The Interaction Effect of Drought and Exogenous Application of Zearalenone on the Physiological, Biochemical Parameters and Yield of Legumes

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Abstract

The effectiveness of exogenously applied zearalenone (ZEN) in alleviating water deficiency stress of pea (*Pisum sativum* L.) and yellow lupine (*Lupinus luteus* L.) was analyzed in the pot experiment. ZEN was applied in the form of spraying in the flowering phase on the first day of induced drought. The effectiveness of ZEN was evaluated based on physiological (electrolyte leakage, greenness, and photosystem II activity) and biochemical (protein, proline, ascorbic acid contents, and antioxidant enzyme activity) parameters after 14 days of drought. The yield and yield quality defined as yield components, total protein, fats, sugars, and antioxidants (tocopherols and β -carotene) were measured in newly formed seeds. ZEN residue in the seeds was analyzed employing UHPLC-MS/MS to exclude its accumulation. The results showed the possibility of reducing the effects of drought stress through the use of ZEN. It was manifested by increased cell membranes stability and antioxidant enzyme activity and above all ZEN increased crop yield, compared to untreated plants. ZEN modified seed composition by inducing the accumulation of fats and antioxidants. There was no accumulation of exogenous ZEN in seeds.

Keywords Zearalenone · Lupine · Pea · Legumes · Drought · Yield composition · PSII photochemical activity · Antioxidants

Introduction

Plant production is one of the main areas of human activity, and the yield is one of the most important indicators of its efficiency. Yielding can fluctuate greatly within the population, hence it is important to determine the stability of yielding under various environmental conditions. Biotic and abiotic stresses accompany each plant, and their effect leads to a number of changes in its functioning (Rejeb et al. 2014). Abiotic stress is the most harmful, for example long-term drought, soil salinity or lack of nutrients. There are two ways to reduce the harmful effects of adverse factors on plant productivity, chose of individual species or cultivars resistant to water shortages, or the use of appropriate

chemicals (e.g., biostimulators) during cultivation (Lawlor and Cornic 2002).

In recent years, there has been an increased interest in compounds exhibiting activity of plant growth and development regulators. These are organic substances, small amounts of which modify plant physiological functions that enhance or inhibit growth and development processes. Plant growth and development regulators are mainly used to protect plants against biotic and abiotic stresses in modern agriculture. Recently, several such substances have been discovered or characterized in more detail (Srivastava et al. 2016). Zearalenone (ZEN), which is a resorcinol acid lactone, is one of them. It has low acute toxicity, however as a non-steroidal estrogen causes hyperestrogenic symptoms in animals at concentrations in the range from 0.1 to 1.0 μ M. ZEN was first isolated in 1962 by Stob et al. from the fungus *Gibberella zeae* (*Fusarium graminearum*) extract (Stob et al. 1962). In the 1980s, its occurrence in the apical meristems of wintering wheat shoots was shown (Li et al. 1980), and its presence has been reported in more than 30 plant species (Meng et al. 1996).

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The results obtained thus far have indicated its influence, among others, on wheat plant thermoinduction, changes in metabolite contents, growth intensity modifications, callus differentiation stimulation, cell suspension growth, haploid embryo induction, increased plant CO₂ assimilation (Meng et al. 1996; Biesaga-Koscielniak et al. 2003).

Legumes are the second most important source of plant food (Vaughan and Geissler 2009). Their seeds contain the highest protein content (20–42%) compared to other crops (1–2% root crops, 9–18% cereals), and its biological value is higher than the cereal protein. However, the cultivation of legumes is relatively difficult, due to the high variability of yield, which is caused by the specific sensitivity to environmental stress, especially water shortage.

The aim of the study was to evaluate the effectiveness of zearalenone in alleviating the symptoms of drought stress of *Fabaceae* due to the fact that there are only few reports on the use of this substance in the cultivation of these plants.

Materials and Methods

Plant Material and Growth Conditions

For the study pea (*Pisum sativum* L.) cultivar Roch and yellow lupine (*Lupinus luteus* L.) cultivar Talar from the Poznań Plant Cultivation Ltd. were selected. The experiment was performed in a fully random system in three replicates. The seeds of the studied plants were sown in pots (50 × 20 × 20 cm), filled with 3.6 kg of soil substrate (horticultural soil:sand; 2:1), 10 plants per pot.

Plants grew in natural day and night length and air temperature conditions for the spring-summer period (April–September), (50°04'10'' N, 19°50'40'' E) in the open vegetation tunnel, protected from rain. The plants during growth were fertilized once a week with Hoagland medium (Hoagland and Arnon 1938). Soil water content was kept at 70% maximum water-holding capacity (mWHC) until the beginning of the flowering phase by adding an appropriate amount of water every day (determined by weighting, and controlled using a MO750 moisture meter; Extech Instruments Corporation, MA). After this, the watering was stopped. Plants at the flowering phase were subject to 25% mWHC drought for a period of 2 weeks. On the first day of drought (25% mWHC reached), plants were sprayed with 2 mg dm⁻³ zearalenone (Sigma-Aldrich, Poznań, Poland) solution in 0.05% ethanol, as described in previous studies (Biesaga-Koscielniak et al. 2003; Biesaga-Koscielniak et al. 2006a, b; Biesaga-Koscielniak and Filek 2010). Control plants were sprayed with distilled water containing 0.05% ethanol. Approximately 75 cm³ of the solution was used for spraying 10 plants. The number of replicates for each cultivar within the treatment was 60 plants.

Measurements of leaf greenness, PSII fluorescence, and electrolyte leakage were performed after 14 days of drought. At that time leaves for the analysis of proline, total protein, total sugar, ascorbic acid contents, and antioxidant enzyme activity were harvested. After that, the plants were subsequently watered to 70% mWHC. Plants were grown for yielding and selected yield components (number of pods per plant, seeds per plant, seed weight per plant and 1000 seed weight) were determined. The content of total proteins, fats, sugars, and content of β-carotene and tocopherols were determined in collected seeds.

