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Effects of zearalenone and 24-*epi*brassinolide on the salt tolerance of selected monocotyledonous crop plants

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Summary

Salinity has an increasing impact on crop production worldwide. Contemporary agricultural practices increasingly use plant biostimulants that protect plants against various environmental stresses. The aim of the work was to investigate whether such stimulants as 24-*epi*brassinolide (EPI) and zearalenone (ZEN) may alleviate effects of salinity in bread and durum wheat, maize, and sorghum plants. Plants were grown in glasshouse, in pots filled with perlite under continuous salinity stress (120 mM of NaCl). Four-week-old plants were treated with the stimulants. The plant responses to salinity were determined analyzing the following parameters: fresh and dry weights of plants, water content, electrolyte leakage, proline, abscisic acid, and the soluble carbohydrate contents in the leaves. The positive effect of ZEN on the studied parameters was more frequently observed than in the case of EPI. ZEN increased the root mass of both wheat species, as well as the stem and root masses of sorghum. This stimulant improved water relations in bread and durum wheat. Both stimulators increased the content of soluble carbohydrates. ZEN elevated significantly the abscisic acid content in sorghum plants as well as it increased strongly the proline level in all studied plant species. ZEN was more effective in alleviation salinity disorders than EPI.

Keywords: abscisic acid; cereals; 24-*epi*brassinolide; proline; salinity; soluble carbohydrates; zearalenone

Introduction

More and more agricultural areas worldwide are experiencing soil salinity problems. In Poland, located in Central Europe, long-lasting drought periods and the progressive reduction in the groundwater level have been observed. Many crops require irrigation, which results in an increase in soil salinity (Walter et al., 2011). According to the Central Statistical Office in Poland, an unprecedented drought which caused huge crop losses, was recorded in 2015. Thus, soil salinity will most likely affect regions that have not previously experienced problems with this environmental stress. In addition to natural conditions, salinity is also evoked by improper irrigation and fertilization. In most cases high soil salinity is the result of salt accumulation over long cultivation periods and deforestation (Brini et al., 2009).

Salinity causes osmotic stress and ion toxicity stress due to the presence of sodium (Na^+) and chloride (Cl^-) ions (Munns and Tester, 2008). A high Na^+ concentration mainly disturbs the osmotic balance and results in an inhibition of water uptake by the plant. The toxic influence of Na^+ may be manifested by the premature death of leaves, disturbances in the cell membrane's structure or functions, and the inhibition of enzymes taking part in many metabolic processes mainly in photosynthesis (Mitsuya et al., 2003). The plant tolerance responses to salt stress involves: the exclusion of salt ions, the controlled uptake of ions by roots and transport into leaves, ion compartmentation, the synthesis of specific osmolytes, and changes in membrane structure, as well as the activation of antioxidant defense system (Munns and Tester, 2008).

The most important criterion indicating salt tolerance is biomass production (Munns and James, 2003). Salt stress causes stomatal closure which limits CO_2 assimilation. Lower rates of photosynthesis disturb the energy balance between light and dark phases, which stimulates the generation of reactive oxygen species. Furthermore, oxygen radicals disturb the photochemical processes in thylakoids and can evoke strong photoinhibition. Thereby, damage to the membrane structure as a result of salinity causes strong membrane depolarization and membrane lipid peroxidation (Yasar et al., 2006).

The sequestration of Na^+ and Cl^- in vacuoles affects cell osmotic pressure, which should be balanced with the accumulation of specific compounds, such as proline and glycine betaine (Flowers et al., 1977; ElSayed et al., 2014). Osmotic potential is regulated mainly by soluble mono- and oligosaccharides. Raffinose and other raffinose family oligosaccharide (RFO) members are recognized as signaling compounds in stresses, such as drought, salinity, and chilling (ElSayed et al., 2014). Disaccharide trehalose, in addition to regulating osmotic pressure under various stresses may serve as a protectant of enzymes and membranes, may elevate salinity stress by decreasing the rate of ion leakage and lipid peroxidation as well as increasing the potassium K^+ / Na^+ ratio (Zeid, 2009). Plant responses to osmotic stress involving stomatal closure and the regulation of cell water potential may be controlled by abscisic acid (ABA), which is often called the osmotic stress hormone. ABA-dependent signaling under salt and drought stresses is well documented (Zhu, 2002; Zhang et al., 2006; Shinozaki and Yamaguchi-Shinozaki, 2007).

Contemporary agricultural practices increasingly use plant biostimulants that enhance the heights and qualities of yields as well as protect plants against various environmental stresses (Ranawake et al., 2013). To minimize the stress effects exogenous plant hormones are often applied (Biesaga-Kościelniak et al., 2003; Janeczko et al., 2003; Kościelniak et al., 2011). The most successful this mitigation are brassinosteroids (BRs), plant steroidal hormones that play roles in a broad spectrum of developmental and physiological processes (Yuan et al., 2010). Many studies have demonstrated BRs ability to enhance plant tolerance to salinity, heavy metal stress, high and low temperature stress, and pathogen attacks (Houimli et al., 2010; Janeczko et al., 2010; Yuan et al., 2010; Kościelniak et al., 2011). Talaat and Shawky (2013) showed the alleviating effects of 24-*epi*brassinolide (EPI) (Fig. 1), which occurred by enhancing the antioxidant system, in wheat plants under salt stress.

