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ORIGINAL PAPER

The effect of zearalenone on PSII photochemical activity and growth in wheat and soybean under salt (NaCl) stress

Janusz Kościelniak · Agnieszka Ostrowska · Jolanta Biesaga-Kościelniak · Władysław Filek · Anna Janeczko · Hazem Mohamed Kalaji · Katarzyna Stalmach

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Abstract The effects of mycotoxin zearalenone (ZEN) on the photochemical activity of photosystem II (PSII) in wheat and soybean leaf discs incubated in ZEN solutions as well as the after-effects of pre-sowing soaking of seeds in solutions containing ZEN on the photochemical activity of PSII and on the seedlings growth under salt stress (NaCl solutions were investigated). The incubation of wheat leaf discs in ZEN solutions strongly inhibited the energy flux per cross section (CS) for absorption (ABS/CS), trapping (TRo/CS) and electron transport (ETo/CS), while the effects of ZEN action on soybean discs were opposite and the values of those parameters significantly increased with the increase in ZEN concentration. Incubation of seeds in a ZEN solution resulted in an increase in photochemical efficiency of PSII in soybean seedlings, but did not induce any response of PSII in those of wheat at medium illuminations. Only at the stronger illumination for both species did ZEN induce an increase in efficiency of excitation energy capture by open PSII reaction centers, photochemical quenching of chlorophyll *a* fluorescence and quantum yield of PSII electron transport. Pre-sowing soaking of seeds in a ZEN solution decreased the photoinhibitory injuries of PSII in wheat and soybean due to safe scattering of the excess excitation energy through an increase in energy-dependent quenching (qE) and state transition quenching (qT). ZEN when added to NaCl solutions during the period of germination contributed to reduction in the growth inhibition of wheat seedlings. The incubation of wheat leaf discs in ZEN solutions strongly inhibited CS, ABS/CS, TRo/CS and ETo/CS. Possible effects of ZEN on some physiological processes in plants have been discussed especially in the context with photochemical activity of PSII and a salt stress.

Keywords Chlorophyll *a* fluorescence · Germination · Photosystem II · Photoinhibition · Salinity stress (NaCl) · Soybean · Wheat · Zearalenone

Abbreviations

ABS	Absorption energy flux
CS	Excited cross-section of leaf
DIo	Dissipation energy flux at the level of the antenna chlorophylls
ETo	Flux of electrons from Q_A^- into the electron transport chain
Fm	Maximum fluorescence in dark-adapted state
F′m	Fluorescence when all PSII reaction centers are 'closed' in light-exposed leaves
Fo	Initial fluorescence during the dark-adapted state
F′o	Fluorescence in leaves previously exposed to light; darkened just before measurement
F′v/F′m	Efficiency of excitation energy capture by open PSII reaction centers
qN	Non-photochemical quenching of chlorophyll *a* fluorescence

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J. Kościelniak (✉) · K. Stalmach
Department of Plant Physiology, University of Agriculture in Krakow, Podluzna 3, 30-239 Kraków, Poland
e-mail: j.koscielniak@ur.krakow.pl

A. Ostrowska · J. Biesaga-Kościelniak · W. Filek · A. Janeczko
Institute of Plant Physiology, Polish Academy of Sciences, Podluzna 3, 30-239 Kraków, Poland

H. M. Kalaji
Department of Plant Physiology, Warsaw University of Life Sciences SGGW, Nowoursynowska 159, 02-776 Warsaw, Poland

OEC	Fraction of O_2 evolving centers PSII in comparison to the control sample
PFD	Photon flux density
PSII	Photosystem II
Q_A	The first stable electron acceptor in PSII
qE	Energy-dependent quenching
qI	Photoinhibitory quenching
qP	Photochemical quenching of chlorophyll *a* fluorescence
qT	State transition quenching
RC	Number of active reaction centers in the state of fully reduced PSII reaction center of PSII
TRo	Excitation energy flux trapped by RC and utilized for the reduction in Q_A to Q_A^-
Zearalenone	2,4-Dihydroxy-6-(10-hydroxy-6-oxo-trans-1-undecenyl)-benzonic acid lactone].
$\Delta Fm =$	$F'm - Fs$
Φ_{PSII}	Quantum yield of PS II electron transport
Ψo	Osmotic potential

