDG	CL C	1.48±0.42a	nd	0.30±0.21a	0.29±0.11a	2.92±0.72ab	94.84±1.50b	98.06±0.90b	290.67±8.90b
	CL Cd	2.27±0.60a	nd	0.55±0.22a	0.53±0.27a	3.55±0.53b	92.87±2.40b	96.96±1.16b	286.24±2.74b
	CL PEG	2.40±0.75a	nd	0.81±0.51ab	0.32±0.18a	2.88±0.50ab	93.59±1.81b	96.79±1.3b	286.84±5.62b
	UV C	1.66±0.43a	nd	0.38±0.10a	0.16±0.09a	2.41±0.22a	95.39±3.70b	97.961±1.99b	291.15±2.41b
	UV+Cd	5.40±1.12b	nd	1.25±0.40b	0.56±0.23a	3.74±0.46b	87.25±2.12a	91.54±0.89a	269.78±5.75a
	UV+PEG	4.77±0.52b	nd	2.05±0.59b	0.64±0.4a	4.18±0.63b	88.29±4.20ab	93.10±2.05a	273.86±4.55a
DGDG	CL C	9.69±0.98ab	nd	1.51±0.41ab	1.24±0.11c	2.43±0.48a	85.02±3.52ab	88.69±2.09a	261.15±5.63ab
	CL Cd	8.26±0.75a	nd	1.15±0.32a	0.501±0.06b	2.76±0.46a	86.43±1.38b	89.69±1.98a	265.30±6.05b
	CL PEG	10.48±1.74ab	nd	2.78±0.12c	1.07±0.20c	2.71±0.21a	82.43±3.55ab	86.38±1.98a	253.76±4.47a
	UV C	10.51±0.81b	nd	2.18±0.48bc	0.20±0.04a	2.31±1.14a	83.79±1.94ab	86.29±1.32a	256.17±5.88ab
	UV+Cd	11.38±1.34b	nd	1.85±0.55ab	0.52±0.08b	2.72±0.97a	82.83±1.89a	86.07±3.69a	254.45±3.56a
	UV+PEG	11.50±1.90b	nd	1.98±0.56abc	1.17±0.57bc	3.16±1.01a	81.74±1.26a	86.07±2.94a	252.72±5.63a
PE	CL C	33.48±3.61b	nd	8.56±2.02c	4.07±1.64c	21.66±1.52a	32.23±2.50a	57.96±4.11a	144.08±9.06a
	CL Cd	34.70±2.23b	nd	7.55±1.90c	3.34±0.65bc	22.39±1.72ab	32.03±2.74a	57.75±5.66a	144.21±6.26a
	CL PEG	30.63±3.65ab	nd	3.42±1.60ab	2.84±0.46b	20.65±2.80a	42.47±3.51b	65.95±4.27a	171.54±5.77bc
	UV C	25.90±2.30a	nd	2.54±0.99ab	2.92±0.55b	30.21±2.36c	38.47±2.86ab	71.57±3.33b	178.73±7.85c
	UV+Cd	28.39±3.02ab	nd	1.45±0.87a	1.79±0.32a	26.21±1.99bc	42.16±3.00b	70.16±5.23b	180.68±9.01c
	UV+PEG	31.24±1.76ab	nd	4.30±1.22bc	2.0±0.56ab	25.43±2.01abc	37.03±2.69ab	64.47±4.13ab	163.97±2.25b
PG	CL C	24.24±1.33cd	13.97±1.31a	12.02±1.15d	2.14±0.26c	2.81±0.16a	44.83±2.58bc	49.77±1.75b	142.22±2.63b
	CL Cd	17.25±1.65ab	24.13±2.01b	5.33±0.026b	3.06±0.96cd	7.08±0.70d	43.16±2.33b	53.29±1.66bc	146.68±5.21bc
	CL PEG	22.44±0.98c	13.59±1.20a	8.74±0.33c	3.50±0.69d	4.74±0.37c	46.99±2.10bc	55.23±1.71c	153.94±5.70c
	UV C	21.07±2.33bc	24.40±2.49b	8.01±0.46c	1.37±0.32b	4.15±0.59b	38.92±0.80a	44.44±0.74a	126.43±7.20a
	UV+Cd	17.06±0.87a	25.66±2.11b	1.91±0.60a	0.48±0.32a	6.12±0.15d	48.78±1.52c	55.37±2.59c	159.04±4.71c
	UV+PEG	25.97±1.26d	21.62±1.82b	5.84±0.75b	3.44±1.20cd	6.27±0.55d	36.86±2.62a	46.57±1.95a	126.56±3.99a