Electrolyte Leakage

The electrolyte leakage was measured for the youngest fully developed leaf. The material was rinsed twice with deionized water, and placed in vials filled with 15 cm³ of deionized water, and shaken (24 h, 50 rpm at 20 °C). The conductance was measured after 24 h (C1317 Elmetron, Poland)—EL1. The samples were then frozen at – 80 °C for 24 h and transferred to room temperature for 24 h on a shaker, after which the conductance (EL2) was recorded. The electrolyte leakage was calculated from the formula: $EL = (EL1/EL2) \times 100\%$.

Leaf Greenness (SPAD)

The chlorophyll content was measured using a SPAD spectrometer (502, Konica—Minolta). The chlorophyll content was measured on the youngest fully developed leaf.

Chlorophyll a Fluorescence

Chlorophyll *a* fluorescence measurement was performed on the first, youngest and fully developed leaf, after 20-min adaptation in the dark using a PEA fluorimeter (Hansatech Ltd. Kings Lynn) and following the manufacturer's instruction. The use of the JIP test allowed to calculate the following parameters based on the energy flux through the photosystem II (Lazar 1999; Lazar and Pospisil 1999; Strasser et al. 2000; Kalaji and Guo 2008):

ABS/RC—light absorption flux per PSII reaction center (RC),

TRo/RC—trapped energy flux per RC,

ETo/RC—electron transport flux per RC,

DIo/RC—dissipated energy flux per RC.

Protein Content

Total soluble proteins were determined according to the Bradford (1976) method in 96-well plate format as reported

by Biesaga-Koscielniak et al. (2014). The absorbance was read at 595 nm using Synergy II (Biotek, USA) Reader.

Proline Content

The content of free proline was determined in plant leaves based on the method of Ting and Rouseff (1979), exactly as described by Marcinska et al. (2013).

Analysis of Antioxidant Enzyme Activity

Plant material was homogenized at 4 °C in 0.05 mM (pH 7) phosphate-potassium buffer containing 0.1 mM EDTA. The activity of the following antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) was determined. After centrifugation, (10000×g, 15 min at 4 °C, 32R, Hettich, Germany) clear supernatant was sub-sampled and assayed for SOD, catalase and peroxidase activity using a UV/VIS spectrophotometer (Lambda Bio 20, Perkin-Elmer, Germany). SOD activity was determined by the cytochrome reduction method (McCord and Fridovic, 1969). CAT activity was measured at 240 nm according to Aebi (1984), as a decrease of H₂O₂ absorbance. The activity of POD was measured using the Lück (1962) method. The increase in oxidized p-phenylenediamine absorbance was monitored at 485 nm. Analyses were conducted as described by Gudys et al. (2018).

Ascorbic Acid Content

Vitamin C determination was performed using the CUPRAC method of Ozyurek et al. (2007) modified by Biesaga-Koscielniak et al. (2014) in multi-well-plate format (Synergy II). The results are expressed in µg mg⁻¹ of sample.

Total Soluble Sugar Determination

Sugars were analyzed by phenol-sulfuric method (Dubois et al. 1951) with modifications reported by Marcinska et al. (2013). Samples were extracted in ultra-pure water and after centrifugation the supernatant aliquot (5 µl) was diluted to 200 µl with water, afterwards, 200 µl of 5% water-phenol solution and 1 ml of concentrated sulfuric acid were added. The samples were incubated for 20 min, and then transferred to 96-well plates and absorbance was measured at 490 nm (Synergy II). The sugar content was estimated using a standard curve prepared for glucose.

Total Fat Determination

The total fat content was estimated in 0.2 g samples according to the method described by Bligh and Dyer (1959). Shortly, after extraction in methanol:chloroform (1:2 v/v),

phases were separated by addition of 1M KCl solution (1:3 v/v), the chloroform phase was collected, evaporated under N₂, and the total fat was calculated by weighing.

Determination of Tocopherols and β-Carotene

The extraction of tocopherols and β-carotene was performed according to the method described by Janeczko et al. (2018). The obtained extract was then used for HPLC analyses. Tocopherol analysis was performed on an Agilent 1200 (Agilent Technologies, Germany) system with a binary pump, autosampler and fluorescence detector. Separation was achieved on Zorbax Eclipse XDB 5 µm, 4.6 mm × 150 mm column (Agilent, USA) at the linear gradient of (A) methanol:acetonitrile:water (2.5:2.5:0.33 v/v) and (B) acetonitrile:dichloromethane (1:1 v/v) (from 95 to 30% A in 15 min) at a flow rate of 1 ml min⁻¹, at 25 °C. Tocopherols were detected at an excitation wavelength of 295 nm and an emission wavelength of 330 nm. The optimal parameters of fluorescent detection were selected according to the absorption and emission spectra of standards (α-, γ-, δ-tocopherol) acquired online. Tocopherols determination was performed for plant material from the pot and field experiment.

β-Carotene was analyzed using an Agilent 1260 UHPLC system (Agilent Technologies, Germany) with a binary pump, thermostated autosampler at 10 °C, and a diode array detector. The sample was injected onto a Spherisorb ODS2, 3 µm, 4.6 mm × 150 mm analytical column (Waters, Ireland) and at 25 °C. A linear gradient of (A) acetonitrile:water (2.5:0.33 v/v) and (B) ethyl acetate was applied (from 0 min to 6.5 min 40% A, then to 8 min 20% (A) at a flow rate of 1 ml min⁻¹. The detector was set up at 450 nm. β-carotene was used as a standard.

