Influence of 24-epibrassinolide on seedling growth and distribution of mineral elements in two maize hybrids

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Abstract

In this study, influence of wide range of 24-epibrassinolide (24-EBL) on early growth potential of two maize hybrids (ZP 434 and ZP 704) was examined. Paper concerns germination, seedling biomass, important chlorophylls content, and redistribution of elements (heavy metals and microelements), in a seedlings of the maize hybrids, as influenced by different 24-EBL concentrations. It was found that hybrids react differently to exogenously applied hormone. The biggest differences between two examined maize hybrids considering the germination level were reached with the lowest values at 86% for ZP 704 and 72% for ZP 434, gained at the highest applied concentration of 24-EBL. Seedlings of hybrid ZP 434 reacted positively moderately in the case of shoot length and biomass under the influence of 24-EBL, but seedlings of hybrid ZP 704 had lower values of these parameters under the influence of the phytohormone. Chlorophyll a/b ratios showed that photosynthetic apparatus of seedlings of the hybrids is not active in this stage of development. It was established that 24-EBL affects seedling growth and re-allocation of naturally present mineral elements in early growth stages and that could be one of the reason for poorer growth of ZP 704 treated with various concentrations of 24-EBL, comparing to control. When applied in lower concentrations, 24-EBL is blocking toxic elements such as chromium and nickel to relocate to vital parts of plant, what was case in hybrid ZP704. In case of ZP 434, lower concentrations of 24-EBL are affecting re-allocation of Cu and Cr and these findings suggest that maize hybrid seedlings treated with lower concentrations of 24-EBL could survive and be successful in polluted areas.

Keywords: 24-epibrassinolide, maize, heavy metals, element redistribution, plant protection.

Maize (*Zea mays* L.) is the world's most widely grown crop, both for human consumption and livestock feeding. It is also an important source of biofuel, animal feed and raw material in industry. Depending on favorable seed content, genetic predisposition, weather conditions and agricultural treatments, seeds have better possibility to grow into plants with improved characteristics [1]. Major maize producers in the world are trying to achieve better production while they are dealing with different environmental stress factors.

Different stress factors and genetic potential of plants conduct their growth and development. Agricultural plants are especially susceptible to external influences during seed germination and seedlings emergence phases [2].

Brassinosteroids (BRs) are important regulators of plant growth and development. They are organic compounds with polyhydroxylated sterols structure, which show multiple effects on plant physiology, growth and development. Plant hormone 24-epibrassinolide (24--EBL, Figure 1), as a member of BR group of hormones, applied in higher concentrations (5.2×10^{-7} and 5.2×10^{-8} M) showed inhibitory effect on the synthesis of phenolics in some maize hybrids [3], enhancement or retardation of root growth, differentiation of xylem vessels, membrane hyperpolarization, increased ATPase activity, and enhanced protein synthesis [4]. Natural BRs identified so far, have a common 5α-cholestane skeleton, and their structural variations come from the kind and orientation of oxygenated functions in rings A and B. These modifications are produced by oxidation and reduction reactions during biosynthesis.

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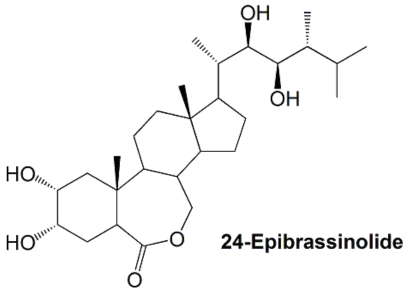

Figure 1. 24-EBL structure.

24-EBL belongs to the group of C_{28} BRs and BRs with 7-membered 7-oxalactone-B-ring and vicinal $2\alpha,3\alpha$-hydroxyl groups. 7-Oxalactone BRs have stronger biological activity than 6-oxo types (such as castasterone) and non-oxidized BRs reveal no activity in biological tests [5]. The application of exogenous BRs can be used as an anti-stress agent in a wide range of biotical and abiotic stress conditions (heavy metals, herbicides and drought stress) [6]. BRs have ability to regulate the uptake of elements into the plant cells and they can be used to reduce the accumulation of heavy metals and radioactive elements in plants. Heavy metals are non-essential elements that do not have any known positive function in plants. Metals such as cadmium, chromium, copper, nickel, and other naturally occurring metallic elements are considered as important pollutants which are affecting environment generally [7]. Cadmium is extremely toxic to plants. It retards biosynthesis of chlorophyll, alters water balance, induces oxidative stresses in plants and slows down the rate of photosynthesis. It was reported that there was an improvement in the shoot emergence and biomass production of mustard plants under the influence of pre-germination seed treatment with epibrassinolide (EBR). Moreover, EBR blocked copper metal uptake and its accumulation in the plants [8]. Another recently discovered aspect of the influence of BRs is their ability to redistribute elements within the plant cell. BRs can be used to reduce the accumulation, not just tolerance to heavy metals and radioactive elements in polluted areas [9] and in salty soils [10]. Although elements are present in low concentrations in seeds, they are necessary, especially zinc, for early growth stages [11].