MGDG: monogalactosyldiacylglycerol; DGDG: digalactosyldiacylglycerol, PE: phosphatidylethanolamine; PG: phosphatidylglycerol; *t*16:1: *trans*-Δ_3-hexadecanoic acid; 16:0: palmitic acid; 18:0: stearic acid; 18:1: oleic acid; 18:2: linoleic acid; 18:3: linolenic acid, nd: not detected.

In the phospholipid PE fraction Cd did not influence the amount of fatty acids detected, while the other stress factors, drought and UV-B, and the combined treatments UV+Cd and UV+PEG decreased the levels of 16:0, stearic acid (18:0) and oleic acid (18:1), but increased the proportion of 18:3 leading to an increase in DBI (Table 1).

Changes in the fatty acid composition of the other phospholipid, PG, showed a different patterns in each of the six treatments (Table 1). Although Cd stress alone decreased the levels of 16:0 and 18:0 and increased that of 18:2, it did not cause pronounced changes in the percentage of unsaturation or DBI. PEG-induced drought stress reduced the amount of 18:0, but increased that of 18:1 and 18:2, with a higher level of unsaturation, while after UV-B treatment the levels of 18:0, 18:1 and 18:3 decreased and that of 18:2 increased, resulting in lower DBI. The combined UV+Cd treatment decreased the amount of saturated fatty acids (16:0 and 18:0) and increased that of unsaturated fatty acids (18:2 and 18:3), with a consequent rise in the percentage of unsaturation and the DBI. Interestingly, the UV+PEG treatment had the same outcome as found for UV-B stress alone, as the unsaturation and DBI were lower than in the control plants, due to the reduction in 18:0 and increased amounts of 18:2 (Table 1). The amount of ~~*trans-*~~*Δ*trans-Δ^{3t}-hexadecanoic acid (*t*16:1), which was only detectable in the leaf PG, increased significantly in all the treatments except for drought stress alone (Table 1).

3.1.2 Changes in root membrane fractions

In contrast to the leaves, significant changes were observed for all the lipid classes examined (Table 2).

Table 2 Effect of cadmium or drought stress with or without UV-B treatment on the fatty acid composition (mol%), double bond index (DBI) and percentage of unsaturation (%unsat) of various lipid classes obtained from wheat roots. Data represent mean values±SD (*n*=3); different letters denote significant differences from control samples taken on the same day at $p<0.05$.