Introduction

Zearalenone [ZEN, 2,4-dihydroxy-6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-benzonic acid lactone] is well known mycotoxin because of its harmful effects on animals fed with fodders obtained from plants that were infected with fungi belonging to genus *Fusarium*. It was isolated for the first time from a fungal culture *Gibberella zeae* by Stob et al. (1962). Christensen et al. (1965) also isolated this substance and named it F-2. ZEN has a strong estrogenic activity due to its competition with β-estradiol in the binding to cytosolic estrogen receptors (Olsen 1989). ZEN estrogenicity causes several physiological alternations in the productive tract in animals (Price et al. 1993; Etienne and Dourmad 1994). Studies revealed that ZEN induces reactions at many levels of animal cell organization (Hassen et al. 2007). It was revealed that ZEN can be also toxic for plants causing chromosome damages and disorders in the synthesis of photosynthetic pigments (Kumar and Sinha 1995) as well as in photosynthesis and growth processes (Kościelniak et al. 2009). On the other hand, it was found that ZEN was an endogenous regulator controlling plant development (Meng et al. 1992), but as yet the mechanism of its action has not been explained. A peak of endogenous ZEN levels occurred during the vernalization of many winter plants, and exogenous ZEN applied at low concentrations could partly replace the low temperature requirement for ear development in winter wheat (Fu and Meng 1994; Fu et al. 2000; Biesaga-Kościelniak 2001; Biesaga-Kościelniak and Filek 2010). ZEN can increase the number of ears, pods and seeds in wheat and soybean (Biesaga-Kościelniak et al. 2006a, b). It can also affect plant growth and development in many ways, as well as in the status and functioning of the photosynthetic apparatus (Kościelniak et al. 2009). The response of the photosynthesis process to ZEN and the participation of ZEN in the response of plants to environmental stress are less known. The aim of our study was to investigate the effect of ZEN on: (1) the photochemical activity of photosystem II (PSII) of wheat and soybean plants, (2) the photoinhibition of the photosynthetic apparatus, and (3) the growth of seedlings under salt (NaCl) stress.

Materials and methods

Plant material and seedlings growth

The experiment was carried out on Polish common wheat (*Tritium aestivum* L.) *cv*. Torka and on soybean (*Glycine max* (L.) Merrill) *cv*. Aldana. Seeds were soaked for 24 h in the solution of ZEN (*Sigma*, Poznań, Poland) at a concentrations of 0.25, 2.0 and 4.0 mg dm^{-3}, as similar to that were applied by Biesaga-Kościelniak (Biesaga-Kościelniak 1998, 2001; Biesaga-Kościelniak et al. 2003, 2006a, b), and separately in water (control). Plants were grown in growth rooms in 5 dm^3 pots filled with a mixture of clay, peat and sand (3:2:1, *v*/*v*/*v*) at a 16-h photoperiod, PFD of 500 µmol(quantum) m^{-2} s^{-1} (provided by high pressure sodium lamps, 400 W; Philips SON-T AGRO, Brussels, Belgium) and at 50–60% of air humidity. During germination (5 days), a temperature of 25°C and that of 25/17°C (day/night) after emergence was kept. Seedlings were watered and fertilized with half diluted Hoagland nutrient solution as required (Hoagland and Arnon 1938).

Seed germination under salinity (NaCl) stress condition

Wheat and soybean seeds were germinated on Petri dishes with different amounts of NaCl and ZEN. The osmotic potentials of NaCl solutions were: 0.00, −0.25, −0.50 and −1.00 MPa (Chirife and Resnik 1984). ZEN solutions (0.0, 0.25, 2.0, 4.0 mg dm^{-3}) were prepared using those with different NaCl concentrations. To avoid an increase in the solution concentration, Petri dishes with germinating seeds were kept at about 100% RH and the solutions in the Petri dishes were changed every 2–3 days. Germination was carried our for 7 days for wheat and 10 days for soybean at 26°C under weak illumination (80 µmol(quantum) m^{-2} s^{-1}). The dry weight (drying at 75°C), and the length of the coleoptile and roots were estimated.