But, what is natural affinity for redistribution of heavy metals and elements, and what is affinity if maize plants were treated with 24-EBL, is the maize suitable for bioremediation, and can maize grow on polluted soils? In presented manuscript, we discuss 24-EBL influence on maize architecture, seed germination and redistribution of important elements at early stage of development.

MATERIALS AND METHODS

Plant materials and growth conditions

The influence of different concentrations of 24-EBL (The product “Epin-Extra”, obtained from “Galenika-Fitofarmacija” a.d. Zemun Company, manufactured in Russia) in concentrations of 5.2×10^{-7}–5.2×10^{-15} M on the initial growth of maize has been examined. Two certified maize hybrids, ZP434 (drought tolerant) and ZP 704 (older generation hybrid, drought sensitive) were tested. The seeds are product of the Maize Research Institute Zemun Polje, Zemun Polje, Serbia.

Seeds (4×50, previously weighted) were germinated in two-liter plastic boxes (each box contains 50 seeds), on filter paper sheets, topped at the beginning of experiment with 60 mL of different concentrations of 24-EBL solution and under the phytothrone (Loške tovarne hladilnikov Škofja Loka, d.d., Slovenia) conditions at 30 (over day) and 20 °C (overnight), with a 12 h of light (110–160 μmol photons m^{-2} s^{-1})/12 h of dark regime [12].

Growth parameters

After seven days, 4×25 uniformly grown seedlings were separated with blades to shoot, root and rest of the seed from the seedlings (RoS). Weight of seedling parts was measured on analytical balance (Radwag, Poland), germination percentage was evaluated, length of seedlings shoots and roots length were measured, and subsequently seedlings were stored at –70 °C until further analyses.

Redistribution of fresh weight in the plant is expressed in modified allometric coefficients (fresh weight of individual plant organs relative to the total fresh weight of the plant). These parameters are: SMR (ratio of fresh weight of shoot (FWs) relative to the total fresh weight of the plant (FWp): g FWs g^{-1} FWp), RMR (ratio of fresh root weight (FWr) relative to the total fresh weight of the plant (FWp): g FWr g^{-1} FWp) [13].

Microwave digestion

A microwave-assisted acid digestion system (Berghof, Speed wave 4, Germany) was used to extract the elements from the samples (shoot, root, RoS). Approximately 0.5 g of dry weight (DW) of previously pooled 25 samples from every part of seedlings treated with 5.2×10^{-9}, 5.2×10^{-12} and 5.2×10^{-15} M were digested. The digestion procedure was based upon recommendations in US EPA method 3051B (with nitric acid, hydrogen chloride and hydrogen peroxide) [14]. Measurements were performed from three replicates.

The ICP-OES determination

The resulted solutions were analyzed by a Spectro Genesis ICP-OES instrument with Smart Analyzer Vision software (Spectro Analytical Instruments GmbH, Boschstr. 10, 47533 Kleve, Germany). The curves were recorded on the basis of individual (Ultra Scientific U.S.A. (concentration of 1 g L^{-1})) and the multi standards (SPS-SW2, LGC, UK) for the target elements, Mn, Na, Zn, Cu, Cr, Ni, Cd, Mo and Mg. The confirmation was carried out with matrix spike samples for a three concentrations (mg kg^{-1}).

Measurements of photosynthetic pigments (chlorophyll a and b and total carotenoids)

Photosynthetic pigments were extracted from frozen plant material with 96% ethanol and the absorbance of ethanol extracts was measured with UV visible spectrophotometer (Agilent 8453, USA) at 3 wave lengths: 470, 648 and 664 nm. The amount of pigments (chlorophylls and carotenoids; mg of pigments kg^{-1} FW of leaves) was attained using methods described by Lichtenthaler (1987) [14]. Measurements were performed from three replicates.

Statistical analyses

The basic statistical parameters of the data were calculated and the results were presented through histograms and tables. The analysis of variance was supported by the Kolmogorov–Smirnov test for the normality of residuals and Levene's test for homogeneity of variance. The data obtained were subjected to analysis of variance (ANOVA). The mean separation of content of mineral elements, content and ratios of photosynthetic pigments, shoot length, SMR, RMR and percentage of germination were accomplished by Tukey's HSD (honest significant difference) test. Significance was evaluated at $P < 0.05$ for all tests. Statistical analyses were conducted by the general procedures of Statistica v.7 (StatSoft, Inc.) and IBM SPSS Statistics v.20 (SPSS, Inc.).