Fraction	Treatment	C16:0	C18:0	C18:1	C18:2	C18:3	%Unsat	DBI
MGDG	CL C	52.18±3.13b	15.22±1.46a	9.26±3.01a	16.24±4.20b	7.10±1.37a	32.60±1.99b	63.04±4.63b
	CL Cd	43.38±1.66a	17.82±0.98a	16.35±1.88b	12.96±3.23ab	9.49±0.78b	38.80±1.75c	70.73±2.39bc
	CL PEG	45.36±2.31a	17.83±0.66a	7.61±0.39a	16.51±1.55b	12.69±2.72b	36.81±4.55bc	78.69±6.82c
	UV C	52.42±2.10b	26.43±2.34b	6.26±1.11a	8.65±2.25ab	6.25±1.13a	21.15±1.64a	42.29±3.21a
	UV+Cd	50.03±1.94b	17.20±2.64a	6.46±2.19a	15.58±3.69ab	10.74±0.99b	32.78±2.67b	69.84±2.37bc
	UV+PEG	47.88±2.73ab	18.90±1.85a	6.24±1.37a	17.61±2.09b	9.38±0.87b	33.22±3.64bc	69.58±5.67bc
DGDG	CL C	39.66±3.45cd	13.47±0.52b	8.48±0.33bc	28.31±1.89b	10.08±0.41a	46.87±3.46b	95.34±5.41b
	CL Cd	40.53±2.82d	12.48±0.12a	7.51±0.68ab	25.49±3.56b	13.99±0.89b	46.99±0.92b	100.47±2.85b
	CL PEG	27.27±0.0b	14.63±0.87b	10.21±1.25c	31.73±2.19b	16.16±0.31c	58.10±0.88d	122.14±7.42c
	UV C	34.760±0.68cd	12.82±0.70ab	7.19±0.55ab	13.50±0.96a	31.72±1.46e	52.42±0.59c	129.36±6.34c
	UV+Cd	44.88±2.72d	22.39±2.11c	9.67±0.92c	14.32±1.28a	8.74±0.94a	32.73±0.64a	64.54±1.62a
	UV+PEG	20.70±1.40a	12.46±0.82a	8.01±0.32ab	39.07±3.08c	19.76±1.54d	66.84±1.55e	145.42±2.61d
PE	CL C	31.87±1.52b	6.12±0.95a	3.23±0.42a	35.86±3.89b	22.91±1.26ab	62.01±0.98b	143.70±1.26b
	CL Cd	30.17±1.37b	5.02±0.64a	5.24±0.15b	34.62±4.02b	24.94±1.96bc	64.81±2.10bc	149.31±6.91bc
	CL PEG	23.02±0.32a	5.58±0.57a	5.33±0.82bc	40.55±4.80b	25.52±1.55bc	71.40±0.80d	162.99±14.52d
	UV C	34.75±0.98c	13.29±1.01c	2.60±0.33a	27.51±1.96a	21.85±0.48a	51.96±2.14a	123.18±0.97a
	UV+Cd	30.98±1.26b	4.75±0.78a	3.02±0.66a	34.92±2.34b	26.33±1.16c	64.27±2.40bc	151.86±3.85c
	UV+PEG	26.58±2.25a	8.27±0.61b	6.13±0.51c	35.32±1.36b	23.69±0.96b	65.15±1.67c	147.85±2.54bc
PG	CL C	42.00±2.23a	9.22±0.95b	9.83±0.95c	19.67±3.81b	19.28±0.69b	48.79±1.38b	107.02±7.13c

	CL Cd	43.32±6.55ab	24.39±1.78c	8.74±0.56c	9.26±2.59a	14.29±0.43a	32.29±4.18a	70.13±10.84a
	CL PEG	41.25±7.51ab	8.24±0.26b	5.67±0.53b	19.29±0.42b	25.56±0.47d	50.51±3.60b	120.92±5.96d
	UV C	46.81±0.81b	6.29±0.20a	4.91±0.45b	19.83±0.02b	20.83±0.70b	46.19±0.85b	107.05±0.87c
	UV+Cd	40.84±3.60a	21.55±1.10c	5.68±0.97b	10.86±1.84a	21.07±0.56c	37.61±2.02a	90.62±1.98b
	UV+PEG	41.34±2.39a	8.49±0.94b	4.02±0.27a	19.55±0.54b	26.59±0.47d	50.16±2.82b	122.89±2.67d

MGDG: monogalactosyldiacylglycerol; DGDG: digalactosyldiacylglycerol, PE: phosphatidylethanolamine and PG: phosphatidylglycerol; *t*16:1: *trans*-Δ_3-hexadecanoic acid; 16:0: palmitic acid; 18:0: stearic acid; 18:1: oleic acid; 18:2: linoleic acid; 18:3: linolenic acid.

