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Review paper

Brassinosteroid phytochormones as regulators of plant growth and modulators of pesticide and fertilizer activity

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SUMMARY

The mode of action of agrochemicals on plants implies the totality of their effect on plant metabolism, growth and development. The effects of different doses of 24-epibrassinolide (24-EBL) as a class of brassinosteroid phytohormones on growth and other physiological processes in maize plants during different development stages are reviewed in order to assess the influence of these agrochemicals on various factors determining the yield of maize as an important agricultural crop. In addition, several examples are given of the effects of these phytohormones on other crops, fruits and vegetables, in terms of their effect on yield, yield quality, and increase in crop resistance to some types of stress. Own results are discussed in the context of other literature data.

Abbreviations: 24-EBL: 24-epibrassinolide; BRs: brassinosteroids; PCZ: propiconazole; Chl a: chlorophyl a; RFW (g g^{-1}): relative fresh weight of different organs (R: radicle; P: plumule; RoS: rest of seed); TDW, TFW (g): total dry and fresh weight of plants; V root (ml): root volume; LMR, RMR, SMR (g g^{-1}): relative dry weight of plant parts (leaves, roots, stem); dH (J mol^{-1} K^{-1}): differential enthalpy of different parts (R: radicle; P: plumule; RoS: rest of seed) of 25 maize seedlings exposed to T(reatments) of different molar concentrations of 24-EBL; ΔG_{105} (J mol^{-1} K^{-1}) differential Gibbs free energy of total maize plant and their parts (R: roots; L: leaves; S: stem) assesed at 105 ^{0}C; ZP434, ZP704, ZP505: maize hybrids; Fv/Fm, , Fv/F_0, ΦPS_2, qP, NPQ, RFD_{730} (all in relative units), ETR (µmol electrons m^{-2} s^{-1}): different Chl a fluorescence parameters; Pphy, Pi: phosphorus bond to phytic acid and free phosphorus available to many cellular biochemical reactions; GSH: reduced form of gluthathyone; K, Ca, Fe, Mg, Zn, Si: different chemical elements.

Keywords: Phytohormones; Brassinosteroids; Plant growth; Plant nutrition; Plant protection

INTRODUCTION

Brassinosteroid phytohormones have a central role in coordinating plant growth and development (Clouse & Sasse, 1998), and a variety of highly diverse processes are controlled by these phytohormones. They decide plant sex (Hartwig et al., 2011) and plant phenotype, or rather its ideotype, (Hong et al., 2004; Sakamoto et al., 2005; Kir, 2010; Schulz et al., 2012), which all reflects on plant yield. These phytohormones are endogenous in plants and occur in very small amounts. Most phytohormones act at concentrations (endogenous or exogenous) of 10^{-6}-10^{-7} M, while brassinosteroids are effective at concentrations that are about 1000-fold lower (endogenous or exogenous), i.e. at 10^{-8} to 10^{-10} M, sometimes even lower. The regulation mechanism and mode of action of these phytohormones occur at three levels at least: a) brassinosteroid synthesis; b) brassinosteroid receptors; c) brassinosteroid signal pathways. All this reveals why practical exogenic application of these phytohormones by foliar treatment or crop seed soaking is not always reliable. An application dose may be effective under a particular set of agroecological conditions and in a particular crop, while the effect may be less than positive or even phytotoxic with the same dose under different agroecological conditions in another crop (Nikolić et al., 2013, 2014; Waisi et al, 2013, 2014, 2015a). Why is this so? Firstly, the concentration of receptors for brassinosteroids varies in different tissues, and most likely depends on whether that tissue is young and developing or it has already been formed with its precise functions (Van Esse et al., 2011, 2012), and secondly, brassinosteroid signal pathways interact with other signal pathways in plants (Kim & Wang, 2010; Clouse, 2011), so that the effectiveness of exogenous application of brassinosteroids greatly depends on agroecological factors that are difficult to control (Vriet et al., 2012). However, it opens a possible third way of manipulation of these phytohormones (disregarding genetically transformed plants with increased contents and/or susceptibility to brassinosteroids, whose practical use is only at a start globally) by manipulating their biosynthesis (Fujioka & Yokota, 2003). Namely, the crucial enzyme in the biosynthesis of brassinosteroids (some 70 compounds are known in this class of phytohormones so far) is cytochrome P450 oxidase, which belongs to a multi-functional class of oxidase, i.e. monooxygenase (Fujioka & Yokota, 2003). During biological research of the mechanisms of action of brassinosteroids, brassinazole was discovered as an inhibitor of brassinosteroid biosynthesis, belonging in the triazole chemical class (Clouse & Sasse, 1998). Those research reports had only a scientific relevance for a long time before a revelation was made in 2012 that the triazole fungicide propiconazole (PCZ) (Hartwig et al., 2012) specifically inhibits the biosynthesis and accumulation of brassinosteroids in the genus *Arabidopsis*, as well as in maize, which opens possibilities for manipulation of endogenous brassinosteroid contents in crops, which is analogous to growth retardants/inhibitors of gibberellic acid biosynthesis (Nikolić et al., 2015). It is noteworthy that inhibitors of gibberellic biosynthesis belong to triazole compounds (Nešković et al., 2003), which adds options to crop growth manipulation, and consequently crop yields.