The direct effect of ZEN on the photochemical activity of PSII

Leaf discs (Ø 5 mm) were cut out from the first (soybean) and third (wheat) leaf 3 weeks after the germination of seeds and incubated in water without ZEN for 72 h. The discs were then placed in aerated vessels filled with a mineral nutrient medium (Appenroth et al. 1996) supplemented with ZEN at concentrations 0, 0.5, 1.0 and 2.0 mg dm^{-3}, chosen on the basis of previous experiments (Biesaga-Kościelniak 2001). The discs were soaked in ZEN solutions for 72 h, at 20°C, and at PFD 150 µmol (quantum) m^{-2} s^{-1}. Before measuring the fluorescence, discs were dried and adapted to darkness for 30 min (clips with a 4-mm diameter hole). The measurements of Chl *a* fluorescence were carried out with a Plant Efficiency Analyzer (*PEA*; *Hansatech Ltd.* Kings Lynn, UK) with an excitation irradiance of 3 mmol m^{-2} s^{-1} (peak 650 nm). Before measurements, the LED-light source of the fluorometer was calibrated using an SQS light meter (*Hansatech Ltd*. Kings Lynn, UK). Fluorescence intensity was measured with a PIN-photodiode after being passed through a long-pass filter. Changes in fluorescence were registered during irradiation of 10 µs to 1 s. During the initial 2 ms, data were collected every 10 µs with 12-bit resolution. After this period, the frequency of measurements was reduced automatically. On the basis of these measurements, parameters: ABS/CS, TRo/CS, ETo/CS, DIo/CS and OEC were calculated based on the theory of energy flow in PSII and using the JIP test (Srivastava and Strasser 1977; Strasser and Strasser 1995; Lazár 1999; Lazár and Pospíšil 1999; Strasser et al. 2000; Appenroth et al. 2001).

The after-effect of ZEN on the photochemical activity of PSII in seedlings

Measurements of *Chl a* fluorescence were carried out at two developmental stages: after the development of the second and fourth leaves. Seedlings developed from seeds soaked in ZEN (4 mg dm^{-3}) solution and water (control) was tested. The measurements were carried in the middle part of well-developed leaves situated at the highest part of the plant. The leaves were illuminated for a period longer than 5 min until stabilization of fluorescence. Photochemical measurements were made with an FMS2 fluorometer (*Hansatech Ltd.* Kings Lynn, UK). The source of the modulation beam (duration pulses 1.8 µs) was the amber LED [peak wavelength 594 nm, 0.05 µmol (quantum) m^{-2} s^{-1}]. Actinic [white light; 400–750 nm; 500 µmol (quantum) m^{-2} s^{-1}] and pulse irradiations were provided by a halogen lamp (*Osram 64255*; 20 W). The saturating pulse intensity amounted about 5,800 µmol (quanta) m^{-2} s^{-1} and lasted 0.9 s. Fo′ was measured after turning off the actinic light, by immediately irradiating the leaf for 3 s with a far red emitting diode (wavelength 735 nm) with about 15 W m^{-2}. The following parameters were estimated: F′v/F′m, qP and Φ_{PSII} (Genty et al. 1989; Schreiber et al. 1994; Lichtenthaler et al. 2005).

Measurements of PSII photoinhibition

The response of PSII to strong illumination was carried out after the development of the second leaf. The fluorescence quenching and current photochemical efficiency of PSII were measured with a FMS2. First, seedlings were adapted (5 min) to darkness to measure the minimum fluorescence (Fo), and then they were illuminated with strong irradiance (ca. 5,800 µmol (quantum) m^{-2} s^{-1}) to determine the maximum level of fluorescence (Fm). Then, leaves were irradiated for 30 min with white light (halogen lamp) at 1,200 and 300 (control) µmol (quantum) m^{-2} s^{-1}. Towards the end of this period, a pulse of saturating light was applied to determine F′m. 2 min later the actinic light was switched off and the recovery of Fm followed for 60 min by applying pulses of saturating light every 1–2 min. The experimental protocol for the estimation of these components of qN was essentially the same as that described by Walters and Horton (1991). The non-photochemical quench qN is based on three major constituents: the energy quenching qE, qT and qI caused by a photoinhibition of PSII units (Fork and Satoh 1986; Horton and Hauge 1988; van Wijk and van Hasselt 1993; Ting and Owens 1994; Ruban and Horton 1995; Owens 1996; Haldrup et al. 2001). qE is thought to occur in PSII antennae and there is evidence that it is regulated by the pH gradient across the thylakoid membrane and by the interconversion of pigments in the xanthophylls cycle (Owens 1996; Ting and Owens 1994; Ruban and Horton 1995). The photoinhibitory quenching qI is caused by the photoinhibition of PSII units (Greer et al. 1986; Baker 1994; Owens 1996). The state transition quenching qT is related to "state 1" → "state 2" transitions of the photosynthetic apparatus including phosphorylation of the mobile light-harvesting protein LHC2.