In the MGDG fraction PEG treatment decreased the level of 16:0 and increased the proportion of 18:3, leading to a rise in DBI. In contrast, UV-B radiation increased the amount of 18:0, but decreased the level of 18:2, resulting in lower unsaturation and DBI.

Cd, alone or in combination with UV-B did not markedly influence the fatty acid pattern (Table 2).

Drought stress caused similar changes in the DGDG as in the MGDG fraction due to the lower 16:0 and the higher 18:3 content (Table 2). The unsaturation was also higher in the root DGDG fraction of UV-B treated plants compared to the control, but mainly because of the decreased proportion of 18:2 and the three-fold increase in the level of 18:3. Although Cd treatment alone did not affect the fatty acid composition, a drastic decrease in DBI was observed when it was applied in combination with UV-B radiation due to an increase in 18:0, together with a decrease in 18:2 and 18:3. PEG and UV-B treatment were found to have an additive effect, leading to a high level of unsaturation (Table 2).

In the PE fraction, ~~similarly~~similar to MGDG, the percentage of unsaturation and the DBI were increased by PEG, decreased by UV-B, while the combined UV+Cd treatment also caused slight increase (Table 2).

Although UV-B treatment alone did not influence the unsaturation or DBI of the PG fraction, the changes in these parameters after the combined treatments (UV+Cd and UV+PEG) were similar to those observed in the case of single stressors, being decreased by Cd, and increased by PEG treatment. Changes in DBI were associated with an increase in 18:0 and a decrease in 18:2 and 18:3 after Cd treatment, but mainly with an increase in 18:3 in the case of PEG-induced drought stress (Table 2).

3.2 Changes in flavonoid contents

The flavonoid levels were measured both in the free and methanol-soluble bound forms (Table 3). The Cd treatment alone did not influence any of the flavonoid contents in the leaves, while drought stress, UV-B radiation, and the combined treatments (UV+Cd and UV+PEG) increased the total rutin, myricetin, and quercetin.

Table 3 Effect of cadmium or drought stress with or without UV-B treatment on the flavonoid content (μg $g^{~~-1~~-1}$ FW) in wheat leaves. Data represent mean values±SD (*n*=5); different letters denote significant differences from control samples taken on the same day at $p<0.05$.

Treatments	Free rutin	Bound rutin	Free myricetin	Bound myricetin	Free quercetin	Bound quercetin
CL C	0.49±0.01a	15.35±8.62a	0.2±0.04a	1.4±0.16a	7.78±1.13a	46.84±11.22a
CL Cd	0.53±0.22ab	22.79±3.24a	0.21±0.1a	1.73±1.26ab	8.11±2.55a	55.11±18.85a
CL PEG	0.73±0.09b	28.68±3.94a	0.49±0.36ab	8.57±0.26d	9.83±3.2a	136.9±2.17bc
UV C	1.8±0.24c	41.73±10.52b	0.37±0.15ab	3.8±1.13b	21.96±5.15b	140.53±18.71c
UV+Cd	1.55±0.12c	47.35±11.52b	0.43±0.39ab	7.43±1.56cd	16.64±3.91b	197.36±33.98d
UV+PEG	1.47±0.35c	40.02±5.68b	0.53±0.08b	6.15±0.32c	21.67±7.82b	125.55±8.12b

The additive effect of the combined UV+Cd stress could be observed in the case of bound myricetin and quercetin (Table 3). In contrast, Cd stress caused significant changes in the roots, where PEG treatment did not influence the flavonoid levels (Table 4). Interestingly, UV-B radiation alone also increased the content of flavonoids in the root, especially that of total quercetin. The additive effect of the combined UV+Cd stress on the contents of free and bound myricetin and quercetin was observed in the roots. The elevated levels of flavonoids were also detected after UV+PEG treatment compared to the individual PEG and UV-B treatments (Table 4).