The present article surveys our hitherto results in research of brassinosteroid effects on maize and other crops which are generally undertaken to achieve practical uses in agriculture.

REVIEW OF BRASSINOSTERIOID INVESTIGATIONS

Treatment of maize seeds and seedlings with brassinosteroids

Changes in dry weight allocation in different organs of maize seedlings (plumula, radicle) show that 24-EBL concentrations ranging from $5.2 \cdot 10^{-12}$ M to $5.2 \cdot 10^{-10}$ M have the greatest effect on the status of radicles in two genotypes, as well as the status of plumula, only in different ways (Tables 1 and 2). Conversely, the top 24-EBL concentration of $5.2 \cdot 10^{-7}$ M had the greatest effect on the RoS in both maize genotypes (Tables 1 and 2), meaning that it inhibits dry weight allocation from the RoS to plumula and radicle in maize seedlings. In contrast to the uniform response of weight allocation and growth process of different organs of maize seedlings under the influence of different 24-EBL concentrations, differential enthalpy, which is a thermodynamic measure of synthetic processes in a system (i.e. reflects thermodynamic and chamical potentials), is highly variable in both genotypes, depending on temperature at which maize seedling organs are dried (Tables 1 and 2).

Chemical reactions in live biological systems depend on water as the universal solvent. The most negative values of differential enthalpy are indicative of completely exothermic and spontaneous processes in organs of maize seedlings (Tables 1 and 2), while enthalpy data determined at different temperatures (during drying of maize organs)

Table 1. Average of 4 measurements of different parameters of maize ZP434 hybrid seeds/seedlings: G (%): percent of germination of 50 maize seeds; RFW (g g^{-1}): relative fresh weight of different organs (R: radicle; P: plumule; RoS: rest of seed) of 25 maize seedlings; dH (J mol^{-1} K^{-1}): differential enthalpy of different organs (R: radicle; P: plumule; RoS: rest of seed) of 25 maize seedlings exposed to T(reatments) of different molar concentrations of 24-EBL: 1: C(ontrol); 2: $5.2 \cdot 10^{-7}$ M; 3: $5.2 \cdot 10^{-8}$ M; 4: $5.2 \cdot 10^{-9}$ M; 5: $5.2 \cdot 10^{-10}$ M; 6: $5.2 \cdot 10^{-11}$ M; 7: $5.2 \cdot 10^{-12}$ M; 8: $5.2 \cdot 10^{-13}$ M; 9: $5.2 \cdot 10^{-14}$ M and 10: $5.2 \cdot 10^{-15}$ M. Bold: Maximal values in a series. Italic: Minimal values in a series. (According to Waisi, 2016)