RESULTS AND DISCUSSION

Difference between two examined maize hybrids was present at the germination level, with the lowest values at 86% for ZP 704 and 72% for ZP 434, gained at the highest applied concentration of 24-EBL (Figure 2). Germination of ZP 434 slightly varied under the influence of different concentrations of 24-EBL, with the highest drop induced by concentration of 5.2×10^{-7} M, comparing to control. In the case of ZP 704, concentrations of 24-EBL higher than 5.2×10^{-12} M inhibit germination. The highest influence has been noted at 24-EBL concentration of 5.2×10^{-7} M, with statistically significant decrease of germination. Control is presented as "0" due to water without 24-EBL in all figures.

Shoot length of ZP 434 and ZP 704 maize hybrids was significantly changed in response to 24-EBL concentrations. Gained results are less than alpha (α), so we can conclude that the difference between a pair of group means is statistically significant. According to results presented in Figure 3, seedlings of hybrid ZP 704 showed values of seedling shoot length similar to control only at 24-EBL concentration of 5.2×10^{-15} M, while all other concentrations had statistically significant inhibitory influence on shoot length. For hybrid ZP 434, the highest values of shoot length were obtained at lower 24-EBL concentrations. 24-EBL applied in 5.2×10^{-7} and 5.2×10^{-9} M concentrations significantly reduced the shoot length. As it can be seen from the Figure 3, hybrid ZP 434 had statistically significant differences at concentration of 5.2×10^{-7}, 5.2×10^{-9} and 5.2×10^{-15} M, while hybrid ZP 704 had higher sensitivity for 24-EBL range, with more statistically significant differences, relative to 24-EBL concentrations. Seed germination and seedling elongation (and in the later stages of plant development) are directly dependent on gibberellins inducing activity of the enzyme α-amylase, one of the key enzymes in the process of degradation of the

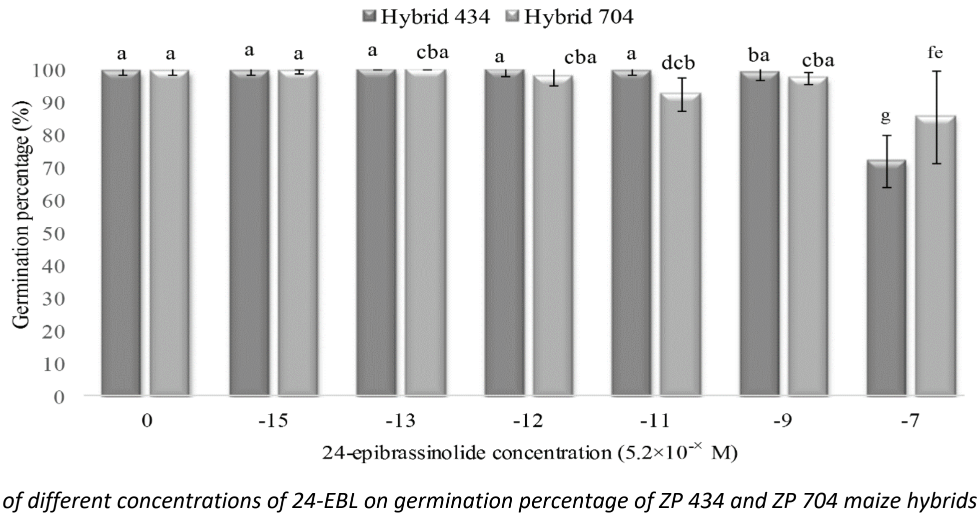

Figure 2. Effects of different concentrations of 24-EBL on germination percentage of ZP 434 and ZP 704 maize hybrids. The values followed by same letters are not significantly different at the P<0.05 level according to Tukey's HSD test.

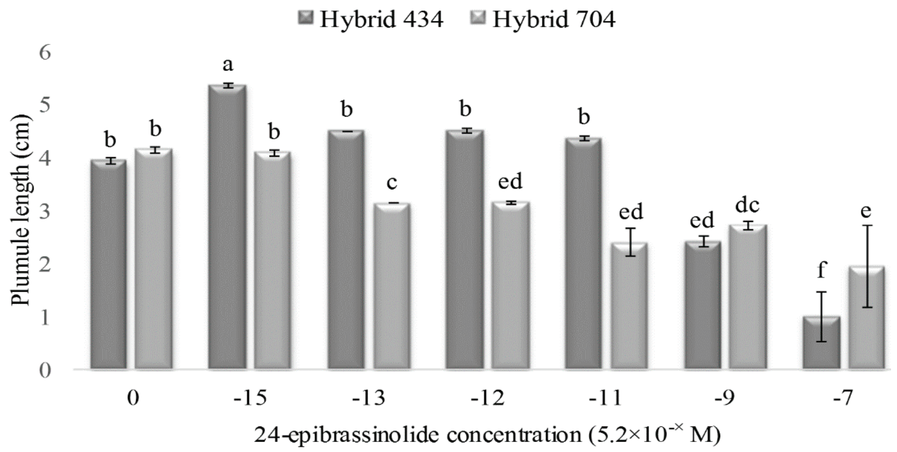

Figure 3. Effects of different concentrations of 24-EBL on seedling shoot length of ZP 434 and ZP 704 maize hybrids. The values followed by same letters are not significantly different at the P<0.05 level according to Tukey's HSD test.

starch reserves during seed germination with which BRs act synergistically [16,17].