Table 4 Effect of cadmium or drought stress with or without UV-B treatment on the flavonoid content (μg g^{-1} FW) in wheat roots. Data represent mean values±SD (n=5); different letters denote significant differences from control samples taken on the same day at p<0.05.

Treatments	Free rutin	Bound rutin	Free myricetin	Bound myricetin	Free quercetin	Bound quercetin
CL C	nd	1.58±0.54a	0.41±0.03a	1.13±0.6a	6.08±1.4a	25.81±4.2a
CL Cd	nd	6.81±1.58b	1.47±0.42c	1.95±0.67a	15.22±2.11b	48.72±3.53b
CL PEG	nd	1.92±0.19a	0.42±0.22a	1.59±0.91a	5.88±1.19a	18.21±4.15a
UV C	nd	3.7±2.13ab	0.85±0.24bc	1.95±0.51a	15.56±3.23b	54.44±10.43b
UV+Cd	nd	6.38±1.24b	2.32±0.17d	4.6±0.45b	42.26±5.11c	76.62±2.55c
UV+PEG	nd	4.98±0.38b	0.75±0.07b	4.92±0.48b	43.29±1.55c	97.53±12.7d

nd: not detected.

4 Discussion

It was demonstrated that UV-B radiation may have either a positive or negative impact on the stress tolerance of wheat, depending on the type of secondary abiotic stress factor: Cd or drought. Although supplemental UV-B light caused visible shoot growth retardation, it prevented the wilting and leaf rolling induced by PEG treatment. In contrast, the combined UV-B and Cd treatment resulted in pronounced oxidative stress, manifested by retarded growth and a yellowish appearance (Kovács et al., 2014).

This is the first study so far to investigate how the combination of UV-B stress with drought or cadmium treatment, as a secondary stress, alters the fatty acid composition of certain membrane lipid fractions in wheat plants. The impact of UV-B radiation on physiological changes has mostly been studied in the leaves, and has not yet been elucidated in the roots, so the data presented here are novel in this respect.

Earlier work showed that the leaves and roots of maize gave different physiological responses to treatment with 50 μM Cd, and that Cd treatment caused a decline in the percentage of unsaturation in the membrane lipid fractions, which can be explained by the damaging effect of Cd (Pál et al., 2007). Drought stress also induced a decrease in the proportion of unsaturated fatty acids in the total lipids content (El Kaoua et al., 2006; Martins Júnior et al., 2008). The percentage of unsaturation in rolled leaves of *Ctenanthe setosa* subjected to water-deficit stress was higher than in control leaves (Ayaz et al., 2001). It was also found that leaf dehydration tolerance was associated with the ability to maintain a relatively higher proportion and level of unsaturated fatty acids in Kentucky bluegrass (Xu et al., 2011). UV-B radiation reduced the saturated fatty acid content and increased that of unsaturated fatty acids in *Spirulina platensis* (Gupta et al., 2008); furthermore, the UV-B sensitive cultivar had a higher level of saturated fatty acids than did the insensitive one in cucumber (Kramer et al,. 1991). However, high UV-B radiation decreased the levels of unsaturated fatty acids (Moorthy and Kathrisan, 1998; Wulff et al., 1999).

Under the present stress conditions, PEG, Cd and UV-B had different effects on the fatty acid composition in the different membrane fractions; furthermore, in some cases the leaves and roots responded differently.

In the MGDG and DGDG galactolipid fractions the initial unsaturation was so high in the leaves that only the combined UV+Cd and UV+PEG treatments caused a significant decrease, and only in MGDG. MGDG is considered to be indispensable for PSII activity, because it regulates the supramolecular structure of PSII complexes (Murata et al., 1990), and a high level of unsaturation of the thylakoid lipids is required to maintain the degree of fluidity necessary for diffusion and the suitable geometry of lipid molecules. Thus, changes in this lipid class clearly show the effect of oxidative stress. In the roots drought stress increased the unsaturation in both fractions, while UV-B radiation decreased it in MGDG, but increased it in DGDG. The additive effect of the combined UV+PEG treatment in DGDG may indicate better adaptive capacity. In contrast, when Cd and UV-B stress were applied together a drastic decrease in unsaturation was observed in the DGDG fraction due to the severe oxidative stress.