Another plant regulator, zearalenone (ZEN), also shows positive effects in plant protection against unfavorable environmental factors. ZEN was isolated for the first time from a fungal *Gibberella zeae* culture by Stob et al. (1962). ZEN [2,4-dihydroxy-6-(10-hydroxy-6-oxo trans-1-undecenyl)-benzoic acid lactone] (Fig. 2) is an F2-toxin produced by fungi belonging to genus *Fusarium*. ZEN possesses estrogenic activity by competing with animal hormones to bind to estrogen receptors. Plants infected by Fusarium fungi producing ZEN demonstrate chromosomal damage and disturbances in chlorophyll

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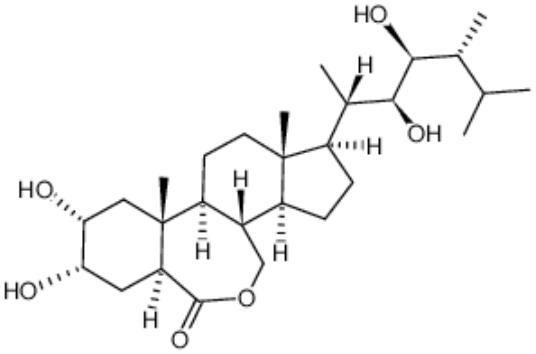

Fig. 1: The structure of 24-*epi*brassinolide (https://www.chemsrc.com/en/cas/78821-42-8_1009735.html)

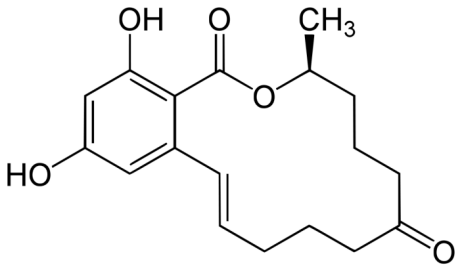

Fig. 2: The structure of zearalenone (Wikipedia public domain)

synthesis and photosynthesis processes (KUMAR and SINHA, 1995; KOŚCIELNIAK et al., 2011). However, ZEN is an endogenous regulator that at hormone concentrations controls plant development. ZEN has a positive role in vernalization processes (FILEK et al., 2010) and the stimulation of ear, pod and seed numbers in wheat and soybean (BIESAGA-KOŚCIELNIAK et al., 2006).

This study investigated whether EPI or ZEN can mitigate the effects of salinity in monocotyledonous crop plants, such as bread wheat (*Triticum aestivum* L.), durum wheat (*Triticum durum* Desf.), maize (*Zea mays* L.), and sorghum (*Sorghum bicolor* L. Moench). Mentioned species belong to the most important crops in all of the world. Plants were grown under continuously salinity stress (120 mM of NaCl), while seeds were sown directly into saline perlite. The plant's responses to salinity stress were determined on the basis of following parameters: fresh and dry weights (FW and DW) of stems and roots, tissue hydration, cell membrane permeability, proline and ABA contents, as well as the quality and quantity of soluble sugars in the leaves.

Materials and methods

Plant material and experimental design

The experiment was conducted under glasshouse conditions (20-24 °C day; 18 °C night) from the beginning of June until the end of August at day light (50°04′10″ N, 19°50′40″ E). The study was performed on *T. aestivum* cv. Banderola, *T. durum* cv. Komnata, *Z. mays* cv. Król, and *S. bicolor* cv. Cukrosorgo. The experiment was a completely randomized design with five replicates for each treatment. Seeds were sown in 22 cm × 22 cm × 22 cm (V = 10 dcm^3) pots filled with perlite. In the experiment perlite was used instead a soil to maintain controlled salinity level. For both wheat species there were 12 plants per pot, and for maize and sorghum there were 4 plants per pot. All of the plants were treated daily with 120 mM NaCl solution supplemented with Hoagland medium (HOAGLAND et al., 1950). The molar concentration of Hoagland's medium was low in comparison with that of the NaCl solution; therefore, we also analyzed the influence of only NaCl. The salinity level used in the experiment was determined in an earlier our study. Non-treated with salt solution plants were the control, while plants growing in saline perlite untreated with EPI or ZEN were used as the saline control. Four week-old plants were sprayed with 40 cm^3 per pot with the solution of the plant regulator EPI (2 mg dm^{-3}) or ZEN (2 mg dm^{-3}). Two mg of 24-*epi*-brassinolide (OlChemim, Olomouc, Czech Republic) or zearalenone (Fermentek, Jerusalem, Israel) were dissolved in 1 cm^3 of 50% ethanol and next filled up with distilled water to 1 dm^3. Control plants were sprayed with distilled water containing 0.05% ethanol.

The applied doses of both regulators were based on previous experiments (BIESAGA-KOŚCIELNIAK et al., 2003; JANECZKO et al., 2003). Four weeks after the stimulator application, plant samples were collected for analyses.

Measurement of FW, DW, and relative water content (RWC)

The FW of aboveground plant parts (shoots) and roots were determined. Next, their DWs were measured after drying for 48 h at 70 °C. Finally, the ratio of the FW of shoots (S) to the whole plant weight (WP) was calculated according to the formula: S × 100 / WP. The RWC of shoots and roots was calculated as (FW-DW) / DW and expressed as g H_2O g^{-1} DW. Analyses were performed in 15 replicates (15 plants) for each plant species and treatment.