According to the results presented in Table 1, ZP 434 was characterized by moderate, non-significant changes of shoot and root weight at 5.2×10^{-15} M as concentration of 24-EBL with non-significant influence on average increase in fresh weight of root and shoot comparing to untreated samples. Opposite to these trends, treatments of 5.2×10^{-9} and 5.2×10^{-7} M concentration of 24-EBL significantly decreased average fresh weight of seedling root and shoot, comparing to untreated samples. One of the important functions of BRs in plant physiology is stimulation of seedling elongation, which could be attributed to activation of BRs--induced genes, with its implicated influence on cell elongation or expansion. Also, five of the BRs-induced genes are either known to be early auxin induced or share homology to auxin induced genes [18,19]. Exogenous application of BRs usually inhibits primary root extension and lateral root formation, with occasional promotions of elongation or adventitious rooting [20,21], which is in accordance with results achieved by application of 5.2×10^{-7} M concentration of 24-EBL at hybrid ZP 434. 24-EBL concentrations higher than 5.2×10^{-12} M had the important impact on ZP 704 accumulation of fresh weight of shoot.

Germination percentage, weight of intact plant and innitial plant height are parameters of quality which affect field establishment and performance [22]. These parameters are used to describe ability of plants to germinate rapidly in the soil and to tolerate various, mostly negative environmental factors. It has been previously demonstrated that BRs can enhance crop yield and crop efficiency [23].

Low values of the ratio (<<3) of photosynthetic pigments (Chl a/Chl b) observed for both hybrids (Table 2) testify poor competence of the shoot photosynthetic apparatus, given that photosynthesis has not yet started, which is not surprising for such an early stage of development of seedlings [24]. However, this means that most assimilates (*i.e.*, and sugar), necessary for growth and development of shoot and root comes from the rest of the seed [25].

In the case of ZP 704, it can be noted that all concentrations of 24-EBL are influencing redistribution of Zn and Mn between shoots and roots. Usually plants are accumulating higher amounts of Zn in the shoots than in the roots, and that is the case with the control

Table 1. Effect of different concentrations of 24-EBL on SMR (g FWs g^{-1} FWp) and RMR (g FWr g^{-1} FWp) of ZP 434 and ZP 704 seedling parts. The values followed by same letters are not significantly different at the P < 0.05 level within the columns for each part of seedling according to Tukey's HSD test

24-EBL concentration, M	SMR, g FWs g^{-1} FWp		RMR, g FWr g^{-1} FWp	
	ZP 434	ZP 704	ZP 434	ZP 704
Control	0,0449^{cb}	0,0419^{dc}	0,0518^{dcb}	0,0482^{fed}
5.2×10^{-15}	0,04771^{ba}	0,0375^{ed}	0,0548^{ab}	0,0456^{gf}
5.2×10^{-13}	0,04473^{cb}	0,0336^{hgfe}	0,0529^{cb}	0,0486^{fedc}
5.2×10^{-12}	0,04573^{cb}	0,0347^{gfe}	0,0531^{cb}	0,0491^{edc}
5.2×10^{-11}	0,04125^{dc}	0,0283^{i}	0,0575^{a}	0,0451^{gf}
5.2×10^{-9}	0,0343^{gfe}	0,0317^{ihgf}	0,0459^{gf}	0,0454^{gf}
5.2×10^{-7}	0,0293^{ihg}	0,0286^{ih}	0,0367^{i}	0,0394^{ih}

Table 2. Measurements of photosynthetic pigments (mg kg^{-1} FW, mean±SD) in seedlings of ZP 434 and ZP 704 maize hybrid treated with different concentrations of 24-EBL. The values followed by same letters are not significantly different at the $P < 0.05$ level within the columns for each part of seedling according to Tukey's HSD test