UHPLC-MS/MS Determination of Zearalenone Content

The grinded seeds (2.5 g) were extracted in 10 ml of acetonitrile and water (80/20 v/v) solution by shaking for 1 h on a rotator (RL-2020, JW Electronic, Poland). Fifty ng of heavy-labeled internal standard ([¹³C₁₈]-ZEN; Sigma-Aldrich) was added to each sample. Samples were centrifuged (10 min 4000×g, 15 min at 4 °C, 32R, Hettich) and 3 ml supernatant aliquot was applied to Bond Elut Mycotoxin columns (3 ml 500 mg, Agilent Technologies), according to the manufacturer's instructions. The column eluate was evaporated under N₂ and the residue was dissolved in 250 µl of acetonitrile in H₂O (50:50 v/v), filtered through a 0.22-µm membrane and analyzed. ZEN was determined using the UHPLC system (Infinity 1260, Agilent Technologies, Germany) with a tandem quadrupole mass spectrometry detector (QQQ 6410, Agilent Technologies, USA). Samples were separated onto a Poroshell 120 Phenyl-Hexyl 2.1 × 5 mm, 2.7 µm analytical

column. The analyzed substances were eluted with a gradient of water (A) and methanol (B) both modified with 0.1% formic acid, from 53 B to 75% in 4.5 min, with a mobile phase flow of 0.3 ml min⁻¹. Multiple reaction monitoring (MRM) transitions after positive ESI ionization were used for identification and quantification (m/z 319>283 for ZEN and m/z 337>301 for [¹³C₁₈]-ZEN). Quantitation was based on calibration curves obtained with authentic ZEN standard taking account of the recovery rates of internal standard used.

Statistical Analysis

Statistical significance was estimated based on the Student's test ($P \leq 0.05$). The objects were compared in pairs: ZEN spraying with proper control. Chemical analysis was conducted in three replicates (with three chemical repeats each). The sample collected from leaves and ground seeds collected from ten randomly selected plants served as one replicate. Statistical analysis of the results was carried out using the Statistica 10 (StatSoft, USA) program.

Results

Spraying with a ZEN solution resulted in a reduction in the plant electrolyte leakage in both species tested compared to control plants (Fig. 1a). ZEN increased the level of tissue greenness and caused a significant increase in proline content by about 30% in lupine leaves (Fig. 1b and c). In pea plants, it slightly decreased leaf greenness and significantly lowered proline concentration.

In addition, ZEN induced an increase in protein content in pea leaves (by about 30%), an increase in sugar content (by about 25%) and a slight increase in vitamin C content (by about 10%) in comparison to control plants (Table 1). In lupine leaves, it only caused a significant reduction in the content of sugars. A marked increase in the activity of antioxidant enzymes was noted under the influence of ZEN both in pea and lupine leaves.

The photochemical activity of pea and lupine PSII system changed to a small extent under ZEN and these changes were negligible in most of the calculated indicators (Table 2). In pea plants, ZEN caused a decrease in radiation absorption by the antenna system (ABS/RC) as well as a reduction in the energy stream reaching the photochemical reaction center (TR₀/RC). ZEN activity, however, faded away at further stages of excitation energy transport. Spraying plants with ZEN did not cause significant changes in the values of measured parameters in lupine leaves.

ZEN applied at the beginning of a 14-day drought clearly stimulated plant yield compared to the control plants, which resulted in a higher number of pods in pea and lupine (by

Fig. 1 Effect of zearalenone (ZEN) applied in the form of spray at the beginning of a 2-week drought induced in the flowering phase on **a** the electrolyte leakage (EL), **b** greenness and **c** proline content in pea and lupine leaves. The values marked with * differ statistically according to the Student's *t* test, $\alpha < 0.05$, $n = 15$

47% and 41%, respectively) (Fig. 2a) and seeds (by 28% and 15%, respectively) (Fig. 2b). ZEN increased seed weight only in lupine by 28% (Fig. 2c), but it did not affect the weight of pea seeds, and therefore, reduced 1000 seed weight by approx. 19% (Fig. 2d).

Seed chemical analysis determining the content of protein, fats, sugars, β -carotene and tocopherols in the seeds obtained from plants subjected to 2-week drought stress and the action of ZEN was carried out. The difference in protein content in both pea and lupine seeds between the treated and control plants was low and these changes were not significant (Fig. 3a). ZEN induced a significant increase

Table 1 Effect of zearalenone (ZEN) applied in the form of spray at the beginning of a 2-week drought induced in the flowering phase on the content of proteins, sugars, vitamin C and antioxidant enzymes activity in pea and lupine leaves

Species	Treatment	Protein [μg/mg d.w.]	Sugars [mg/mg d.w.]	Vitamin C [μg/mg d.w.]	POX [U/mg protein]	SOD [U/mg protein]	CAT [U/mg protein]
Pea	ZEN _{2.00}	58.15*	0.185*	3.176	77.466*	3.986*	0.080
	Control	48.30	0.148	2.866	59.460	2.940	0.030*
Lupine	ZEN _{2.00}	12.89	0.108*	1.344	168.731*	8.797*	0.341*
	Control	13.03	0.138	1.513	98.388	4.521	0.170

The values marked with * differ statistically according to Student's *t* test, $\alpha < 0.05$, $n = 15$

SOD superoxide dismutase, CAT catalase, POD peroxidase

Table 2 Effect of zearalenone (ZEN) applied in the form of spray at the beginning of a 2-week drought induced in the flowering phase on the photosynthetic activity of pea and lupine leaves (ABS/RC—light absorption flux per PSII reaction center (RC); TRo/RC - trapped energy flux per RC; ETo/RC - electron transport flux per RC; DIo/RC - dissipated energy flux per RC)

Species	Treatment	Activity of a single reaction center (RC)			
		ABS/RC	TRo/RC	ETo/RC	DIo/RC
Pea	ZEN _{2.00}	3.011*	2.524*	1.123	0.488
	Control	3.194	2.706	1.156	0.484
Lupine	ZEN _{2.00}	2.278	1.881	1.024	0.397
	Control	2.327	1.916	1.047	0.411

The values marked with * differ statistically according to Student's *t* test, $\alpha < 0.05$, $n = 15$

in fat concentration in pea and lupine (15% on average) (Fig. 3b). The sugar content increased only in lupine by 34% (Fig. 3c). The analysis of ZEN content in the obtained seeds was carried out due to the fact that ZEN is a known fungal toxin. Plant treatment with ZEN solution did not significantly affect the increase in ZEN concentration in the obtained yield (Fig. 3d). Trace amounts of zearalenone were detected both in the seeds of control plants (untreated ZEN) and those treated with ZEN. ZEN content did not exceed 2 μg per kg of seeds. These concentrations are ten times lower than the lowest acceptable food contamination (20 μg per kg of product in the case of infant formulas).