Electrolyte leakage

Ion leakage, which indicates the plasma membrane's integrity level, was measured in the 3rd upper, well-expanded leaves. Leaf discs (Ø = 1 cm) were washed in distilled water, put into plastic vials containing 13 cm^3 deionized water and shaken for 24 h (50 rpm) at 20 °C. Next, ion conductivity was measured (EL_1) using a conductometer (CI 317, Elmetron, Poland). Then, the samples were frozen at -80 °C for 24 h. After thawing, they were shaken again for 24 h, and the total ion leakage (EL_2) was measured. Membrane permeability was expressed as a percentage of EL_2, (EL_1 × 100 / EL_2). The measurements were performed in 10 replicates for each plant species and treatment.

Proline content

The free proline content in leaves was determined as described by BATES et al. (1964). Samples were homogenized in 3% (w/v) sulfosalicylic acid to precipitate proteins, and centrifuged at 14,000 × *g* for 10 min. The reaction mixture contained 2 cm^3 glacial acetic acid, 2 cm^3 ninhydrin reagent (2.50% w/v ninhydrin in 60% v/v 6 M phosphoric acid) and 2 cm^3 of supernatant. The incubation lasted for 1 h at 90 °C. After stopping the reaction with ice, 4 cm^3 of toluene was added and mixed by vortexing. The upper toluene phase was decanted into a glass cuvette, and the absorbance was measured using an Ultrospec 2100 pro spectrophotometer (Biosciences Amersham, Sweden) at λ = 520 nm. The concentration was assayed using proline as the calibration standard. Each assay was performed in five replicates, with five leaves from different plants for each treatment. The content of proline was expressed as μg g^{-1} DW.

ABA content

From five plants of each species and treatment the 3rd upper, well-expanded leaves were collected. Lyophilized samples were pulverized then extracted with 1 cm^3 of Bieleski buffer (BIELESKI, 1964). Extracts were evaporated, re-dissolved in 1 M formic acid, cleaned-up on Oasis MCX SPE cardridges (Waters, Ireland) and used for high-performance liquid chromatography (HPLC) analyses according

to Żur et al. (2015). A chromatograph coupled to a triple quadruple mass spectrometer (6410 Triple Quad LC/MS, Agilent, USA) controlled by MassHunter software was used. Quantization was based on calibration curves obtained for the pure standards of ABA, taking into account the recovery of the internal standard ([2H6]ABA). Phytohormone standards were obtained from Olchemim (Olomouc, Czech Republic), while other chemicals were from Sigma-Aldrich.

Soluble carbohydrate content

Sugars were analyzed according to the method reported by Janeczko et al. (2013). Approximately 10 mg of freeze-dried and pulverized samples were extracted in 1 cm^3 of ultrapure water by shaking for 15 min at 30 Hz (MM 400, Retsch, Germany). Then, samples were centrifuged for 5 min at 21,000 × *g* (Universal 32R, Hettich, Germany). Supernatants were collected, diluted with acetonitrile 1:1 (v/v), and filtered (0.22 µm nylon membrane, Costar Spin-X, Corning, USA). Samples were analyzed by HPLC (Agilent 1200). An ESA Coulochem II electrochemical detector with a 5040 Analytical Cell (ESA, USA) coupled to an analog-to-digital converter (Agilent, China) was used. The separation of soluble sugars (glucose, fructose, sucrose, maltose, trehalose, and raffinose) was achieved on a RCX-10 (7 µm; 250 × 4.1 mm) column (Hamilton, USA) at a flow rate of 1.5 $cm^3\ min^{-1}$ in gradient mode. The injection volume was 0.01 cm^3, and the column temperature was 40 °C. Pulse amperometric detection was employed (analytical potential of 200 mV; oxidizing potential of 700 mV, and reducing potential of -900 mV, with reference to a palladium electrode) on a gold electrode.

Statistical analyses

All of the data were analyzed with Statistica 10.0 software (Statsoft, OK, USA) using the MANOVA method to evaluate statistical differences between treatments. The percentage data were transformed according to the formula $arcsin\ \sqrt{x}$. All data were presented as means ± standard errors (SEs). The differences between means were additionally analyzed according to Duncan's multiple range test at $p < 0.05$.

Results

Visual symptoms of salinity effect, FW, DW and water relations

Among the studied plant species, the most visible symptoms of the applied salinity level (120 mM NaCl) were observed on maize plants, which demonstrated slight leaf drying and a reddening of the stems. Leaf blades of both bread and durum wheat dried at the ends, while sorghum plants underwent the drying of older leaf blades. All of the salt treated plants showed strong growth inhibition. The most sensitive to salinity were plants of maize and sorghum demonstrating a decrease in the fresh weights of shoots by 75% and 85%, respectively, while bread and durum wheat were more tolerant showing 47% decline of shoot FW (Tab. 1). The applied salinity affected the considerable decrease in shoot DW of all studied plant species, but maize and sorghum demonstrated greater reduction (67%) of this parameter than bread and durum wheat (~30%). Fresh weights of roots of bread and durum wheat, as well as of maize and sorghum decreased by 35%, 39%, 38% and 56%, respectively, while dry weights by 61%, 58%, 29% and 28%, respectively. These data demonstrate that in the case of maize and sorghum the salinity affected more the shoots than

Tab. 1: Influence of 24-*epi*brasinolid (EPI) and zearalenone (ZEN) on fresh (FW) and dry weight (DW) of shoots (S) and roots (R), as well as percentage participation of shoots' FW to whole plant (WP) fresh weight of *Triticum aestivum*, *T. durum*, *Zea mays* and *Sorghum bicolor* plants grown at 120 mM of NaCl. Means (n=15) ± SE within a column for each plant species marked with the same lowercase do not differ significantly (multiple range Duncan's test; $p < 0.05$).