24-EBL concentration, M	Chl a	Chl b	Chl a/Chl b ratio	Total chl	Total Carr
Hybrid ZP 434					
Control	1903^{a}±31	3513^{a}±35	0.54	5416	2516^{d}±6
5.2×10^{-15}	1687^{b}±6	3267^{b}±15	0.52	4954	3323^{a}±15
5.2×10^{-12}	1503^{c}±38	2737^{c}±12	0.55	4240	2826^{b}±29
5.2×10^{-9}	1357^{d}±40	2517^{d}±21	0.54	3874	2680^{c}±10
Hybrid ZP 704					
Control	1817^{a}±71	2350^{a}±70	0.77	4167	1187^{b}±6
5.2×10^{-15}	1407^{a}±49	2203^{ab}±55	0.64	3610	2363^{a}±59
5.2×10^{-12}	1377^{a}±15	2190^{b}±44	0.63	3567	2363^{a}±49
5.2×10^{-9}	1300^{b}±20	2237^{ab}±67	0.58	2537	2283^{a}±15

samples. Maize seedlings treated with lower concentration of 24-EBL, had significantly decreased content of Zn in all parts of plant. These could be one of the reasons for poorer emergence of shoots (Table 1) for hybrid ZP 704 treated with various concentrations of 24-EBL and it could be linked with higher Zn concentrations for hybrid ZP 434 in growing tissues [26]. Mn is almost equally distributed in roots of both control and treated ZP 704 hybrids, while other parts of seedlings were submissive to the influence of 24-EBL. Cu is the constituent of cytochrome oxidases and most of the enzymes in which Cu is a component are reacting with O_2 which results in producing H_2O or H_2O_2, and beside that function, Cu is involved in redox systems in plants [27]. There was an improvement in the shoot emergence and plant biomass production in the case of *Brassica juncea* L. seeds if they were treated before germination with 24-EBL (10^{-7}, 10^{-9} and 10^{-11} M) and submitted to Cu stress [28]. In the case of lowest applied 24-EBL concentration, obvious blocking of distribution of Cu was observed within both hybrids, which might indicate that maize plants could achieve optimum of growth in polluted soils. It is well known that 24-EBL can reduce the toxic effect of Cd [29]. In case of seedlings treated with the lowest concentrations of 24-EBL it is obvious that the accumulation of Cr and Ni is probably blocked in the seeds. Similar inhibitory role of BRs on the uptake of Ni was also reported by Sharma *et al*. [30]. These important insides to redistribution of highly toxic elements are leading to conclusion that maize treated with 5.2×10^{-15} M 24-EBL could survive much polluted soils due to its ability to block toxic elements before they reach roots and shoots, what could protect plants in stressed conditions. Mo is a highly mobile element translocated between various plant tissues [31] and that fact could be the reason why changes of Mo concentrations in shoot were not affected on treatments with 24-EBL. From the Table 3, it can be concluded that lower concentrations of 24-EBL has weak influence on the concentration of Mg in shoot of maize seedlings.

Also, Mo has important role in primary mechanisms of assimilation of nitrogen as a co-factor of nitrate reductase, crucial enzyme in the process of transformation of inorganic nitrate form of nitrogen, assimilated from the soil. Both Mo and Mn are extremely important in photosynthetic water-splitting process [32]. The content of elements such as Ni (except for RoS in concentration of 24-EBL at 5.2×10^{-15} M), Mo (except for RoS in concentration of 24-EBL at 5.2×10^{-9} M) and Mg was not affected with applied 24-EBL concentrations in case of ZP 434 hybrid (Table 3), while the content of Zn and Na was partially influenced. Results showed that redistribution of important elements stayed in a normal range, while the accumulation of potentially toxic elements was blocked in seeds, which could enable seedlings normal growth and development, and protection against toxic metals. As a confirmation of such hypothesis, lower concentrations of 24-EBL (5.2×10^{-13} and 5.2×10^{-12} M) had stimulatory effect on ZP 434 maize seedlings length (Figure 3), while the weight of the shoot remained unchanged. Treatment of seedlings of both hybrids with various concentrations of 24-EBL is affecting balance of Cu (Table 3), so we can assume that 24-EBL have protective effect in terms of avoidance of possible toxic effect of Cu.

Transport of Na under saline conditions is still poorly understood but it is suggested that plants could have compartments for reserving Na. This probably is helping plants to overcome environmental stress such as salinity [33]. Considering the fact that 24-EBL is influencing relocation of Na into root, especially in the case of ZP 704 and in the case of ZP 434, treated with 5.2×10^{-12} M concentration of 24-EBL (Table 3), we can assume that maize seedlings treated with lower concentrations of 24-EBL could have better chances to emerge in saline habitats [34]. 24-EBL applied in lower concentrations is also influencing redistribution of Cu

Table 3. Effect of different concentrations of 24-EBL on content of mineral elements (mg kg^{-1} DW) in seedling parts of ZP 704 and ZP 434 hybrid. <0.05 represents content below the detectable value. The values followed by same letters are not significantly different at the P < 0.05 level within the columns for each part of seedling according to Tukey's HSD test