The concentration of β-carotene (Fig. 4a) increased by 27% in pea and by as much as 52% in lupine. Moreover, ZEN increased the concentration of α-tocopherol in pea (nearly 40%) (Fig. 4b), δ-tocopherol by 23% in pea and 69% in lupine (Fig. 4c), γ-tocopherol by 34% in lupine, while in pea there was a 20% decrease in its content (Fig. 4d).

Discussion

Stress caused by water scarcity is one of the most important factors limiting crop yields. Reports on biochemical and physiological studies of drought resistance are regularly

summarized in the reviews (Foyer and Noctor 2009; Jogaiah et al. 2013; Wani et al. 2016; Kerchev et al. 2019). It is generally accepted that maintaining cell membranes integrity and stability under water stress conditions is a major component of drought tolerance in plants (Bajji et al. 2001; Quan et al. 2004; Liu et al. 2019). In the current study, the use of ZEN during drought caused a reduction in membrane permeability in both species tested compared to control plants. Our unpublished data also showed a decrease in electrolyte leakage during low temperature stress in wheat under ZEN action. It can, therefore, be assumed that ZEN contributes to cell membranes stabilization during stress. Other plant hormones, such as brassinosteroids exert a similar effect to ZEN (Houimli et al. 2010; Gruszka 2013; Dehghan et al. 2020).

The reduction of chlorophyll content is another symptom indicating the sensitivity of plants to stress and primarily, water shortage (Baryla et al. 2001; Wang et al. 2008; Ostrowska et al. 2019). Thus, chlorophyll content can be an indicator of the current viability of plants and their response to changing environmental conditions (Zobayed et al. 2005). Plants lose their green chlorophyll tissues under environmental stresses. The present study found an increase in tissue greenness under ZEN influence, as compared to control plants. Probably such changes in the content of this pigment are the result of specific ZEN stimulation of metabolic processes occurring in the plant, leading to an increase in the content of substances, such as proteins or sugars in the leaves.

Drought stress not only affects the chlorophyll content but also damages the photosynthetic apparatus. The functioning of photosystem II (estimated by the JIP test) provides indirect information about the physiological condition of plants during stresses. The current study demonstrated only small differences in the photosynthetic activity of plants treated with ZEN, as compared to control plants. In drought conditions, ZEN reduced absorbed energy flux (ABS) and trapped energy flux (TRo) for single PSII reaction centers (RCs) while maintaining the level of the electron transport per active reaction center (ETo/RC) at the level of control plants. This demonstrates the more efficient use of energy for photochemical processes in drought stress in plants treated

Fig. 2 Effect of zearealenone (ZEN) applied in the form of spray at the beginning of a 2-week drought induced in the flowering phase on **a** the number of pods per plant, **b** seeds per plant, **c** seed weight per plant and **d** 1000 seed weight in pea and lupine. The values marked with * differ statistically according to the Student's *t* test, $\alpha < 0.05$, $n = 15$

with ZEN. Koscielniak et al. (2009) showed that pre-sowing soaking of soybean and wheat seeds in ZEN solution caused a higher PSII efficiency under salt stress and, as a result, an increase in their yield. However, scarce data available in the literature indicate that the stimulation of the photosynthetic process by ZEN probably does not occur in all species. It

was discovered in the experiments with chickpea (*Cicer arietinum* L.) and mustard (*Brassica juncea* L.) that seed incubation in ZEN caused such effects as strong inhibition of the synthesis of chlorophylls a, b and carotenoids (Kumar and Sinha 1995). The latter response was explained by the disturbance of pigment synthesis by restricting growth

Fig. 3 Effect of zearealenone (ZEN) applied in the form of spray at the beginning of a 2-week drought induced in the flowering phase on the **a** protein, **b** fat, **c** sugar and **d** zearealenone contents in pea and lupine seeds. The values marked with * differ statistically according to the Student's *t* test, $\alpha < 0.05$, $n = 15$

hormone-induced synthesis of RNA, DNA and proteins in the leaf. ZEN changes the distribution of cell membrane charges, and thus it can affect the chloroplast membrane reconstruction in plants (Filek et al. 2007). The general opinion may suggest that its action seems to be significant for chloroplast (and PSII) function. In addition, it is possible

that changes in photosynthesis intensity directly after cessation of a 2-week drought were not fully visible. It should also be noted that plant response to bioregulators depends on the species and even on the cultivar (Srivastava et al. 2016).

Plant tissues have an efficiently functioning defense system against active forms of oxygen, consisting of enzymes

Fig. 4 Effect of zearalenone (ZEN) applied in the form of spray at the beginning of a 2-week drought induced in the flowering phase on the content of **a** β -carotene, **b** α -tocopherol, **c** δ -tocopherol and **d** γ -tocopherol in pea and lupine seeds. The values marked with * differ statistically according to the Student's *t* test, $\alpha < 0.05$, $n = 15$.

that neutralize reactive oxygen species and interact with antioxidants. Excessive proline production is often observed in plants experiencing various types of stresses, especially drought, which is associated with a decrease in cell turgor (Kishor et al. 2005; Ashraf and Foolad 2007; Gadzinowska et al. 2019; Meena et al. 2019). Despite numerous studies,

there are still many uncertainties about the role of proline in the reaction of plants to abiotic factors. Hanson and Hitz (1982) considered increased proline accumulation only as a symptom of the stress conditions impact on the plant, not necessarily indicating plant adaptation to these conditions. Prevailing interpretation of the role of proline is osmotic

adaptation, cell structure stabilization or scavenging of free radicals (Meena et al. 2019). The experiments presented in this article demonstrated that ZEN caused a reduction in the proline concentration in pea. Different results were obtained in yellow lupine plants, where ZEN increased the content of free proline in leaf cells. The reduction of proline content in plants subjected to ZEN spraying may indicate its effect on alleviating the effects of drought. Increased content of free proline can be perceived as a sign of plant adaptation to drought, which would confirm the theory of some researchers that the increase in free proline content is analogous to stress degree experienced by the plant. This effect is also exerted by some regulators (e.g., brassinosteroids), contributing to proline increase in plant tissues subjected to various stresses (Fariduddin et al. 2011; Płażek et al. 2017).