Species/regulator	FW of S [g]	DW of S [g]	FW of R [g]	DW of R [g]	S / WP [%]
Triticum. aestivum cv. Banderola					
Control	42.08±4.05^a	5.08±0.60^a	5.02±0.45^b	2.82±0.25^a	89.3±5.1^a
Saline control	22.30±3.35^b	3.66±0.29^b	3.26±0.29^c	1.10±0.91^b	87.2±5.0^a
EPI	13.37±2.05^c	2.76±0.33^b	3.04±0.23^c	1.01±0.82^b	81.5±5.1^a
ZEN	22.02±3.30^b	4.33±0.45^a	10.29±0.93^a	2.83±0.24^a	68.1±3.8^b
Triticum. durum cv. Komnata					
Control	30.64±3.95^a	4.57±0.55^a	7.90±0.95^a	4.21±0.37^a	79.5±6.8^a
Saline control	16.24±1.95^b	3.16±0.38^b	4.82±0.58^b	1.77±0.21^c	77.1±6.9^a
EPI	10.62±1.17^c	2.76±0.33^b	5.53±0.66^b	1.82±0.20^c	80.7±8.1^a
ZEN	11.12±1.45^c	4.33±0.52^a	7.12±0.85^a	2.39±0.28^b	57.4±5.2^b
Zea mays cv. Król					
Control	423.10±21.15^a	43.57±3.49^a	29.32±2.35^a	4.45±0.36^a	93.5±7.5^a
Saline control	105.75±2.29^b	14.38±1.15^b	18.18±1.45^c	3.16±0.25^b	85.3±6.8^b
EPI	106.85±5.34^b	14.47±1.16^b	22.26±1.78^b	3.66±0.31^b	82.8±6.6^b
ZEN	90.73±4.53^c	12.92±1.03^b	19.81±1.58^b	3.19±0.26^b	82.1±6.6^b
Sorghum bicolor cv. Cukrosorgo					
Control	227.87±20.51^a	13.45±1.21^a	21.29±1.56^a	2.44±0.22^a	91.5±8.3^a
Saline control	34.18±3.08^c	4.44±0.39^c	9.37±1.04^b	1.76±0.16^b	77.9±7.0^b
EPI	28.68±2.58^c	4.03±0.36^c	7.67±0.99^c	1.89±0.17^b	78.9±7.0^b
ZEN	46.56±4.19^b	7.46±0.99^b	10.59±2.51^b	2.51±0.23^a	81.5±7.3^b

Control – plants untreated with NaCl and stimulants; Saline control – plants grown at 120 mM NaCl

roots. Salinity decreased S/WP ratios also only in the case of maize and sorghum. On the basis of these data maize and sorghum could be recognized as more salt sensitive than both studied wheat species. Neither EPI nor ZEN relieved the visual symptoms of salt stress in the wheat species, however in the case of bread wheat ZEN increased DW of shoots and roots to the level of salt untreated plants (control) and enhanced two-fold FW of roots compared with these control plants. Similar effect of ZEN was observed in DW of shoot and FW of roots of durum wheat. Thus S/WP ratios of both wheat species under ZEN impact was significantly lower when compared with that of the both control and the EPI-treated plants. The EPI decreased the FW of bread and durum wheat's shoots compared with the saline control plants, but the S/WP ratios of plants under EPI influence did not differ considerably from that of the saline control.

ZEN and EPI alleviated the visual symptoms of salinity mainly in sorghum and maize plants, respectively, but only in the case of sorghum ZEN increased the FW and DW of shoots as well as DW of roots compared with the saline control. However, this effect did not influence the S/WP ratio. Maize plants responded to the ZEN treatment by decreasing the FW and DW of the shoots, however, the S/WP ratio was similar for saline control and EPI treated plants.

The studied plant species differed considerably in natural (in control plants) tissue hydration (Tab. 2). Maize and sorghum plants of C_4 photosynthesis type demonstrated ca. 7-fold and two-fold, respectively, higher RWC values of shoots than bread and durum wheat. Salt stress decreased RWC values of shoots and roots of both wheat species by 30% and 50%, respectively. In maize plants RWC of shoots decreased by 27%, while RWC of roots did not change, similarly as in the case of the sorghum plants. The most visible reduction of this parameter at 120 mM of NaCl was observed in sorghum shoots (60%). The impact of applied stimulants on RWC changes in shoots and roots was specific for each plant species. ZEN decreased the RWC of shoots in all studied plant species, while EPI in bread wheat and sorghum. This stimulant increased RWC value of shoots in maize plants to that of the control plants which were not grown under salt stress. ZEN increased the RWC values in the roots of bread and durum wheat, while in sorghum it declined the roots' RWC, compared to the saline control. Generally, EPI did not change root RWC of studied plants compared with that of the saline control, with exception of the sorghum roots, where it decreased the values of this parameter.