T	G (%)	RFW (g g^{-1}) R	RFW (g g^{-1}) P	RFW (g g^{-1}) RoS	dH_{105-60}			$dH_{130-105}$			dH_{130-60}		
					R	P	RoS	R	P	RoS	R	P	RoS
1	86.0	0.27	0.20	0.53	-9.77	-8.44	**-8.33**	-6.93	-7.19	-13.85	-14.96	-13.84	-18.84
2	*41.5*	*0.14*	*0.09*	**0.76**	-8.16	-7.58	-6.94	**-10.24**	-7.41	-15.67	**-15.90**	-13.16	-18.85
3	75.0	0.19	0.11	0.68	-8.68	-7.91	-7.55	-7.47	-6.83	-13.60	-14.30	-13.04	-17.87
4	89.5	0.21	0.12	0.64	*-7.91*	*-7.35*	*-6.85*	-9.33	-11.63	-12.70	-14.96	-16.17	-16.50
5	77.0	0.25	0.15	0.60	-9.09	-8.17	-8.34	-8.81	-9.76	-12.59	-15.74	-15.54	-17.88
6	91.5	0.33	0.17	0.53	-8.53	-8.08	-6.99	-8.40	-11.44	**-16.96**	-14.86	**-16.75**	**-19.89**
7	90.0	**0.34**	**0.24**	*0.42*	-10.34	-8.77	-8.10	-6.42	-6.08	-10.15	-15.14	-13.32	-15.77
8	92.0	0.28	0.20	0.52	**-10.42**	-8.76	-8.22	-5.46	-7.61	-9.97	-14.48	-14.48	-15.76
9	87.0	0.30	0.23	0.47	-10.37	**-8.83**	-8.31	*-3.80*	*-5.34*	*-8.66*	*-13.16*	*-12.82*	*-14.85*
10	**92.5**	0.30	0.23	0.47	10.40	-8.67	-7.75	-5.10	-7.14	-10.66	-14.19	-14.04	-15.82

Table 2. Average of 4 measurements of different parameters of maize ZP704 hybrid seeds/seedlings: G (%): percent of germination of 50 maize seeds; RFW (g g^{-1}): relative fresh weight of different organs (R: radicle; P: plumule; RoS: rest of seeds) of 25 maize seedlings; dH (J mol^{-1} K^{-1}): differential enthalpy of different organs (R: radicle; P: plumule; RoS: rest of seeds) of 25 maize seedlings exposed to T(reatments) of different molar concentrations of 24-EBL: 1: C(ontrol);2: $5.2 \cdot 10^{-7}$ M; 3: $5.2 \cdot 10^{-8}$ M; 4: $5.2 \cdot 10^{-9}$ M; 5: $5.2 \cdot 10^{-10}$ M; 6: $5.2 \cdot 10^{-11}$ M; 7: $5.2 \cdot 10^{-12}$ M; 8: $5.2 \cdot 10^{-13}$ M; 9: $5.2 \cdot 10^{-14}$ M and 10: $5.2 \cdot 10^{-15}$ M. Bold: Maximal values in a series. Italic: Minimal values in a series. (According to Waisi, 2016)