The induction of SOD activity and subsequent up-regulation of other antioxidant enzymes is one of the main ways to overcome oxidative stress (Alscher et al. 2002). There are many studies in the literature on the activity of antioxidant enzymes in numerous plant species, e.g., magnolia (Wojtania et al. 2016), tomato (Mittova et al. 2000), sugar beet (Bor et al. 2003), wheat (Szechyńska-Hebda et al. 2012) and barley (Gudys et al. 2018). In the present study, we observed an increased activity of antioxidant enzymes under ZEN during a 2-week drought. It can, therefore, be concluded that ZEN, by elevating the activity of antioxidant enzymes, induces an increase in stress tolerance in the plants. Many studies indicated increased antioxidant enzyme activity and higher biosynthesis of antioxidant molecules in various types of stresses by plant hormones (Osman 2015).

According to our previous work, zearalenone can be used in agricultural practice as a regulator increasing yield, and above all, preventing a decrease of wheat or soybean yield under drought conditions (Biesaga-Koscielniak et al. 2007; Dziurka et al. 2007). ZEN treatment of legumes also prevented yield reduction after the occurrence of drought. This

action of ZEN is very important, because, it is estimated that the action of various abiotic stress factors can reduce crop yields even several times (Kaydan et al. 2007). The mechanism of the protective ZEN effect is dependent on the species, because it can stimulate the number of pods and seeds set, as well as the weight of seeds (lupine) or the number of pods and seeds (pea). Płażek et al. (2017) have tested ZEN and 24-epibrassinolide (EBR) as regulators in the alleviation salt stress of selected cereals. In that research, ZEN had a greater positive impact on stress alleviation on tested crop plants than EBR. Our observations show that ZEN can have important role in alleviation of drought. ZEN action is connected with stimulation of the antioxidative system, osmotic adjustments, and the cell membrane integrity simultaneously maintaining PSII energy use efficiency. All of that increases the yield, compared to control plants. This can be considered as a piece of pictorial evidence for drought alleviation (summarized in Fig. 5). ZEN not only increased the yield but also influenced the chemical composition of seeds by increasing the contents of β -carotene, tocopherols, fats (pea and lupine) and sugars (lupine), but did not change protein content in both species. The metabolic mechanism of ZEN action on the content of chemical components in seeds is not known; it can be assumed that ZEN is involved in the plant defense mechanism triggered during stress, especially stimulating the content of antioxidant substances. Other regulators and biostimulants also exhibit this type of activity (Osman 2015).

The demonstrated properties of ZEN offer the possibility of its practical use, but the application of ZEN on a larger scale should be monitored due to the potential environmental risk (Videmann et al. 2008; Gajecka et al. 2012; Gao et al. 2018; Dellaflora et al. 2020). Although zearalenone with toxic properties is present in the environment, it poses a potential risk to animal and human health only if it is absorbed in large quantities or during

Fig. 5 Proposed model of ZEN potential role in drought alleviation in tested legume plants. Foliar spraying of ZEN increases PSII energy use efficiency, retains cell membrane integrity, stimulates enzymatic ROS scavenging activity, and increases osmotically active substances. The effect is the alleviation of drought manifested in maintaining or increasing yield quantity and quality compared to control plants.

the long-term exposure (Zinedine et al. 2007). The content of ZEN varies in the range of 0.001–8.04 mg kg⁻¹ in wheat, 0.016–0.095 mg kg⁻¹ in oat and 0.004 ± 15 mg kg⁻¹ in barley (Placinta et al. 1999). Chełkowski et al. 2012 found that Fusarium-damaged kernels of wheat were contaminated with amounts of ZEN, from 0.035 to 4.48 mg/kg. The not infested kernels fraction contain about 20 times less ZEN than fusarium-damage kernels. Report of Rolli et al (2018) proves that wheat plants are able to absorb up to 200 µg of ZEN per plantlet and metabolize it. The JECFA (Joint Expert Committee on Food Additives) data show that the average consumption of ZEN ranges from about 1 ng kg⁻¹ body weight per day (Italy) to 29 ng kg⁻¹ body weight per day (France). It is assumed that TDI (tolerable daily intake) for ZEN is 200 ng kg⁻¹ body weight, thus the average consumption of ZEN ranges from 1 to 58% TDI. The results of the analyses indicate that ZEN does not accumulate in newly set seeds. According to our work, small amounts of zearalenone act as factors stimulating the development of plants and can serve as a plant hormone in the induction of various physiological processes.

The results allowed to draw two important conclusions. First of all, they demonstrated the possibility of alleviating the drought stress in legumes by zearalenone application as well as using zearalenone to increase the yield and modify the chemical composition of new seeds. ZEN increased the level of antioxidants in the seeds, which is important from a nutritional perspective. Therefore, we suggest that zearalenone can be use in agricultural practice. All the more that we proved ZEN does not accumulate in seeds. At the same time, we indicated certain mechanisms of ZEN action on plant metabolism. It seems that, similarly as other plant hormones, ZEN also acts on a group of protective mechanisms, i.e., it stimulates plant antioxidant system. In addition, ZEN improves seed nutritional value by increasing the content of antioxidants in newly produced seeds. These findings are important and can be a starting point for further, more detailed research.