Tab. 2: Influence of 24-*epi*brassinolide (EPI) and zearalenone (ZEN) on relative water content (RWC) in shoots and roots of *Triticum aestivum*, *T. durum*, *Zea mays* and *Sorghum bicolor* plants grown at 120 mM of NaCl. Means (n=15) ± SE within a column for each plant species marked with the same lowercase do not differ significantly (multiple range Duncan's test; $p < 0.05$).

Species/regulator	RWC of shoots [g H_2O g^{-1} DW]	RWC of roots [g H_2O g^{-1} DW]
Triticum. aestivum cv. Banderola		
Control	7.39±0.88^{a}	3.94±0.47^{a}
Saline control	5.10±0.57^{b}	1.97±0.24^{c}
EPI	3.84±0.42^{c}	2.03±0.25^{c}
ZEN	4.08±0.44^{c}	2.64±0.32^{b}
Triticum. durum cv. Komnata		
Control	5.58±0.67^{a}	3.51±0.39^{a}
Saline control	3.96±0.41^{bc}	1.72±0.18^{c}
EPI	4.05±0.42^{b}	1.59±0.14^{c}
ZEN	3.39±0.29^{c}	2.54±0.21^{b}
Zea mays cv. Król		
Control	38.25±3.42^{a}	4.95±0.54^{a}
Saline control	27.92±3.35^{b}	4.75±0.52^{a}
EPI	37.74±4.51^{a}	5.08±0.48^{a}
ZEN	19.91±2.38^{c}	5.21±0.62^{a}
Sorghum bicolor cv. Cukrosorgo		
Control	16.78±2.01^{a}	4.10±0.38^{a}
Saline control	6.71±0.80^{b}	4.51±0.41^{a}
EPI	5.51±0.66^{c}	3.06±0.27^{b}
ZEN	5.24±0.65^{c}	3.21±0.28^{b}

Control – plants untreated with NaCl and stimulants; Saline control – plants grown at 120 mM NaCl

Electrolyte leakage (EL)

Maize plants demonstrated the highest EL from leaf cells under 120 mM NaCl; it amounted to 236% comparing to the control plants (Tab. 3). It was three times greater than that noted in bread wheat plants and more than two times greater than those in durum wheat and sorghum. EPI significantly increased the EL in bread wheat leaves, while in maize it caused a 27% reduction of the EL, in relation to that of the saline control. In other plants this BR did not considerably change the membrane permeability in comparison with that of the saline control. ZEN increased the EL in the leaves of durum wheat, while the ion outflow in maize leaves was decreased under ZEN treatment, however it was still higher than in the salt-untreated plants.

Proline content

Salt stress decreased proline content in the leaves of bread and durum wheat by 33%, while in sorghum and maize leaves proline amount increased comparing to the plants grown under non-salt stress conditions (Tab. 4). Sorghum plants grown at 120 mM of NaCl showed higher proline contents in the leaves than the other studied plant spe-

Tab. 3: The influence of 24-*epi*brassinolide (EPI) and zearalenone (ZEN) on percentage electrolyte leakage (EL) [%] from the leaf cells of *Triticum aestivum*, *T. durum*, *Zea mays* and *Sorghum bicolor* plants grown in perlite at 120 mM NaCl. Means (n=10) ± SE in the rows for each species marked with the same lowercase do not differ significantly (multiple range Duncan's test; $p < 0.05$).

Species/cultivar	Control	Saline control	EPI	ZEN
Triticum. aestivum cv. Banderola	3.01±0.29^{c}	5.33±0.48^{b}	7.15±0.64^{a}	5.91±0.42^{b}
Triticum. durum cv. Komnata	3.63±0.32^{c}	7.02±0.63^{b}	6.63±0.53^{b}	10.31±0.91^{a}
Zea mays cv. Król	6.84±0.75^{d}	16.15±1.78^{a}	12.69±1.39^{b}	9.16±0.83^{c}
Sorghum bicolor cv. Cukrosorgo	4.53±0.36^{b}	7.97±0.72^{a}	8.35±0.92^{a}	9.76±1.07^{a}

Control – plants untreated with NaCl and stimulants; Saline control – plants grown at 120 mM NaCl

cies. Treatment with ZEN increased the amount of proline in all of the studied plants, with the exception of sorghum. The largest effect of ZEN was observed in the case of durum wheat, in which this regulator increased the proline content 9-fold in relation to the saline control plants and 6-fold compared to the salt-untreated plants. In the case of bread wheat and maize, the proline amount in the leaves was 6 times and 1.7 times higher, respectively, after the ZEN treatment than in the saline control plants. In the wheat species, the EPI effect was much lower than that of ZEN; however, EPI increased the proline content in the leaves of both wheat species to the level noted in the control plants, non-treated with the salt. In maize leaves the EPI treatment did not change the proline level in comparison with that in the both control plants, while in the sorghum leaves, under this BR influence the proline content was the lowest.

ABA content

Among the control plants, the highest ABA level was noted in bread wheat leaves (Tab. 5). Salinity enhanced considerably content of this hormone in the leaves of all studied plant species. The ZEN increased evidently the ABA content in the leaves of durum wheat and sorghum by 1.9-fold and 2.3-fold, respectively, compared to that of the saline control. ZEN and EPI reduced the ABA content in the leaves of bread wheat, while EPI decreased its amount in the maize leaves to that of the non-salt treated plants.