T	G (%)	RFW (g g^{-1}) R	RFW (gg^{-1}) P	RFW (gg^{-1}) RoS	dH_{105-60}			$dH_{130-105}$			dH_{130-60}		
					R	P	RoS	R	P	RoS	R	P	RoS
1	99.5	0.23	**0.18**	*0.59*	-8.51	*-7.37*	*-6.49*	-9.33	**-12.79**	-14.02	-15.55	-17.09	-17.14
2	*86.0*	*0.16*	0.08	0.76	-8.77	-7.99	-6.75	-9.83	-10.42	**-14.92**	**-16.19**	-15.87	-18.09
3	**100.0**	0.21	*0.08*	0.69	-9.19	**-9.50**	-7.21	-5.23	-2.79	-14.92	-13.13	-11.52	**-18.55**
4	97.5	0.21	0.10	0.69	*-8.26*	-8.16	-6.61	-7.23	-6.71	-13.02	-13.70	-13.20	-16.50
5	97.5	0.24	0.09	0.66	-8.54	-8.22	-6.68	-7.24	-8.70	-13.03	-13.99	-14.78	-16.57
6	92.5	0.20	0.08	0.72	-10.47	-8.98	-7.67	-2.21	-3.93	-4.26	-12.04	-11.88	-10.85
7	96.0	**0.25**	0.12	0.64	-9.77	-8.91	-8.35	-2.33	-3.41	-3.98	-11.44	-11.42	-11.30
8	98.0	0.24	0.11	0.66	-10.52	-9.32	-7.96	-0.49	*-0.51*	-3.32	-10.77	-9.60	-10.40
9	97.5	0.19	0.12	0.69	-8.35	-7.74	**-9.45**	0.04	-1.98	*1.65*	*-8.23*	*-9.16*	*-8.08*
10	99.8	0.21	0.14	0.65	-8.71	-8.02	-9.02	*0.05*	-2.22	-0.12	-8.56	-9.63	-9.01

are associated with different water fractions in the plant: free, apoplastic water; cytoplasmatic water; and chemically bound water (Sun, 2002). Based on relevant data, the enthalpy of free, apoplastic water was found to be mostly influenced by low concentrations of 24-EBL, $5.2 \cdot 10^{-13}$ and $5.2 \cdot 10^{-14}$ M, acting on biochemical processes in the radicle and plumule, while $5.2 \cdot 10^{-9}$ M of 24-EBL was the least suitable concentration for processes taking place in seedlings of the ZP434 genotype (Table 1). Regarding changes in the enthalpy of cytoplasmatic (dH130-60) and chemically bound (dH130-105) water in seedlings of the maize genotype ZP434, optimal concentrations of 24-EBL that may have effect on biochemical reactions are completely different (Table 1). Notably, changes in the free water enthalpy of plumules of ZP704 genotype of maize seedlings were reverse from what was found in seedlings of the genotype ZP434 (Table 2). What was the cause of the observed differences in germination, redistribution of weight and capacity for synthetic biochemical reactions (differential enthalpy: dH) in different organs of seedlings of the mentioned maize genotypes under different 24-EBL concentrations?

We analyzed the content of photosynthetic pigments in fresh tissue, as well as some sugars (Tables 3 and 4) and polyphenols (data not presented) in dry organ tissue of maize seedlings. Low data of the ratios of photosynthetic pigments (Tables 3 and 4) indicate a poor competence of the photosynthetic plumule apparatus, which is not surprising considering the early stage of seedling development (Babani & Lichtenthaler, 1996). It means that the greatest part of assimilates (sugars primarily) that are required for plumule and radicle growth and development come from the RoS (Thomas & Rodriguez, 1994).

Contents of trehalose, sugars important for plant tolerance to stress (Paul et al., 2008), arabinose sugar, and the cellulose constituent of cell walls in grasses (Carpita, 1996) were analysed, as well as contents of important glycoproteins (Fincher et al., 1983), glucose and fructose, sugars important for primary metabolism and obligate monomers of important polysaccharides in higher plants (Duffus & Duffus, 1984), and succrose, the most important transport sugar in higher plants (Komor, 2000). To sum up, the contents of these sugars in seedling organs of maize were observed to increase with higher 24-EBL concentrations, and decrease with

Table 3. Average data (3 measurements) of different biochemical parameters determined in different organs (R: radicle; P: plumule; RoS: rest of seeds) of 25 maize ZP434 hybrid seedlings exposed to T(reatments) of different molar concentrations of 24-EBL: 1: C(ontrol 2: $5.2 \cdot 10^{-7}$ M; 3: $5.2 \cdot 10^{-8}$ M; 4: $5.2 \cdot 10^{-9}$ M; 5: $5.2 \cdot 10^{-10}$ M; 6: $5.2 \cdot 10^{-11}$ M; 7: $5.2 \cdot 10^{-12}$ M; 8: $5.2 \cdot 10^{-13}$ M; 9: $5.2 \cdot 10^{-14}$ M and 10: $5.2 \cdot 10^{-15}$ M. Bold: Maximal values in a series. Italic: Minimal values in a series. (According to Waisi, 2016)