Authors Contribution AM, AO and MD carried out the experiment, data analysis and the preparation of manuscript. JBK performed critical revision of the manuscript. MD participated in biochemical analysis. AO and AM participated in physiological analysis. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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References

- Aebi H (1984) Catalase in Vitro. In: Packer L (ed) Methods in enzymology. Academic Press, San Diego, pp 21–126
- Alscher RG, Erturk N, Heath LS (2002) Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. J Exp Bot 53:1331–1341. <https://doi.org/10.1093/jexbot/53.372.1331>
- Ashraf M, Foolad MR (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Exp Bot 59:206–216. <https://doi.org/10.1016/j.envexpbot.2005.12.006>
- Bajji M, Lutts S, Kinet JM (2001) Water deficit effects on solute contribution to osmotic adjustment as a function of leaf ageing in three durum wheat (*Triticum durum* Desf.) cultivars performing differently in arid conditions. Plant Sci 160:669–681. [https://doi.org/10.1016/S0168-9452\(00\)00443-X](https://doi.org/10.1016/S0168-9452(00)00443-X)
- Baryla A, Carrier P, Franck F, Coulomb C, Sahut C, Havaux M (2001) Leaf chlorosis in oilseed rape plants (*Brassica napus*) grown on cadmium-polluted soil: causes and consequences for photosynthesis and growth. Planta 212:696–709. <https://doi.org/10.1007/s004250000439>
- Biesaga-Koscielniak J, Dziurka M, Ostrowska A, Mirek M, Koscielniak J, Janeczko A (2014) Brassinosteroid improves content of antioxidants in seeds of selected leguminous plants. Aust J Crop Sci 8:378–388
- Biesaga-Koscielniak J, Filek M (2010) Occurrence and physiology of zearalenone as a new plant hormone. In: Lichtfouse E (ed) Sociology, organic farming, climate change and soil science. sustainable agriculture reviews, vol 3. Springer, Dordrecht, pp 419–435
- Biesaga-Koscielniak J, Janeczko A, Dziurka M, Filek M, Adamska A, Koscielniak J, Lukaszewska GS (2007) Evaluation of grain yield and composition in wheat cultivars under natural drought conditions - impact of zearalenone. Acta Physiol Plant 29:S39–S40
- Biesaga-Koscielniak J, Janeczko A, Filek M, Dziurka M, Koscielniak J (2006a) Effect of zearalenone on the growth and productivity of crop plants I. Effectiveness of application of zearalenone on wheat production. Bibliotheca Fragmenta Agronomica 11:53–54
- Biesaga-Koscielniak J, Janeczko A, Filek M, Dziurka M, Koscielniak J (2006b) Effect of zearalenone on the growth and productivity of crop plants. II. Effectiveness of application of zearalenone on soybean production. Bibliotheca Fragmenta Agronomica 11:55–56
- Biesaga-Koscielniak J, Marcinska I, Wedzony M, Koscielniak J (2003) Effect of zearalenone treatment on the production of wheat haploids via the maize pollination system. Plant Cell Rep 21:1035–1039. <https://doi.org/10.1007/s00299-003-0622-9>
- Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Phys 37:911–917
- Bor M, Ozdemir F, Turkan I (2003) The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L. and wild beet *Beta maritima* L. Plant Sci 164:77–84. [https://doi.org/10.1016/S0168-9452\(02\)00338-2](https://doi.org/10.1016/S0168-9452(02)00338-2)
- Bradford MM (1976) Rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of