Soluble carbohydrate contents

Salinity affected the total soluble carbohydrate content in the leaves of all studied plant species (Tab. 6). It increased by 26% in bread wheat, 14% in durum wheat, 24% in maize, whereas by 26% in sorghum. Under salt impact the increase of glucose, fructose and maltose amount was noted in all studied plants, while sucrose amount enhanced only in maize leaves. Raffinose level did not change under salinity impact, whereas salinity increased trehalose amount only in the sorghum leaves.

In most cases, EPI and ZEN significantly influenced particular sugar contents in the leaves of the studied species. Generally, each plant species responded specifically to the regulator applications. In the case of bread wheat, EPI and ZEN increased all type of sugars without raffinose and trehalose in comparison with the saline control. In durum leaves, the glucose and fructose levels were greater after EPI and ZEN treatments than that of the saline control. Additionally, in this plant species ZEN increased the sucrose level 5-fold. The both regulators decreased the maltose content compared with the saline control level, while EPI also decreased the sucrose content. In maize plants, EPI considerably increased the fructose, sucrose, and trehalose amounts, while ZEN elevated only trehalose content. In sorghum leaves, the ZEN treatment increased the sucrose and maltose contents, which were 3.6 and 5 times, respectively, higher than that of the saline control. Raffinose was detected only in maize plants under the ZEN influence. Both regulators' effects on particular sugars influenced the total sugar amount. EPI treatment significantly enhanced the total soluble sugar content in the leaves of bread wheat and maize compared to the both control plants. Similar effect of the ZEN treatment was observed in bread and durum wheat leaves, as well as those of sorghum in which total sum of soluble sugars increased 2.4-fold compared to the control (untreated with salt) and 1.8-fold compared to the saline control plants.

Discussion

In the most sensitive plant species, high salinity causes premature leaf senescence, necrosis or the reddening of stems, which could be the result of a phosphorus metabolism disturbance (Sonneveld and Kreij, 1999). In the present investigation, we observed similar symptoms on the studied plants. As demonstrated in our previous study, a salinity level greater than 100 mM NaCl causes significant reductions in the FW and DW of bread and durum wheat shoots (Płażek et al., 2013). Reductions in the FW and DW of shoots and roots of barley under rising salinity levels were observed also by El-Tayeb (2005). Treatments with EPI or ZEN did not alleviate the visual symptoms of

Tab. 4: The influence of 24-*epi*brassinolide (EPI) and zearalenone (ZEN) on free proline content [μg g^{-1} DW] in the leaves of *Triticum aestivum*, *T. durum*, *Zea mays* and *Sorghum bicolor* plants grown in perlite at 120 mM NaCl. Means (n=5) ± SE in the rows for each species marked with the same lowercase do not differ significantly (multiple range Duncan's test; $p < 0.05$).

Species/cultivar	Control	Saline control	EPI	ZEN
Triticum aestivum cv. Banderola	3.9±0.35^{b}	2.6±0.25^{c}	4.1±0.38^{b}	15.1±1.42^{a}
Triticum durum cv. Komnata	3.1±0.28^{b}	2.1±0.17^{c}	3.3±0.29^{b}	19.8±1.91^{a}
Zea mays cv. Król	2.3±0.15^{c}	2.7±0.18^{b}	2.4±0.21^{c}	4.7±0.38^{a}
Sorghum bicolor cv. Cukrosorgo	3.1±0.28^{b}	3.4±0.31^{ab}	2.5±0.18^{c}	3.9±0.32^{a}

Control – plants untreated with NaCl and stimulants; Saline control – plants grown at 120 mM NaCl

Tab. 5: The influence of 24-*epi*brassinolide (EPI) and zearalenone (ZEN) on abscisic acid (ABA) content [ng mg^{-1} DW] in the leaves of *Triticum aestivum*, *T. durum*, *Zea mays* and *Sorghum bicolor* plants grown in perlite at 120 mM NaCl. Means (n=5) ± SE in the rows for each plant species marked with the same lowercase do not differ significantly (multiple range Duncan's test; $p < 0.05$).

Species/cultivar	Control	Saline control	EPI	ZEN
Triticum. aestivum cv. Banderola	0.26±0.03^{c}	0.37±0.05^{a}	0.33±0.03^{b}	0.31±0.02^{b}
Triticum. durum cv. Komnata	0.18±0.01^{d}	0.24±0.03^{c}	0.36±0.04^{b}	0.45±0.05^{a}
Zea mays cv. Król	0.23±0.02^{c}	0.34±0.04^{a}	0.26±0.03^{b}	0.32±0.03^{a}
Sorghum bicolor cv. Cukrosorgo	0.15±0.01^{c}	0.19±0.03^{b}	0.22±0.02^{b}	0.44±0.04^{a}

Control – plants untreated with NaCl and stimulants; Saline control – plants grown at 120 mM NaCl

Tab. 6: The influence of 24-*epi*brassinolide (EPI) and zearalenone (ZEN) on soluble carbohydrates' content [mg g^{-1} DW] in the leaves of *Triticum aestivum*, *T. durum*, *Zea mays* and *Sorghum bicolor* plants grown in perlite at 120 mM NaCl. Means (n=5) ± SE within a column for each species marked with the same lowercase do not differ significantly (multiple range Duncan's test; $p < 0.05$); LOD – limit of detection.