T	Chl a/b ratio	Chla/carr ratio (x+c)	Trehalose content (μg/ 0.25 g of dry matter)			Arabinose content (μg/ 0.25 g of dry matter)			Glucose content (μg/ 0.25 g of dry matter)			Fructose content (μg/ 0.25 g of dry matter)			Sucrose content (μg/ 0.25 g of dry matter)		
			RoS	P	R	RoS	P	R	RoS	P	R	RoS	P	R	RoS	P	R
1	0.54	**0.75**	73.9	43.9	62.4	**283.1**	81.4	55.2	**1046.3**	222.6	68.5	261.2	271.3	254.4	130.2	649.2	842.1
2	*0.50*	*0.45*	*39.7*	127.9	**83.1**	40.2	56.6	21.6	894.4	**2386.6**	141.7	149.7	**2698.7**	432.3	**495.4**	2103.8	**4385.5**
3	-	-	70.2	**198.5**	74.2	134.4	14.6	26.6	874.8	469.6	**221.2**	160.1	667.9	**549.4**	225.7	135.4	3688.2
4	0.54	0.50	84.8	115.7	32.2	4.4	21.0	**108.6**	430.5	184.6	72.4	117.1	252.2	110.0	45.9	*88.6*	1210.0
5	-	-	83.3	62.9	61.9	6.4	24.6	21.8	*275.7*	143.7	43.8	*77.6*	261.4	107.6	76.2	100.0	2785.1
6	**0.55**	0.51	207.7	*14.7*	31.5	7.0	40.9	15.9	563.6	113.8	39.7	79.4	286.9	243.9	*38.8*	**3174.9**	2993.6
7	-	-	226.9	20.2	22.3	13.9	32.8	14.9	560.8	*41.1*	49.9	116.7	112.2	114.6	87.8	2599.5	1338.2
8	0.54	0.56	180.5	36.1	30.6	1.54	67.5	23.6	544.5	251.9	120.8	128.5	*101.5*	199.5	98.1	2977.4	2089.3
9	-	-	140.7	32.7	*3.0*	3.1	63.9	*0.9*	583.4	286.5	*2.7*	121.0	156.4	*24.8*	119.0	2622.7	*29.9*
10	0.52	0.51	**333.8**	34.9	29.7	5.6	59.5	19.0	624.8	212.4	38.6	**162.6**	101.5	178.5	178.8	2298.5	2169.6

Table 4. Average data (3 measurements) of different biochemical parameters determined in different organs (R: radicle; P: plumule; RoS: rest of seeds) of 25 maize ZP704 hybrid seedlings exposed to T(reatments) of different molar concentrations of 24-EBL: 1: C(ontrol); 2: $5.2 \cdot 10^{-7}$ M; 3: $5.2 \cdot 10^{-8}$ M; 4: $5.2 \cdot 10^{-9}$ M; 5: $5.2 \cdot 10^{-10}$ M; 6: $5.2 \cdot 10^{-11}$ M; 7: $5.2 \cdot 10^{-12}$ M; 8: $5.2 \cdot 10^{-13}$ M; 9: $5.2 \cdot 10^{-14}$ M and 10: $5.2 \cdot 10^{-15}$ M.. Bold: Maximal values in a series. Italic: Minimal values in a series. (According to Waisi, 2016)