- protein-dye binding. *Anal Biochem* 72:248–254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
- Chefkowski J, Gromadzka K, Stępień L, Lenc L, Kostecki M, Berthiller F (2012) Fusarium species, zearalenone and deoxynivalenol content in preharvest scabby wheat heads from Poland. *World Mycotoxin J* 5(2):133–141. <https://doi.org/10.3920/WMJ2011.1304>
- Dehghan M, Balouchi H, Yadavi A, Zare E (2020) Improve wheat (*Triticum aestivum*) performance by brassinolide application under different irrigation regimes. *S Afr J of Bot* 130:259–267. <https://doi.org/10.1016/j.sajb.2020.01.013>
- Dellafiora L, Oswald IP, Dorne J-L, Galaverna G, Battilani P, Dall'Asta C (2020) An in silico structural approach to characterize human and rainbow trout estrogenicity of mycotoxins: proof of concept study using zearalenone and alternariol. *Food Chem* 312:126088. <https://doi.org/10.1016/j.foodchem.2019.126088>
- Dubois M, Gilles K, Hamilton JK, Rebers PA, Smith F (1951) A colorimetric method for the determination of sugars. *Nature* 168:167–167. <https://doi.org/10.1038/168167a0>
- Dziurka M, Biesaga-Koscielniak J, Janeczko A, Adamska A, Filek M, Koscielniak J, Łukaszewska GS, Janeczko Z (2007) Effect of zearalenone on yield of soybean. *Acta Physiol Plant* 29:S52–S52
- Fariduddin Q, Yusuf M, Chalkoo S, Hayat S, Ahmad A (2011) 28-homobrassinolide improves growth and photosynthesis in *Cucumis sativus* L. through an enhanced antioxidant system in the presence of chilling stress. *Photosynthetica* 49:55–64. <https://doi.org/10.1007/s11099-011-0022-2>
- Filek M, Biesaga-Koscielniak J, Macháčková I, Krekule J (2007) Generative development of winter rape (*Brassica napus* L.)—the role of vernalization. *Int J Plant Dev Biol* 1:57–63
- Foyer CH, Noctor G (2009) Redox regulation in photosynthetic organisms: signaling, acclimation, and practical implications. *Antioxid Redox Signal* 11:861–905. <https://doi.org/10.1089/ars.2008.2177>
- Gadzinowska J, Ostrowska A, Hura K et al (2019) Physiological traits determining high adaptation potential of sweet briar (*Rosa rubiginosa* L.) at early stage of growth to dry lands. *Sci Rep* 9:19390. <https://doi.org/10.1038/s41598-019-56060-3>
- Gajęcka M, Rybarczyk L, Jakimiuk E, Zielonka Ł, Obremski K, Ziwerchowski W, Gajęcki M (2012) The effect of experimental long-term exposure to low-dose zearalenone on uterine histology in sexually immature gilts. *Exp Toxicol Pathol* 64:537–542. <https://doi.org/10.1016/j.etp.2010.11.009>
- Gao X, Xiao Z, Li C, Zhang J, Zhu L, Sun L, Zhang N, Khalil MM, Rajput SA, Qi D (2018) Prenatal exposure to zearalenone disrupts reproductive potential and development via hormone-related genes in male rats. *Food Chem Toxicol* 116:11–19. <https://doi.org/10.1016/j.fct.2018.04.011>
- Gruszka D (2013) The brassinosteroid signaling pathway - new key players and interconnections with other signaling networks crucial for plant development and stress tolerance. *Int J of Mol Sci* 14:8740–8774. <https://doi.org/10.3390/ijms14058740>
- Gudys K, Guzy-Wrobelska J, Janiak A, Dziurka MA, Ostrowska A, Hura K, Jurczyk B, Zmuda K, Grzybkowska D, Srobka J et al (2018) Prioritization of candidate genes in QTL regions for physiological and biochemical traits underlying drought response in Barley (*Hordeum vulgare* L.). *Front. Plant. Sci.* 9:1–26. <https://doi.org/10.3389/fpls.2018.00769>
- Hanson AD, Hitz WD (1982) Metabolic responses of mesophytes to plant water deficits. *Annu Rev Plant Phys* 33:163–203. <https://doi.org/10.1146/annurev.pp.33.060182.001115>
- Hoagland DR, Arnon DI (1938) The water culture method for growing plants without soil. *Circ—Calif Agric Exp Stn* 347:1–39
- Houimli SIM, Denden M, Mouhanded BD (2010) Effects of 24-epibrassinolide on growth, chlorophyll, electrolyte leakage and proline by pepper plants under NaCl-stress. *Eurasia J Biosci* 4:96–104
- Janeczko A, Dziurka M, Pocięcha E (2018) Increased leaf tocopherol and β -carotene content is associated with the tolerance of winter wheat cultivars to frost. *J Agron Crop Sci* 204:594–602. <https://doi.org/10.1111/jac.12287>
- Jogaiah S, Govind SR, Tran LSP (2013) Systems biology-based approaches toward understanding drought tolerance in food crops. *Crit Rev Biotechnol* 33:23–39. <https://doi.org/10.3109/07388551.2012.659174>
- Kalaji HM, Guo P (2008) Chlorophyll fluorescence: a useful tool in barley plant breeding programs. In: Sánchez A, Gutierrez SJ (ed) *Photochemistry Research Progress*, Nova Science Publishers, New York, pp. 439–463
- Kaydan D, Yagmur M, Okut N (2007) Effects of salicylic acid on the growth and some physiological characters in salt stressed Wheat (*Triticum aestivum* L.). *TarimBilimDerg* 13:114–119
- Kerchev P, van der Meer T, Sujeeth N, Verlee A, Stevens CV, Breusegem FV, Gechev T (2019) Molecular priming as an approach to induce tolerance against abiotic and oxidative stresses in crop plants. *Biotechnol Adv*. <https://doi.org/10.1016/j.biotechadv.2019.107503>
- Kishor PBK, Sangam S, Amrutha RN, Laxmi PS, Naidu KR, Rao KRSS, Rao S, Reddy KJ, Theriappan P, Sreenivasulu N (2005) Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. *Curr Sci India* 88:424–438
- Koscielniak J, Biesaga-Koscielniak J, Janeczko A, Filek W, Kalaji HM (2009) Can the Gibberella zeae toxin zearalenone affect the photosynthetic productivity and increase yield formation in spring wheat and soybean plants? *Photosynthetica* 47:586–594. <https://doi.org/10.1007/s11099-009-0084-6>
- Kumar N, Sinha KK (1995) Effect of zearalenone on some physiological and biochemical processes of gram and mustard seeds. *Recent Adv Phytopathl Res* 149–162
- Lawlor DW, Cornic G (2002) Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant Cell Environ* 25:275–294. <https://doi.org/10.1046/j.0016-8025.2001.00814.x>
- Lazar D (1999) Chlorophyll a fluorescence induction. *BBA-Bioenergetics* 1412:1–28. [https://doi.org/10.1016/s0005-2728\(99\)00047-x](https://doi.org/10.1016/s0005-2728(99)00047-x)
- Lazar D, Pospisil P (1999) Mathematical simulation of chlorophyll a fluorescence rise measured with 3-(3',4'-dichlorophenyl)-1,1-dimethylurea barley leaves at room and high temperatures. *Eur Biophys J Biophys* 28:468–477. <https://doi.org/10.1007/s002490050229>
- Li JL, Zhu TX, Zhang C, Li BR, Deng Z, Li YS, Meng F (1980) Studies on zearalenone. *Acta Agriculturae Universitatis Pekinensis* 1:13–28
- Liu X, Ma D, Zhang Z, Wang S, Du S, Deng X, Yin L (2019) Plant lipid remodeling in response to abiotic stresses. *Ecotoxicol Environ Saf* 165:174–184
- Luck H (1962) *Methoden der Enzymatischen Analyse*. In: von Bergmeyer HU (ed) *Verlag Chemie, GMBH Weinheim*
- Marcinska I, Czyczyło-Mysza I, Skrzypek E, Filek M, Grzesiak S, Grzesiak MT, Nowakowska A, Quarrie SA (2013) Impact of osmotic stress on physiological and biochemical characteristics in drought-susceptible and drought-resistant wheat genotypes. *Acta Physiol Plant* 35:451–461. <https://doi.org/10.1007/s11738-012-1088-6>
- Meena M, Divyanshu K, Kumar S, Swapnil P, Zehra A, Shukla V, Yadav M, Upadhyay RS (2019) Regulation of L-proline biosynthesis, signal transduction, transport, accumulation and its vital role in plants during variable environmental conditions. *Heliyon* 5:e02952. <https://doi.org/10.1016/j.heliyon.2019.e02952>
- McCord JM, Fridovic I (1969) Superoxide dismutase an enzymic function for erythrocyte (hemocytin). *J Biol Chem* 244:6049–6055
- Meng FJ, Han YZ, Fu YF, Guo FL (1996) Zearalenone in higher plants. *Flower Newslett* 22:54–57