Species/cultivar	Glucose	Fructose	Sucrose	Maltose	Raffinose	Trehalose	Total sum of soluble sugars
Triticum aestivum cv. Banderola							
Control	9.43±0.81^{c}	5.25±0.60^{d}	1.12±0.09^{c}	0.43±0.05^{d}	< LOD	0.04±0.01^{a}	16.27±1.11^{c}
Saline control	12.67±1.10^{b}	7.39±0.62^{c}	1.08±0.09^{c}	0.57±0.06^{c}	< LOD	0.05±0.01^{a}	21.96±1.76^{b}
EPI	16.60±1.38^{a}	11.60±0,99^{a}	2.81±0.22^{b}	1.76±0.15^{b}	< LOD	0.04±0.01^{a}	32.81±2.62^{a}
ZEN	11.94±1.06^{b}	8.95±0.80^{b}	7.25±0.63^{a}	3.60±0.31^{a}	< LOD	0.05±0.01^{a}	31.79±2.86^{a}
Triticum durum cv. Komnata							
Control	9.52±0.73^{c}	6.61±0.51^{c}	2.35±0.21^{b}	0.79±0.07^{b}	< LOD	0.05±0.01^{a}	19.32±1.35^{c}
Saline control	10.87±0.77^{b}	8.21±0.77^{b}	2.32±0.21^{b}	0.98±0.09^{a}	< LOD	0.05±0.01^{a}	22.43±1.72^{b}
EPI	12.74±1.11^{a}	9.99±0.83^{a}	1.02±0.09^{c}	0.68±0.07^{b}	< LOD	0.04±0.01^{a}	24.43±1.72^{b}
ZEN	12.71±1.09^{a}	9.48±0.80^{a}	11.94±0.95^{a}	0.53±0.06^{c}	< LOD	0.04±0.01^{a}	34.70±2.15^{a}
Zea mays cv. Król							
Control	4.26 ±0.39^{b}	1.65±0.14^{c}	16.55±1.54^{b}	0.17±0.02^{b}	< LOD	0.01±0.0^{b}	22.64±2.08^{c}
Saline control	6.61±0.58^{a}	3.01±0.25^{b}	19.63±1.53^{b}	0.28±0.03^{a}	< LOD	0.01±0.0^{b}	29.54±2.09^{b}
EPI	7.50±0.69^{a}	5.74±0.49^{a}	23.41±1.75^{a}	0.29±0.03^{a}	< LOD	0.04±0.01^{a}	36.98±3.04^{a}
ZEN	3.93±0.30^{b}	1.55±0.09^{c}	21.97±1.69^{ab}	0.12±0.01^{c}	0.09	0.03±0.0^{a}	27.69±2.26^{c}
Sorghum bicolor cv. Cukrosorgo							
Control	7.14±0.60^{c}	2.64±0.25^{c}	7.86±0.61^{b}	0.29±0.03^{d}	< LOD	0.02±0.0^{c}	17.95±1.61^{c}
Saline control	11.49±1.00^{a}	5.28±0.47^{a}	6.75±0.55^{c}	0.41±0.04^{c}	< LOD	0.06±0.01^{a}	23.99±1.95^{b}
EPI	9.17±0,88^{b}	4.07±0.39^{b}	8.99±0.72^{b}	1.35±0.12^{b}	< LOD	0.02±0.0^{c}	23.60±1.94^{b}
ZEN	10.99±1.08^{a}	5.99±0.54^{a}	24.47±2.01^{a}	2.12±0.19^{a}	< LOD	0.03±0.0^{b}	43.60±2.87^{a}

Control – plants untreated with NaCl and stimulants; Saline control – plants grown at 120 mM NaCl

salinity on bread and durum wheat plants, but sorghum plants treated with ZEN did not show visible symptoms of salt stress, while EPI evoked the same effect in maize plants. In many cases, the stimulators used did not restore the value of the studied parameters under salinity stress to that determined in the control plants not treated with salt. However, in many times we have observed positive effects of ZEN and EPI on investigated physiological and biochemical indicators. ZEN increased the root masses of both wheat species and maize. Additionally, in sorghum, it considerably improved the FW and DW values of the stems.

In our experiment, all studied plants at 120 mM NaCl demonstrated lower hydration level in the shoots and roots compared to the control plants untreated with salt, which could be the effect of a very low water potential in soil caused by the high ion concentration. A sign of salt tolerance is the stable maintenance of the RWC. However, toxic effect of NaCl, which appears at high salt concentrations, results in a high water influx through damaged membranes and could increase RWC values (Flowers et al., 1977; Munns and Tester, 2008). In the investigation, applied stimulants resulted in unique responses of each plant species. ZEN decreased the RWC in the cells of leaves of both bread and durum wheat, but in roots increased the RWC, compared to the saline control plants. This result indicate that positive effect of this regulator may be to reduce the transport of Na^{+} ions between roots and shoots. A similar effect was observed by El-Tayeb (2005) in barley seedlings treated with salicylic acid. The mechanism of plant tolerance to salinity is related to salt ion excretion, control of root ions' uptake and transport to leaves, ion compartmentalization as well as osmolyte synthesis (Munns and Tester, 2008). In contrast to ZEN, EPI increased the RWC in the shoots of maize plants. In general, this exogenously applied hormone was less active than ZEN. This effect may seem strange when compared with the results obtained by other researchers. Yuan et al. (2010) demonstrated that EPI increased the RWC, stomatal conductance, net photosynthesis rate, antioxidant enzyme activities and ABA concentration of tomato plants under drought stress.