Under the present conditions all the treatments except for Cd stress increased the percentage of unsaturation and/or DBI in the leaf PE fraction, while different changes were detected in the root PE, where unsaturation was increased by PEG treatment and decreased by UV-B radiation, while the UV+PEG treatment had no influence, suggesting that the effects of the two stressors offset each other. Although Cd alone caused no significant increase, the UV+Cd treatment raised the level of unsaturation.

PG is the only phospholipid present in thylakoid membranes and is a negatively charged molecule at neutral pH, thus mediating indispensable interactions with the components of photosynthetic complexes in these membranes. PG plays an important role in the oligomerization of the light harvesting complex and the dimerization of PSII (McCourt et al., 1985; Krupa et al., 1987; Kruse et al., 2000). It is also required for both the electron-acceptor side and the donor side of PSII (Sakurai et al., 2007). The DBI was increased by drought stress, but decreased by UV-B radiation in leaf PE. It is reported that fatty acid unsaturation in thylakoid membrane lipids is required for the repair of the damaged D1 (Takami et al.,

2010). Thus, the changes detected in PG in the present work may be responsible for the shoot growth inhibition induced by UV-B (Kovács et al., 2014). In addition, when drought stress was applied in combination with UV-B, the decreasing effect of UV-B on the unsaturation of leaf PG counteracted the impact of PEG treatment, leading to similar values to those found in the case of UV-B stress alone, with a lower value than in the control. The amount of *t*16:1 in the leaf PG increased after Cd stress and in plants grown under UV-B radiation, but did not change during drought stress. In the case of combined stress the effect of UV-B radiation was dominant. In the root PG fraction unsaturation was decreased by Cd and increased by PEG, while it was not influenced by UV-B. In the case of the combined stresses, the impacts of Cd and PEG prevailed.

Although unsaturation increased in PE, the decrease detected in PG and MGDG fractions in the leaves of UV+PEG-treated plants, compared to the control, may explain the fact that the shoot height was lower than in plants treated with PEG alone and that no leaf rolling or wilting was observed. Previous finding also revealed that UV-B pre-treatment alleviated the wilting and leaf rolling induced by PEG treatment under the same conditions (Kovács et al., 2014) could be related to the alteration detected in the fatty acid composition of leaf PG. Changes in the PE fraction may have an adaptive role in counterbalancing the membrane-rigidifying effect of increased saturation (Thompson et al., 1998). The increased proportion of unsaturated fatty acids, especially 18:3, in the MGDG, DGDG and PG fractions in the roots of UV+PEG-treated plants resulted in a substantial increase in DBI in the PG and DGDG fractions, with an even more pronounced increase in the unsaturation of DGDG compared to that recorded when PEG or UV-B were applied alone. These changes may have led to increased water permeability as an adaptive mechanism during water deficit, which is in accordance with the observation that the relative water content was positively correlated with DBI in Kentucky bluegrass under drought stress (Xu et al., 2011).

By comparison, in the UV+Cd treatment the reduction in unsaturation in the leaf MGDG fraction, together with the probable increase in Cd translocation due to changes in the PE and PG fractions in the leaves, may be the cause of the retarded growth and yellowish appearance of the plants (Kovács et al., 2014). The combined UV+Cd treatment drastically decreased the percentage of unsaturation and the DBI in both the PG and DGDG fractions in the roots, which is more likely to be the manifestation of the severe injury induced by combined stress than an adaptive response aimed at reducing membrane permeability and hence metal ion uptake, as reported in the case of Al (Chaffai et al., 2005).