T	Chl a/b	Chla/carr (x+c)	Trehalose (μg/ 0.25 g of dry matter)			Arabinose (μg/ 0.25 g of dry matter)			Glucose (μg/ 0.25 g of dry matter)			Fruktose (μg/ 0.25 g of dry matter)			Sucrose (μg/ 0.25 g of dry matter)		
			RoS	P	R	RoS	P	R	RoS	P	R	RoS	P	R	RoS	P	R
1	**0.77**	**1.53**	104.5	52.2	89.5	20.7	13.0	33.3	1041.4	79.6	55.9	215.3	110.5	63.4	47.6	*228.2*	**2527.6**
2	0.59	*0.57*	95.8	*31.5*	57.1	23.4	*7.6*	37.2	971.0	151.7	**2203.7**	329.3	165.6	**2724.6**	161.0	351.1	2469.6
3	-	-	124.1	458.4	68.6	**26.9**	220.6	37.2	**1271.3**	**1193.0**	85.3	427.9	648.7	154.3	**206.4**	**5884.2**	2097.7
4	*0.58*	*0.57*	*90.5*	471.1	74.8	18.0	**230.9**	23.9	866.1	1070.4	100.2	322.7	**602.0**	69.8	157.4	4749.3	2208.7
5	-	-	110.0	452.0	33.1	13.1	214.9	26.4	873.2	866.8	30.6	**469.4**	479.2	67.9	112.3	4269.4	2369.9
6	*0.58*	0.58	178.6	365.8	181.8	13.7	150.5	47.1	780.0	1076.5	1000.6	335.5	600.4	1000.3	76.3	5051.2	887.3
7	-	-	186.7	276.4	217.3	12.0	155.1	**53.0**	808.5	1054.5	595.3	409.5	590.6	573.0	61.2	4911.6	652.4
8	0.64	0.63	148.9	308.2	65.0	12.3	141.8	39.0	672.5	540.8	163.5	355.4	288.6	144.7	53.6	3331.4	1824.6
9	-	-	**248.4**	862.8	*1.1*	3.1	86.8	*1.8*	*127.3*	176.9	*2.5*	60.7	93.5	*1.9*	8.6	1556.1	*45.9*
10	0.64	0.59	208.8	**1002.4**	**442.1**	*2.9*	67.7	31.1	179.3	*33.7*	112.1	*42.1*	*28.8*	35.0	*4.7*	1169.4	2193.0

lower concentrations of the phytohormone (Tables 3 and 4). We believe that lower 24-EBL concentrations speed up biochemical and metabolic processes in the plumule and radicle of maize seedlings, which grow fast (attention to germination), while seedlings did not suffer from osmotic stress (low trehalose). The effect of BRs on germination and early vegetative growth of maize seedlings was also analyzed and an interesting correlation was revealed in the altered metabolism of sugars and polyphenols under the influence of BRs, which corresponds with high vigour of maize seedlings (Waisi et al, 2015a; Waisi et al., 2017a). The same reports (Waisi et al., 2015a; Waisi, 2017a) revealed that BRs contribute to an irregular distribution of different classes of polyphenols in seedlings as the water-soluble polyphenols primarily occur in the radicle, while lipophyllic polyphenols are primarily associated with the plumule, which may affect the tolerance of maize seedlings to unfavourable environmental conditions during their early vegetative growth. Besides, the effects of BRs on redistribution of micronutrients and heavy metals in maize seedling organs were also analyzed, and a conclusion was made that they prevent their uptake and translocation to key organs (plumule and radicle) of young maize plants, which indicates that these phytohormones may be adequately used in recultivation processes in technogenically degraded soils (Waisi et al., 2017a).

The used methods are explained in detail by Waisi (2016) and Waisi et al. (2015a, 2017a, 2017b).