Relaxation of qN was assumed to consist of kinetically distinct phases: rapid, medium and slow (Walters and Horton 1991). Slow relaxing qN was estimated by plotting ΔFm/F′m (nomenclature Johnson et al. 1993) on a logarithmic scale and extrapolating the slow phase of recovery back to the '*y*' axis. The '*y*' intercept represent the amount of qN due to the slowly relaxing component. This value was then subtracted from the remaining points and the process repeated to obtain the values of qN due to the medium and fast phases. The fast, medium and slow components are defined as: (ΔFm/F′m)f, an estimate of qE

quenching (ΔFm/F′m)m, an estimate of qT and qE quenching, and (ΔFm/F′m)s, estimates of longer term photoinhibitory quenching. The measurements were carried on seedlings which were grown up from seeds incubated in water and in a ZEN solution (4 mg dm^{-3}).

Statistical analysis

All data were analyzed using Statistica 8.0 software (*Statsoft Inc*., Tulsa, OK, USA). Appropriate numbers of replications and tests used are indicated in tables and their descriptions. The significance of differences of mean values taken from data with normal arrangement (Shapiro–Wilk test) was tested using the Student's *t* test and multiple Duncan's test. For data that did not respond to that criterion, the Mann–Whitney *U* test was used.

Results

The direct effect of ZEN on the photochemical activity of PSII in leaf discs

A slow decrease in the photochemical activity of PSII in leaf discs for the first 48 h of immersing them in water, and a significant one after 72 h were being seen (Table 1). That is why in the next series of measurements leaf discs were incubated in ZEN solutions only for 48 h (Table 2). The treatment of wheat leaf discs with ZEN at concentrations 0.5, 1 and 2 mg dm^{-3} decreased the density of active reaction centers per cross section (RC/CS) and energy fluxes for absorption (ABS/CS), trapping (TRo/CS) and electron transport (ETo/CS). This was accompanied by the inhibition of the activity of the O_2 evolving centers (OEC) by ZEN. The energy flux in PSII was inhibited most significantly by ZEN at a concentration of 0.5 mg dm^{-3} (a decrease in the values of ABS/CS, TRo/CS and ETo/CS by about 33–51%), while ZEN at the lowest concentration (0.25 mg dm^{-3}) showed the lowest effect. The effects of ZEN action on soybean were opposite to those observed for wheat. The values RC/CS, ABS/CS, TRo/CS and ETo/CS significantly increased with the increase in ZEN concentration, reaching a maximum at 1 mg dm^{-3}. The increase in the values of these JIP parameters at the optimum ZEN concentration amounted from ca. 30 to 86% in comparison with the control. The incubation of leaves in ZEN solutions also resulted in an increase in the activity of OEC by about 29%.

Table 1 The influence of the incubation time of wheat and soybean leaf discs in water (without ZEN) on parameters obtained from the JIP test per excited cross section (CS)

Time (hours)	RC/CS	ABS/CS	TRo/CS	ETo/CS	DIo/CS
Wheat					
0[a]	822.2 a	1,970.2 a	1,480.2 a	845.2 a	490.0 a
24	769.6 b	1,850.1 ab	1,389.7 ab	763.8 b	460.4 ab
48	740.6 b	1,841.9 b	1,371.3 b	757.7 b	470.6 ab
72	574.3 c	1,215.1 c	765.4 c	375.3 c	449.7 b
Soybean					
0[a]	733.3 a	1,833.9 a	1,335.1 a	712.9 a	498.8 a
24	715.3 ab	1,787.3 a	1,302.9 ab	685.3 b	484.4 a
48	704.6 b	1,761.3 a	1,273.4 b	668.5 b	487.9 a
72	528.8 c	1,109.4 b	687.8 c	323.4 c	421.6 b

Means followed by the same letters within columns (separately for wheat and soybean) do not differ significantly according to the Duncan's multiple range test ($\alpha = 0.05$), $n = 18$–24

[a] The measurement performed just before the immersion of discs in water

The after-effect of ZEN on the photochemical activity of seedlings

The pre-sowing seed incubation in ZEN solution did not induce statistically significant after-effects in efficiency of excitation energy capture by open PSII reaction centers (F′v/F′m), photochemical quenching of chlorophyll *a* fluorescence (qP) and the quantum yield of PS II electron transport (Φ_{PSII}) in wheat seedlings (Table 3). The lack of influence of ZEN on the photochemical activity of PSII in the light was observed at both phases, i.e. the second and fourth leaf. In contrast, the incubation of soybean seeds in ZEN solutions increased the values of F′v/F′m by about 20% and that of Φ_{PSII} by more than 22% for the both developmental stages.