Flavonoids have been reported to possess strong antioxidant activity, inhibiting non-enzymatic lipid peroxidation and playing an important role in protecting plants from UV-B due to their high UV-B absorption (Hernández et al., 2009). One of the first identified and best characterized photomorphogenic responses to UV-B is the biosynthesis of flavonoids. The synthesis of flavonoids is stimulated by very low doses of UV-B within a few hours, although this response is not specific to UV-B exposure, and there are considerable differences both between species and between the levels of flavonoid compounds regulated by UV-B (Jenkins, 2008). Mutant plant genotypes with impaired flavonoid biosynthesis are more susceptible to damage by UV-B (Kaiserli and Jenkins, 2007). Interestingly, in *Arabidopsis* it was found that the roots, rather than other organs, perceive the UV-B signal (Tong et al., 2008). In the present study flavonoid content was influenced to the greatest extent by UV-B and PEG alone, while in the roots, besides the expected effect of Cd, UV-B stress was also detected. The additive effect of UV+Cd was observed in the bound myricetin and bound quercetin contents of the leaves, and in the total myricetin and quercetin contents of the roots. Similarly to the present results, it was reported that drought and UV-B stress caused an increase in flavonoid levels, with the largest quantities in seedlings exposed to combined stress, although the magnitude of MDA accumulation varied as a function of treatments and cultivars, and also depended on the age of the plants (Feng et al., 2007). In another study, Ni treatment caused a decline in flavonoid content, while UV-B treatment increased it significantly in the leaves of pea plants, but no additive effect was observed in the combined treatment. The MDA content, however only increased significantly in UV-B stressed plants, and was not influenced by Ni, either alone or in combination with UV-B (Mishra and Agrawal, 2006; Singh et al., 2009). A significant positive correlation was reported between the flavonoid products and MDA levels in the desert plant *Caryopteris mongolica* (Meiling et al., 2012). The changes observed in the flavonoid contents in the present work are in agreement with previous results on lipid peroxidation under the same experimental conditions. Namely, the PEG, UV and UV+PEG treatments increased the MDA content, the combined UV+PEG treatment had an additive effect on the root MDA content, and Cd-induced oxidative stress was further aggravated by UV-B stress, as manifested in both the leaves and roots of wheat plants (Kovács et al., 2014).

In the present study greater increase in flavonoid content was found in the leaves than in the roots, which may have been due to the localization of the biosynthesis of these phenolic compounds or their precursors in the roots and their transport to the leaves. These results are in accordance with a previous study on the same wheat varieties under the same conditions, where the activity of phenylalanine ammonia lyase (PAL), an enzyme crucial for the plant metabolism and for the synthesis of lignins, flavonoids, anthocyanins and the plant hormone salicylic acid, increased in the roots, especially after PEG, UV-B and UV+PEG treatment, while it showed only slight changes in the leaves. Although changes in PAL activity in the roots were accompanied by changes in the root levels of salicylic acid and its precursor, an increase in the levels of these compounds in the leaves was not accompanied by a pronounced rise in leaf PAL activity (Kovács et al., 2014).

5 Conclusion

Changes in the flavonoid contents revealed that oxidative stress was induced by drought mainly in the leaves, and by Cd especially in the roots. Interestingly, UV-B radiation induced the synthesis of flavonoids in both the leaves and roots. The additive effects of combined stresses were also detected in the roots. The changes induced by UV-B in the unsaturation of leaf PG fractions, together with flavonoid accumulation in the leaves, may play a role in preventing drought-induced wilting. Despite the increased flavonoid content in roots exposed to Cd, pronounced injury was observed in the roots, manifested as a substantial decrease in the unsaturation of the leaf MGDG and root PG and DGDG fractions. The present results suggest a relationship between the capacity of plants to remodel their fatty acid composition and their resistance to various stress factors.

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Highlights

- UV-B influences flavonoid contents and fatty acid composition both in the leaves and roots.
- UV-B has different impact on drought and Cd stress induced changes.
- UV-B radiation might have either a positive or negative impact under the same conditions.

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