- Mittova V, Volokita M, Guy M, Tal M (2000) Activities of SOD and the ascorbate-glutathione cycle enzymes in subcellular compartments in leaves and roots of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii*. *Physiol Plantarum* 110:42–51. <https://doi.org/10.1034/j.1399-3054.2000.110106.x>
- Osman HS (2015) Enhancing antioxidant–yield relationship of pea plant under drought at different growth stages by exogenously applied glycine betaine and proline. *Ann Agric Sci* 60:389–402
- Ostrowska A, Tyrka M, Dziurka M, Hura K, Hura T (2019) Participation of Wheat and Rye genome in drought induced senescence in Winter Triticale (X Triticosecale Wittm.). *Agronomy* 9:195. <https://doi.org/10.3390/agronomy9040195>
- Ozyurek M, Guclu K, Bektasoglu B, Apak R (2007) Spectrophotometric determination of ascorbic acid by the modified CUPRAC method with extractive separation of flavonoids-La(III) complexes. *Anal Chim Acta* 588:88–95. <https://doi.org/10.1016/j.aca.2007.01.078>
- Placinta CM, D’Mello JPF, Macdonald AMC (1999) A review of worldwide contamination of cereal grains and animal feed with Fusarium mycotoxins. *Anim Feed Sci Tech* 78:21–37. [https://doi.org/10.1016/s0377-8401\(98\)00278-8](https://doi.org/10.1016/s0377-8401(98)00278-8)
- Plažek A, Tatrzńska M, Maciejewski M, Dziurka M, Waligórski P, Dubert F (2017) Effects of zearalenone and 24-epibrassinolide on the salt tolerance of selected monocotyledonous crop plants. *J Appl Bot Food Qual* 90:280–287. <https://doi.org/10.5073/JABFQ.2017.090.035>
- Quan RD, Shang M, Zhang H, Zhao YX, Zhang JR (2004) Engineering of enhanced glycine betaine synthesis improves drought tolerance in maize. *Plant Biotechnol J* 2:477–486. <https://doi.org/10.1111/j.1467-7652.2004.00093.x>
- Rejeb IB, Pastor V, Mauch-Mani B (2014) Plant responses to simultaneous biotic and abiotic stress: molecular mechanisms. *Plants-Basel* 3:458–475
- Rolli E, Righetti L, Galaverna G, Suman M, Dall’Asta C, Bruni R, (2018) Zearalenone uptake and biotransformation in micropropagated *Triticum durum* Desf. plants: a xenobiotic approach. *J Agric Food Chem* 66:1523–1532. <https://doi.org/10.1021/acs.jafc.7b04717>
- Srivastava AK, Pasala R, Minhas PS, Suprasanna P (2016) Plant bioregulators for sustainable agriculture: integrating redox signaling as a possible unifying mechanism. *Adv Agron* 137:237–278. <https://doi.org/10.1016/bs.agron.2015.12.002>
- Stob M, Baldwin RS, Gillette KG, Andrews FN, Tuite J (1962) Isolation of an anabolic, uterotrophic compound from corn infected with *Gibberella zeae*. *Nature* 196:1318. <https://doi.org/10.1038/1961318a0>
- Strasser RJ, Srivastava A, Tsimilli-Michael M (2000) The fluorescence transient as tool to characterize and screen photosynthetic samples. In: Yunus M, Pathre U, Mohanty P (eds) Probing photosynthesis: mechanism, regulation and adaptation. Taylor and Francis, Bristol, pp 45–483
- Szechynska-Hebda M, Skrzypek E, Dabrowska G, Wedzony M, van Lammeren A (2012) The effect of endogenous hydrogen peroxide induced by cold treatment in the improvement of tissue regeneration efficiency. *Acta Physiol Plant* 34:547–560. <https://doi.org/10.1007/s11738-011-0852-3>
- Ting SV, Rouseff RL (1979) Proline content in Florida frozen concentrated orange juice and canned grapefruit juice. *Proc Fl State Hortic* 92:143–145
- Vaughan JG, Geissler C (2009) The new Oxford Book of Food Plants: a guide to the fruit, vegetables, herbs and spices of the world. In: Vaughan JG, Geissler C (ed) New York: Oxford University Press
- Videmann B, Mazallon M, Tep J, Lecoeur S (2008) Metabolism and transfer of the mycotoxin zearalenone in human intestinal Caco-2 cells. *Food Chem Toxicol* 46:3279–3286. <https://doi.org/10.1016/j.fct.2008.07.011>
- Wang CR, Yang AF, Yin HY, Zhang JR (2008) Influence of water stress on endogenous hormone contents and cell damage of maize seedlings. *J Integr Plant Biol* 50:427–434. <https://doi.org/10.1111/j.1774-7909.2008.00638.x>
- Wani SH, Kumar V, Shriram V, Sah SK (2016) Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. *Crop J* 4(3):162–176. <https://doi.org/10.1016/j.cj.2016.01.010>
- Wojtania A, Skrzypek E, Gabryszewska E (2016) Morphological and biochemical responses to gibberellic acid in magnolia x ‘spectrum’ in vitro. *Acta Biol Cracov Bot* 58:103–111. <https://doi.org/10.1515/abcsb-2016-0010>
- Zinedine A, Soriano JM, Juan C, Mojemmi B, Molto JC, Bouklouze A, Cherrah Y, Idrissi L, Aouad R, Manes J (2007) Incidence of ochratoxin A in rice and dried fruits from Rabat and Sale area, Morocco. *Food Addit Contam A* 24:285–291. <https://doi.org/10.1080/02652030600967230>
- Zobayed SMA, Afreen F, Kozai T (2005) Temperature stress can alter the photosynthetic efficiency and secondary metabolite concentrations in St John’s wort. *Plant Physiol Bioch* 43:977–984. <https://doi.org/10.1016/j.plaphy.2005.07.013>

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