The effects of ZEN on different physiological processes under stress conditions

Photoinhibition of PSII

The plants used for photoinhibition studies were in a good form and that is why the maximum photochemical efficiency of PSII (Fv/Fm) was high (from 0.808 to 0.809). All components of non-photochemical quenching (qI, qT and qE) for wheat and soybean were several times higher during strong illumination (1,200 μmol(quantum) m^{-2} s^{-1}) than that during weak one (300 μmol(quantum) m^{-2} s^{-1}) (Table 4). The influence of the pre-sowing seed incubation in ZEN solutions on the non-photochemical quench and the efficiency of photochemical reactions in PSII (F′v/F′m, qP and Φ_{PSII}) was not observed during weak illumination, but only during that of strong one for both species. The incubation of wheat kernels in ZEN induced, at the high PFD, a decrease in the value of the quenching

Table 2 The influence of the incubation of wheat and soybean leaf discs in ZEN solutions on parameters obtained from the JIP test per excited cross section (CS)

ZEN (mg dm^{-3})	RC/CS	ABS/CS	TRo/CS	ETo/CS	DIo/CS	OEC
Wheat						
0.00	686.7	2,066.2	1,467.9	849.3	598.3	100.0
Changes in value expressed as percentages of the control						
0.25	+5.1	−6.9	−7.4	−12.6*	−5.4	−28.6**
0.50	−32.0**	−33.5**	−41.9**	−50.7**	−12.7*	−48.1**
1.00	−16.6**	−20.1**	−26.5**	−30.7**	−4.5	−30.5**
2.00	−18.1**	−19.3**	−24.7**	−27.3**	−6.1	−12.0*
Soybean						
0.00	698.8	2,388.0	1,840.4	1,399.5	547.6	100.0
Changes in value expressed as percentages of the control						
0.25	+12.9*	+2.7	+12.4*	+39.1**	−12.1*	+80.0**
0.50	+26.7**	+5.9	+17.1*	+26.9**	−11.3**	−0.7
1.00	+71.3**	+29.2**	+54.0**	+86.0**	−8.7	+28.8**
2.00	+57.0**	+21.4**	+44.3**	+66.8**	−13.8*	+12.4*

The discs were incubated in water (control) and in ZEN solutions for 48 h. The statistically significant differences (*$P < 0.05$ and **$P < 0.001$) in comparison with the control have been marked with an asterisks—on the basis of the Student′s t test, $n = 20$

Table 3 The after-effect of preceding sowing incubation of wheat and soybean seeds in ZEN solution (4 mg dm^{-3}) on the parameters of photochemical activity of PSII at different developmental stages of seedlings

Developmental phase	ZEN (mg dm^{-3})	F′v/F′m	qP	Φ_{PSII}
Wheat				
2 leaves	0.0	0.452	0.354	0.160
	4.0	0.463	0.335	0.155
	%%[a]	+2.4	−5.4	−3.1
4 leaves	0.0	0.412	0.432	0.178
	4.0	0.435	0.421	0.183
	%%	+5.6	−2.5	+2.9
Soybean				
2 leaves	0.0	0.408	0.305	0.124
	4.0	0.487	0.318	0.155
	%%	+19.4*	4.3	+24.5*
4 leaves	0.0	0.407	0.313	0.127
	4.0	0.493	0.317	0.156
	%%	+21.1*	1.3	+22.6*

Measurements carried out on the highest well-developed leaf at PFD 500 μmol(quantum) m^{-2} s^{-1}

[a] The change in the value expressed as percentage of the control (seedlings grown from seeds soaked in water). A statistically significant differences ($P < 0.05$) have been marked with an asterisks- on the basis of the Student′s t test, $n = 8$

component qI by 16.9% in comparison with the control (plants incubated in water). The lower value of qI can be a result of a lower level of photoinhibitory injuries to PSII. The consequence of this could be a considerable decrease in the inhibition of photochemical reactions in PSII caused by ZEN. Due to this effect, the values of F′v/F′m, qP and Φ_{PSII} were significantly higher in plants incubated in ZEN than in the control. The decrease in the photoinhibitory injuries to PSII was possibly due to safe scattering of the energy excess which was reflected as an increase in qT value by 17.2% and qE by 17.3%. A similar pattern of plant response to ZEN was also observed for soybean. At strong illumination, quenching qI was, after the incubation of seeds in ZEN, by 26.2% lower than that in the control. The protective action of ZEN could be explained by the 29.5% increase in the dissipation of energy through the component qE. Similarly as in wheat, the efficiency of PSII in soybean was, due to the weakening of photoinhibitory injuries, higher than for the control. This resulted in plants maintaining higher values of F′v/F′m, qP and Φ_{PSII} at strong illumination.