Concentration of 24-EBL, M	Mn	Na	Zn	Cu	Cr	Ni	Cd	Mo	Mg
ZP 704									
Control shoot	3.1^{a}± 0.65	109.5^{a}± 11.32	980.3^{a}± 175.51	59.77^{a}± 7.90	5.83^{a}± 0.68	0.33^{a}± 0.04	0.28^{a}± 0.02	0.07^{a}± 0.02	252.87^{ab}± 38.25
5.2×10^{-15} shoot	1.8^{b}± 0.09	82.2^{a}± 10.55	51.7^{b}± 3.63	1.43^{b}± 0.3	0.17^{b}± 0.02	0.04^{c}± 0.00	0.14^{b}± 0.06	0.06^{a}± 0.01	207.45^{b}± 15.38
5.2×10^{-12} shoot	2.7^{ab}± 0.07	90.1^{a}± 8.21	58.9^{b}± 6.17	1.99^{b}± 0.20	0.31^{b}± 0.08	0.13^{b}± 0.02	0.17^{b}± 0.01	0.06^{a}± 0.01	230.54^{ab}± 20.01
5.2×10^{-9} shoot	2.5^{ab}± 0.26	101.5^{a}± 13.89	86.3^{b}± 13.26	3.27^{b}± 0.49	0.67^{b}± 0.01	0.26^{a}± 0.04	0.21^{ab}± 0.06	0.07^{a}± 0.01	285.30^{a}± 32.31
Control root	1.8^{b}± 0.2	137.1^{a}± 13.85	407.6^{a}± 46.60	23.26^{a}± 2.86	2.42^{a}± 0.46	0.26^{b}± 0.01	0.21^{a}± 0.01	0.05^{b}± 0.01	116.5^{c}± 14.70
5.2×10^{-15} root	2.5^{b}± 0.5	144.4^{a}± 6.81	47.0^{b}± 9.31	1.86^{b}± 0.18	0.38^{b}± 0.06	0.28^{b}± 0.06	0.23^{a}± 0.04	0.04^{b}± 0.01	131.23^{bc}± 12.19
5.2×10^{-12} root	3.8^{a}± 0.36	155.3^{a}± 32.83	67.2^{b}± 6.09	2.71^{b}± 0.41	0.45^{b}± 0.05	2.28^{a}± 0.02	0.22^{a}± 0.03	0.08^{a}± 0.01	174.51^{b}± 26.40
5.2×10^{-9} root	2.3^{b}± 0.29	162.2^{a}± 16.34	82.4^{b}± 5.18	2.45^{b}± 0.07	0.57^{b}± 0.07	0.13^{c}± 0.02	0.14^{b}± 0.01	0.04^{b}± 0.01	236.51^{a}± 25.50
Control RoS	2.9^{b}± 0.37	194.3^{a}± 45.22	80.7^{a}± 12.25	4.29^{a}± 0.95	1.09^{c}± 0.07	0.39^{b}± 0.07	0.23^{b}± 0.07	0.06^{b}± 0.02	429.5^{b}± 73.88
5.2×10^{-15} RoS	5.6^{a}± 0.48	64.2^{b}± 2.55	57.1^{b}± 4.24	1.78^{c}± 0.27	2.17^{b}± 0.12	1.97^{a}± 0.38	0.17^{b}± 0.03	0.09^{ab}± 0.01	501.29^{ab}± 99.68
5.2×10^{-12} RoS	5.5^{a}± 0.38	113.2^{b}± 12.04	50.2^{b}± 6.30	3.57^{ab}± 0.17	2.78^{a}± 0.20	1.51^{a}± 0.18	0.69^{a}± 0.24	0.12^{a}± 0.02	761.52^{a}± 146.63
5.2×10^{-9} RoS	4.7^{a}± 0.32	108.94^{b}± 9.04	77.6^{a}± 2.48	2.54^{bc}± 0.29	0.43^{d}± 0.03	0.18^{b}± 0.04	0.30^{b}± 0.08	0.08^{b}± 0.01	752.31^{a}± 59.22
ZP 434									
Control shoot	3.94^{a}± 0.39	351.41^{a}± 31.00	312.80^{a}± 18.03	21.52^{a}± 2.31	2.04^{a}± 0.09	0.75^{a}± 0.10	<0.05	0.12^{a}± 0.03	277.47^{a}± 14.89
5.2×10^{-15} shoot	2.72^{b}± 0.09	207.41^{c}± 21.59	68.21^{b}± 9.58	2.49^{b}± 0.19	0.21^{c}± 0.01	0.66^{a}± 0.02	<0.05	0.13^{a}± 0.03	240.8^{a}± 19.56
5.2×10^{-12} shoot	3.28^{ab}± 0.16	286.59^{b}± 6.06	78.73^{b}± 1.25	3.18^{b}± 0.10	0.42^{b}± 0.01	0.85^{a}± 0.14	<0.05	0.15^{a}± 0.02	227.75^{a}± 26.36
5.2×10^{-9} shoot	2.88^{b}± 0.59	374.52^{a}± 26.86	98.31^{b}± 16.78	3.59^{b}± 0.16	0.47b± 0.03	0.75^{a}± 0.01	<0.05	0.14^{a}± 0.01	268.04^{a}± 29.10
Control root	1.96^{a}± 0.28	427.06^{b}± 43.73	209.96^{a}± 27.54	12.58^{a}± 0.92	1.32^{a}± 0.04	0.75^{a}± 0.04	<0.05	0.13^{a}± 0.01	123.13^{a}± 11.16
5.2×10^{-15} root	1.34^{b}± 0.13	265.12^{c}± 27.25	56.83^{b}± 8.05	2.24^{b}± 0.12	0.29^{ab}± 0.05	0.53a± 0.05	<0.05	0.12a± 0.03	106.87^{a}± 6.69
5.2×10^{-12} root	1.82^{ab}± 0.27	710.01^{a}± 92.93	61.83^{b}± 10.94	2.65^{b}± 0.09	0.21^{c}± 0.01	0.72^{a}± 0.17	<0.05	0.14^{a}± 0.02	131.09^{a}± 19.39
5.2×10^{-9} root	1.51^{ab}± 0.11	250.96^{c}± 35.42	54.92^{b}± 4.36	2.33^{b}± 0.05	0.32^{b}± 0.02	0.66^{a}± 0.05	<0.05	0.10^{a}± 0.02	114.81^{a}± 11.19
Control RoS	4.44^{a}± 0.52	154.57^{b}± 21.06	97.53^{a}± 5.48	4.55^{a}± 0.49	0.69^{a}± 0.02	0.68^{ab}± 0.10	<0.05	0.13^{b}± 0.01	609.61^{a}± 38.76
5.2×10^{-15} RoS	4.15^{a}± 0.16	146.68^{b}± 4.18	52.75^{c}± 2.23	1.93^{c}± 0.06	0.17^{d}± 0.02	0.59^{b}± 0.05	<0.05	0.13^{b}± 0.01	549.93^{a}± 46.54
5.2×10^{-12} RoS	4.64^{a}± 0.30	162.89^{ab}± 8.04	59.19^{bc}± 5.41	2.45^{bc}± 0.03	0.32^{c}± 0.06	0.72^{ab}± 0.04	<0.05	0.15^{ab}± 0.01	619.1^{a}± 99.53
5.2×10^{-9} RoS	4.12^{a}± 0.55	209.02^{a}± 30.69	71.08^{b}± 5.25	2.77^{b}± 0.16	0.43^{b}± 0.03	0.79^{a}± 0.07	<0.05	0.17^{a}± 0.01	569.32^{a}± 58.61