EL from the plant cells is a physiological indicator of cell membrane damage from salt stress (Ashraf and Harris, 2004). Kathar and Kuhad (2000) observed an increase in the EL from bread wheat leaf cells under the influence of applied NaCl at 0-200 mM. Salinity in the same NaCl range caused considerable EL from rhizomes of *Miscanthus* × *giganteus* (Płażek et al., 2014). In our previously investigation (Płażek et al., 2013) a salinity of 125 mM NaCl caused a two-fold increase in ion leakage from leaves of the bread wheat 'Banderola' and durum wheat 'Komnata'. In the present experiment, 'Banderola' showed the lowest EL value from leaf cells of both control plants compared with those of other plant species in the study. EPI applications evoked different responses in the studied plants in this respect. Leaves of bread wheat showed a greater ion efflux after EPI application, while maize plants underwent a significant EL reduction comparing to the saline control plants. A 30% reduction in the membrane permeability, as an alleviating salt stress effect of EPI in pepper plants, was observed by Houimli et al. (2010). ZEN stabilized cell membrane permeability under salt stress only in maize. Many plants accumulate proline as a protective osmolyte under saline conditions (Flowers et al., 1977). Khatkar and Kuhad (2000) as well as Khan et al. (2003) observed a large increase in the proline

content in wheat plants grown under moderate salinity. In an earlier investigation (PŁAŻEK et al., 2013) control plants of durum cultivars, non-treated with salt stress, were characterized by a two-fold higher proline content compared with the bread wheat cultivars, but 125 mM NaCl salinity did not increase the proline content in the leaves of both wheat species. In the present experiment, in the leaves of both wheat species under 120 mM NaCl a decline of proline content was observed. EPI significantly increased the proline amount in the leaves of both bread and durum wheat, but this was lower than after the ZEN treatment, which increased the level 5.8-fold in bread wheat leaves and 9.4-fold in durum leaves. TALAAT and SHAWKY (2013) showed that EPI alleviated the inhibition of wheat productivity in saline soil by increasing proline accumulation. Especially high proline synthesis levels under salt stress were observed in *M.* × *giganteus* leaves (PŁAŻEK et al., 2014). At a salinity level of 150 mM NaCl, the level of free proline was 17 times as high as that of the control. Some authors have reported that proline cannot be used as biochemical indicator of salt tolerance (AZIZ et al., 1998; ASHRAF and HARRIS, 2004). AZIZ et al. (1998) described a negative relationship between the proline content in the leaves and the salt tolerance of tomato plants.

A key role of ABA in drought and salt tolerance as a long-distance signaling phytohormone is well documented (ZHU, 2002; ZHANG et al., 2006; YUAN et al., 2010). Moreover, the role of ABA in drought and salt stress is two-fold: water balance and cellular dehydration tolerance. In the studied plants, an increase in ABA, as an effect of the ZEN treatment, was noted in durum wheat and sorghum plants. YUAN et al. (2010) stated the amelioration of the drought stress in tomato seedlings by the EPI-induced elevation of endogenous ABA concentrations.

The accumulation of soluble carbohydrates in plants has been widely reported as a response to salinity or drought (ASHRAF and HARRIS, 2004). ASHRAF and TUFAIL (1995) found higher concentration of sugars in salt tolerant lines of sunflower than in less tolerant lines. Generally, in the studied plant species grown at 120 mM NaCl, an increase in the total sum of soluble sugars, and in particular mono- and disaccharides, under EPI and ZEN treatments was observed. A similar effect occurred with EPI, which increased the soluble carbohydrate content in grains of wheat, was noted by JANECZKO et al. (2010).

Trehalose is a disaccharide that plays a special role in plants under osmotic stress (ZEID, 2009). This sugar accumulates at very low concentrations, which was corroborated by our investigation. According to GARG et al., (2002), trehalose levels remain well below 1 mg g^{-1} FW, and its accumulation correlates with the higher soluble carbohydrate levels and elevated photosynthesis efficiency under stress and nonstress conditions. ZEID (2009) found that maize seedlings grown at high salinity, and treated with exogenous trehalose, demonstrated a stabilization of the plasma membranes, decreases in ion leakage and the lipid peroxidation rate, as well as an increase in the K^+ / Na^+ ion ratio in the leaves. In the described experiment EPI and ZEN elevated the amount of trehalose only in maize leaves of plants grown at 120 mM NaCl. This effect was observed along with a simultaneous decrease in ion leakage from the leaf cells.

Conclusions

In bread and durum wheat as well as in maize and sorghum plants, ZEN had a greater influence on the studied parameters than EPI. The ZEN-alleviating effects on salinity stress could be the result of the improved root mass, which is a typical effect of plant tolerance response to soil drought. In this plant species ZEN caused a reduction of ion transport between roots and shoots. Moreover, this stimulator increases the abscisic acid, proline as well as soluble carbohydrate content in plant leaves, the compounds which play key roles in defence reactions to osmotic stress.

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