Growth of seedlings during salt stress

At its highest concentrations ZEN (2 and 4 mg dm^{-3}) stimulated the coleoptile and root elongation, but decreased mass accumulation in roots during wheat germination in water without NaCl (control) (Table 5). For soybean grown under the same conditions ZEN treatment at higher concentrations led consequently to increased rate of coleoptile elongation and of mass accumulation. When values of the osmotic potential (Ψo) were gradually decreased by the addition of NaCl, a stronger inhibition of the growth of seedlings was observed for both wheat and soybean. ZEN at some concentrations decreased the level of growth

Table 4 The influence of seed incubation in ZEN solution (4 mg dm^{-3}) on the photochemical efficiency of the reaction in PSII in wheat and soybean leaves as dependent on the intensity of plant illumination (30 min) before measurements

	1,200 μmol(quantum) m^{-2} s^{-1}			300 μmol(quantum) m^{-2} s^{-1}		
	ZEN (mg dm^{-3})		Significant of difference	ZEN (mg dm^{-3})		Significant of difference
	4.0	0.0		4.0	0.0	
Wheat						
qI	0.657	0.791	*	0.122	0.121	n.s.
qT	0.751	0.641	*	0.184	0.178	n.s.
qE	1.788	1.524	*	0.316	0.321	n.s.
Total	3.196	2.956	*	0.622	0.620	n.s.
F′v/F′m	0.530	0.455	*	0.723	0.725	n.s.
qP	0.515	0.475	**	0.812	0.815	n.s.
Φ_{PSII}	0.273	0.216	*	0.587	0.591	n.s.
Soybean						
qI	0.698	0.946	**	0.117	0.109	n.s.
qT	0.575	0.557	n.s.	0.034	0.049	n.s.
qE	1.637	1.264	*	0.213	0.222	n.s.
Total	2.910	2.767	*	0.364	0.380	n.s.
F′v/F′m	0.517	0.434	*	0.723	0.728	n.s.
qP	0.520	0.451	*	0.904	0.899	n.s.
Φ_{PSII}	0.269	0.195	*	0.654	0.654	n.s.

Measurements were carried out on second well-developed leaves of wheat and soybean. The statistically significant ($*P < 0.05$, $**P < 0.001$) differences obtained at ZEN concentrations 4 and 0.0 mg dm^{-3} have been marked with asterisks—on the basis of the Student′s t test, $n = 9$

n.s. differences statistically not significant

inhibition by NaCl in wheat. At Ψo values of −0.25 and −0.5 MPa, ZEN at the highest concentrations weakened the inhibition of mass accumulation and elongation of coleoptiles and the roots of wheat. At the lowest osmotic potential (−1.0 MPa), ZEN limited the decrease in the mass accumulation in wheat coleoptile, but did not have any effect on the root mass and elongation of coleoptile and roots. Within the range of NaCl concentrations tested, ZEN at the concentration of 2.0 mg dm^{-3} exerted the strongest effect protecting wheat against salt stress. In its presence the mass of coleoptile and roots was on average about 103% higher than in the control, and the length of coleoptile and roots was higher by about 91 and 36%, respectively.

For soybean, the effects of ZEN under salt stress were more complicated. At some values of Ψo, ZEN did not affect the mass accumulation in roots (−0.25 MPa) and coleoptile elongation (−0.5 and −1.0 MPa). In addition, at the most negative osmotic potential, ZEN at some concentrations (2 and 4 mg dm^{-3}) inhibited the ability of coleoptile and roots of soybean to accumulate mass and the elongation of roots. On an average, for all NaCl concentrations only ZEN at a concentration of 2.0 mg dm^{-3} showed a protective effect on the increment of soybean coleoptile mass. All the ZEN concentrations tested showed a protective effect on the mass increase and elongation of roots. In the presence of ZEN at those concentrations, the mass of coleoptile and roots and the length of roots were higher than that for the control by 40, 107 and 19%, respectively.

Discussion

Photochemical activity of PSII

Owing to the lack of information on the mechanisms of ZEN action in plants, yet it is not possible to formulate clear and reliable view about the causes of the different responses of wheat and soybean leaf discs to zearalenone, as the photochemical activity of PSII was concerned (Table 2). As it is known ZEN contains several aromatic rings in its molecule (Urry et al. 1966) and that is why it is the most hydrophobic structure among such hormones as IAA, 2-4-D, kinetin, zeatin (Gzyl et al. 2004). It may be supposed that the leaf disc surfaces of both species tested differ in their hydrophobic interactions and therefore the different absorption rate of ZEN by cells occured. Another cause of the differing responses of PSII to ZEN in wheat and soybean could be its various affinity to receptors, that may be either non-specific (Olsen 1989) or specific ones (Wang and Meng 1993). In addition, the low solubility of