and Cr in the case of ZP 434 (Table 3), and it could be used as a parameter for choosing right type of chemical treatments for overcoming influence of polluted areas.

Despite that measurements of element uptake from the soil solution was not carried out, results are indicating that 24-EBL, when applied in lower concentrations, is blocking distribution of heavy metals into the vital parts of the plant at the very beginning of the seedling growth and development. These results are implicating, together with above mentioned literature and foundlings of other scientists, that 24-EBL is involved in plant protection [35] and that maize treated with various concentrations of 24-EBL could also be successfully planted in various polluted areas. Considering the fact that germination remained stable for both hybrids, together with higher initial growth (in case of hybrid ZP 704) comparing to control, and the evidence that 24-EBL blocked heavy metals allocation into the vital organs, candidates these hybrids as suitable for the cultivation on polluted soils. Maize is widely used in industry and represents high biomass producing plant [36]. Considering that, ZP 434 and ZP 704 maize hybrids could be valuable for the elimination of toxic elements from the polluted areas. Based on the presented results, it could not be concluded that 24-EBL positively influenced the distribution of potentially toxic elements into the shoots and roots of maize hybrids, so its potential in phytoremediation is not confirmed.

CONCLUSION

Considering the fact that maize is one of the most widely grown crops, producers in the world are trying to achieve better production while they are dealing with different stress factors. In these study, influence of 24-EBL on germination, early plant growth, photosynthetic pigments and redistribution of elements was examined. It was found that 24-EBL is influencing both germination and growth of maize hybrids ZP 704 and ZP 434 at lower concentrations of phytohormone. Hybrids reacted divergently to exogenous application of 24-EBL. Lower concentrations have stimulating effect on growth and germination while high concentrations of 24-EBL have inhibitory effect on these processes. Considering that germination percentage, mass of all plant and innitial plant height are parameters of plant quality, seedlings treated with various concentrations of 24-EBL will probablly have better chance for growth and field establishment. It was found that 24-EBL is affecting redistribution of elements in young plants. Elements could be linked with initial growth and it was assumed that in the case of hybrid ZP 704, poorer emergence of shoots is influenced with lower Zn concentration, since it is known that the Zn and plant growth are correlated.