Table 5 The influence of different concentrations of ZEN on the early growth of wheat and soybean seedlings as dependent on the osmotic potential of NaCl solutions

Osmotic potential (MPa)	ZEN (mg dm^{-3})	Dry weight (mg)		Length (cm)	
		Coleoptile	Roots	Coleoptile	Roots
Wheat					
0.00	0.00	13.32 a	22.57 a	9.77 b	10.05 b
	0.25	12.77 a	20.74 ab	10.10 b	10.84 ab
	2.00	14.13 a	17.03 b	13.32 a	11.35 a
	4.00	13.90 a	17.44 b	14.26 a	12.32 a
−0.25	0.00	5.83 b	11.02 b	4.01 b	5.44 a
	0.25	7.02 ab	19.47 a	4.06 b	5.47 a
	2.00	8.64 a	18.63 a	4.93 a	5.79 a
	4.00	8.69 a	19.52 a	4.94 a	5.49 a
−0.50	0.00	0.38 d	5.02 c	0.25 c	1.84 c
	0.25	1.02 c	9.47 b	0.47 c	2.67 bc
	2.00	3.77 a	14.07 a	3.31 a	4.06 a
	4.00	1.78 b	11.93 ab	1.59 b	3.13 b
−1.00	0.00	0.02 b	0.02 a	0.08 a	0.03 a
	0.25	0.27 a	0.05 a	0.15 a	0.03 a
	2.00	0.23 a	0.04 a	0.08 a	0.08 a
	4.00	0.20 a	0.43 a	1.25 a	1.25 a
Mean for: −0.25, −0.5 and −1.0	0.00	2.08 d	5.35 c	1.45 b	2.44 b
	0.25	2.77 c	9.66 b	1.56 b	2.72 b
	2.00	4.21 a	10.91 a	2.77 a	3.31 a
	4.00	3.56 b	10.63 a	2.59 a	3.29 a
Soybean					
0.00	0.00	6.64 c	12.98 a	1.67 c	13.17 a
	0.25	7.83 bc	13.96 a	2.39 bc	13.01 a
	2.00	11.79 a	12.64 a	3.97 a	13.88 a
	4.00	10.03 ab	13.63 a	2.90 b	13.18 a
−0.25	0.00	3.31 b	10.24 a	0.73 b	9.61 b
	0.25	3.45 ab	11.46 a	0.82 ab	11.72 a
	2.00	4.25 a	11.27 a	0.89 a	10.19 ab
	4.00	3.17 b	10.03 a	0.79 ab	10.04 ab
−0.50	0.00	3.10 bc	6.20 b	0.61 a	4.46 b
	0.25	2.74 c	8.35 a	0.61 a	7.00 a
	2.00	4.17 a	8.62 a	0.61 a	7.18 a
	4.00	3.21 b	8.80 a	0.61 a	7.04 a
−1.00	0.00	2.52 b	4.97 a	0.46 a	2.61 a
	0.25	2.24 b	4.12 ab	0.53 a	2.38 a
	2.00	4.05 a	3.05 c	0.49 a	1.76 b
	4.00	1.94 c	3.71 bc	0.51 a	2.37 a
Means for: −0.25, −0.5 and −1.0	0.00	2.98 b	3.72 b	0.60 a	5.56 c
	0.25	2.81 b	7.98 a	0.65 a	7.03 a
	2.00	4.16 a	7.65 a	0.66 a	6.38 b
	4.00	2.77 b	7.51 a	0.64 a	6.48 b

Means followed by the same letters within columns (separately for each osmotic potential) do not differ significantly according to the Duncan's multiple range test ($\alpha = 0.05$), $n = 15$–20

ZEN in water is the cause of its transformation by the cell to a soluble form—glycoside. This phenomenon was observed in *Arabidopsis thaliana* (Berthillet et al. 2007). Yet it is, unfortunately, unknown whether this is the form of ZEN occurring in the studied species. The energy flow through photosystems involves chloroplast membranes and it is impossible to exclude the possibility that the cause of the observed ZEN effects could result from its interactions with those structures (Vianello and Macri 1978; Filek et al. 2002, 2007).