In case of the lowest concentration of 24-EBL, hormone is obvious blocking distribution of Cu, and these could mean that maize plants could achieve optimum of growth in polluted soils. Low values of ratio of photosynthetic pigments for both hybrids are confirming photosynthetic inactivity. Results are implicating from our findings that maize treated with 24-EBL could be grown at soil polluted with heavy metals due to its ability to remove or block accumulation of toxic elements, especially in shoot.

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IZVOD

UTICAJ 24-EPIBRASINOLIDA NA RASTENJE KLIJANACA I DISTRIBUCIJU MINERALNIH ELEMENATA KOD DVA HIBRIDA KUKURUZA

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(Naučni rad)

Kukuruz (*Zea mays* L.) je jedan od najviše gajenih useva u svetu i koristi se kako za ljudsku, tako i životinjsku ishranu, pri čemu ima široku primenu u različitim granama industrije. Cilj važnih svetskih proizvođača ovog useva jeste da optimizuju uslove njegovog gajenja u uslovima brojnih i različitih stresnih faktora sredine. Poznato je da fitohormoni brasinosteroidnog tipa, pored ostalih imaju i zaštitnu ulogu kod biljaka, pomažući u prevazilaženju posledica stresnih uslova na stanje useva. Ispitivan je uticaj širokog opsega koncentracija ezogeno dodatog 24-epibrasinolida na početne faze rastenja i razvića dva hibrida kukuruza različitih karakteristika. Ispitivani hibridi (ZP 434 i ZP 704) u uslovima polja različito reaguju odnosno promenljivih sredinskih uslova. Ispitivanja su obuhvatila analizu klijavosti, akumulaciju biomase i rastenje različitih delova klijanaca, akumulaciju hlorofila, redistribucije teških metala i mikroelemenata između delova klijanaca pomenutih hibrida kukuruza, zavisno od koncentracije egzogeno dodatog 24-epibrasinolida. Najveće promene u klijavosti između ispitivanih hibrida su dostigle najniže vrednosti od 86% za ZP 704 i 72% za ZP 434, pod uticajem najnižih koncentracijama fitohormona. Utvrđeno je da ispitivani hibridi različito reaguju na koncentracioni opseg dodatog fitohormona. Klijavost predmetnih hibrida kukuruza se uglavnom ne menja (osim na visokim koncentracijama 24-epibrasinolida), ali klijanci hibrida ZP434 reaguju uvećanjem dužine stabla i akumulacijom biomase pri dodavanju 24-epibrasinolida, dok, nasuprot tome klijanci hibrida ZP 704 imaju snižene vrednosti vrednosti navedenih parametara pri dodavanju predmetnog fitohormona. Promene u akumulaciji i odnosima hlorofila a i b pokazuju da fotosintetički aparat klijanaca hibrida kukuruza još uvek nije aktivan i da se biljka u ovom razvojnom stadijumu još uvek snabdeva materijom i energijom iz ostatka semena. Zapaženo je i da 24-epibrasinolid, pored uticaja na rastenje klijanaca kukuruza, utiče i na redistribuciju mineralnih elementata prisutnih u sistemu. Ta činjenica pruža moguće objašnjenje za slabije rastenje klijanaca hibrida ZP 704, tretiranog predmetnim fitohormonom. Pri tretiranju klijanaca kukuruza nižim koncentracijama, 24-epibrasinolid deluje kao blokator ulaska potencijalno toksičnih elemenata (npr. hrom i nikl) u vitalne delove klijanaca, što je slučaj kod hibrida ZP 704. To znači da ovaj fitohormon deluje protektivno na klijance ovih hibrida kukuruza, pri stresu teškim metalima. Kod klijanaca hibrida ZP 434, niže koncentracije 24-epibrasinolida utiču na redistribuciju bakra i hroma, što ukazuje da bi biljke ovog hibrida kukuruza, tretirane nižim koncentracijama 24-epibrasinolida mogle opstati i uspešno rasti na području zagađenom pomenutim teškim metalima. Imajući u vidu da su klijavost, biomasa i visina biljke u ranim stadijumima rastenja i razvića, parametri koji presudno utiču na kvalitet i preživljavanje biljke u kasnijim stadijumima, možemo pretpostaviti da bi kukuruz tretiran 24-epibrasinolidom imao veće šanse da preživi uslove stresa usled zagađenosti zemljišta teškim metalima. Činjenica da je usvajanje teških elemenata blokirano, pre ulaska u vitalne delove biljke, čini kukuruz tretiran 24-epibrasinolidom mogućim kandidatom za rastenje na zagađenom području, ali ga to ne čini pogodnim kandidatom za fitoremedijaciju tog zagađenog zemljišta.

Ključne reči: 24-epibrasinolid • Kukuruz • Teški metali • Preraspodela elemenata • Zaštita bilja