The increase in photochemical activity of PSII in soybean seedlings after soaking of seeds in ZEN solutions (Table 3) proved similar to that in leaf discs incubated in ZEN solutions. These results are consistent with the earlier observations (Kościelniak et al. 2009) that ZEN treatment of plants was the resulted increase in photochemical activity of PSII at the early developmental stages in soybean. ZEN inhibited photochemical reactions in wheat discs, but did not affect PSII activity in wheat seedlings. The disappearance of the inhibitory photochemical reactions in wheat seedlings could be associated with ZEN 'dissolving' during the growth. The light conditions may be recognized as the decisive factor for the occurrence of positive responses of seedlings to ZEN, as photochemical changes in PSII in wheat and soybean were observed during strong illumination—1,200 μmol(quantum) m^{-2} s^{-1} (Table 4). On the other hand, an illumination of 500 μmol(quantum) m^{-2} s^{-1}, the stimulatory effect disappeared in wheat, and at 300 μmol(quantum) m^{-2} s^{-1} also in soybean.

The stimulation of the activity of the photosynthesis by ZEN probably does not occur in all species. In the experiments with gram (*Cicer arietinum* L.) and mustard (*Brassica juncea* L.) it was discovered that seed incubation caused after-effects such as a strong inhibition in the synthesis of chlorophylls *a*, *b* and carotenoids (Kumar and Sinha 1995) and such a response was explained as a result of the disturbance in the pigment synthesis by restricting the growth hormone-induced synthesis of RNA, DNA and proteins in the leaf. Similarly, strong metabolism responses to ZEN were also observed in animals (Mueller et al. 2004, Boehme et al. 2009).

Photoinhibitory reactions of PSII

The data showing that preceding sowing incubation of seeds in ZEN solutions resulted in an increase in the non-photochemical quenching (qE, qT) during strong illumination for both species (Table 4) are consistent with the opinions of other authors (Fork and Satoh 1986; Horton and Hauge 1988; van Wijk and van Hasselt 1993; Ting and Owens 1994; Ruban and Horton 1995; Owens 1996; Haldrup et al. 2001) that such response may be recognized as the action of the first defense line for the photosynthetic apparatus against photoinhibitory injuries and other photooxidative ones. In our experiment it was observed that ZEN prevented photoinhibitory injuries during strong illumination due to the safe dissipation of the excess of absorbed light energy through mechanisms related to the development of pH gradient (qE) in thylakoids and phosphorylation of LHC2 (qT). A decrease due to the influence of ZEN on the photoinhibitory quenching enabled the maintenance of a more efficient course of reaction in PSII than in plants incubated before sowing in water.

The seedlings growth under salt stress

The stimulation of growth by ZEN has been also found earlier for wheat callus (Biesaga-Kościelniak 2001; Szechyńska-Hebda et al. 2007) as well as in wheat and rape seedlings (Biesaga-Kościelniak 2001). On the other hand, it was reported by Kumar and Sinha (1995) that in their experiment the same ZEN concentrations as those used in our experiment caused inhibition the elongation of the coleoptile and root in gram and mustard, and this response increased with ZEN concentration. The salt stress strongly limited the growth of seedlings, as was also reported in other papers concerning wheat and soybean (Ashraf and McNeily 1988; Luo et al. 2005; Soltani et al. 2006). ZEN protected via after-effects the process of wheat growth against the salt stress through decreasing the level of inhibition to the elongation process and mass accumulation in coleoptile and roots. The complicate nature of ZEN action under salt stress was revealed especially by the measurements in soybean. Depending on the water potential, ZEN did not affect the growth, inhibited it or protected this process against the stress consequences. The complex response of growth to the excess of NaCl can result from the fact that salt stress inhibits physiological processes by limiting the amount of water in tissues (Rauf et al. 2007) and through the toxic effects of Na^+ and Cl^- ions (Yang and Blanchar 1993; Lacan and Durand 1996; Kurniadie and Redmann 1999) and ionic imbalance in leaves (Yeo et al. 1985). For the germinating soybean, Cl^- ion is more toxic than Na^+ (Luo et al. 2005). It seems that the positive effect of ZEN on the intensity of seedling growth at the earlier phases of growth could be explained, among other, by its ability to increase the activity of α-amylase and *β*-glucosidase (Vianello and Macri 1982).

Conclusion

On the basis of the results presented and discussed, it may be claimed that mycotoxin ZEN, which is harmful to many organisms, can stimulate the course of photochemical reactions in PSII as well as to support the process of growth under salt stress and to protect the photosynthetic apparatus of wheat and soybean seedlings against the consequences of strong illumination. As the use of ZEN in practice is concerned, it is necessary to consider the fact that the incubation of soybean seeds in ZEN solutions can be risky in salty soils with low water potential (ca. −1 MPa) because it can decrease the growth vigor. On the other hand in wheat ZEN could be harmful for the course of seed

germination in non-stress conditions because it can inhibit the growth of roots. The consequence of those phenomena could be a decrease in plant productivity.

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