Treatment of maize plants at the vegetative stage (trials in vegetation pots)

Another type of trials was conducted to examine the effects of 24-EBL ($\approx 10^{-7}$ M) and propiconazole ($\approx 10^{-6}$ M) (inhibitor of BR biosynthesis in plants; Hartwig et al., 2012) on growth, dry weight allocation, accompanying termodynamic changes and changes in photosynthetic parameters in maize plants at the vegetative development stage, simultaneously exposed to manipulation of root status during trial (Tables 5 and 6). PCZ treatment was found to affect the volume of so-called "5" plant roots (Table 5). Changes in Gibbs free energy of whole plant (ΔG_{105tot}) were also observed to be the highest in "5" plants, while PCZ treatment (Table 5) intensified changes in Gibbs free energy in "5→11" plants. It means that "5" plants have higher contents of Gibbs free energy, which further indicates their greater susceptibility to stress, even though the reaction may be modulated

Table 5. Average values of parameters of maize hybrid ZP505 plant growth and matter partitioning and thermodinamic changes during manipulation of root status and plant content of BRs. T – Treatments; P – Parameters: 1: FW (g) leaves; 2: DW (g) leaves; 3: ΔG_{105} leaves (J mol^{-1} K^{-1}); 4: LMR (g g^{-1}); 5: FW (g) stem; 6: DW (g) stem; 7: ΔG_{105} stem (J mol^{-1} K^{-1}); 8: SMR (g g^{-1}); 9: FW (g) root; 10: DW (g) root; 11: ΔG_{105} root (J mol^{-1} K^{-1}); 12: RMR (g g^{-1}); 13: V root (ml); 14: TFW (g); 15: TDW (g); 16: ΔG_{105} tot (J mol^{-1} K^{-1}). FW, DW: Fresh and dry weight of plant parts. 5L, 11L, 5L→11L: plants grown in pots of 5L and 11L volume, and plants first grown in 5L pots and then transferred to 11L pots. Start, End: Start and end of trial. 24-EBL, PCZ: Treatments of plants with 24-EBL ($\approx 10^{-7}$ M) and propiconazole ($\approx 10^{-6}$ M). LMR, SMR, RMR: Relative weight (gg^{-1}) of plant parts: leaf, stem and root. ΔG_{105}: Differential Gibbs energy (J mol^{-1} K^{-1}) of plant parts or whole plant. Bold: Maximal values in a series. Italic: Minimal values in a series (According to Nikolić et al., 2014).

P/T	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Start K 5	*4.94*	*0.46*	0.35	0.56	*3.24*	*0.23*	0.16	0.28	*0.68*	*0.13*	**0.61**	0.16	-	*8.86*	*0.82*	0.31
Start K 11	9.13	0.81	**0.51**	0.59	6.45	0.35	**0.27**	*0.25*	2.28	0.22	0.35	0.16	-	17.86	1.38	0.25
End K 5→11	29.28	2.83	0.39	0.57	34.42	1.52	0.17	0.31	4.50	0.64	0.51	0.13	3.9	68.2	4.99	0.24
End K 5	14.06	1.96	0.36	0.49	15.01	1.16	0.16	0.29	8.09	0.86	0.52	0.22	5.5	37.16	3.98	0.36
End K 11	36.18	3.19	0.52	0.58	38.95	1.61	0.25	0.30	5.09	0.66	0.36	0.12	*3.8*	80.22	5.46	0.22
End 24-EBL 5→11	28.74	3.11	*0.30*	0.57	31.94	1.56	0.14	0.29	5.15	0.78	0.52	0.14	4.8	65.83	5.45	*0.20*
End 24-EBL 5	13.38	2.04	0.32	0.48	15.85	1.22	0.14	0.29	**9.11**	**0.98**	0.48	**0.23**	3.9	38.34	4.24	**0.37**
End 24-EBL 11	**38.58**	3.49	0.47	0.56	**46.01**	**1.98**	0.25	0.32	4.79	0.73	0.35	**0.12**	4.4	**89.38**	**6.20**	0.23
End PCZ 5→11	29.41	3.12	0.29	0.59	31.2	1.53	*0.13*	0.29	3.61	0.64	0.44	0.12	4.3	64.22	5.29	0.27
End PCZ 5	12.80	1.91	0.31	*0.46*	16.45	1.36	0.23	**0.32**	8.66	0.91	0.67	0.22	**6.8**	37.91	4.18	0.37
End PCZ 11	30.65	**3.55**	0.29	**0.61**	32.41	1.69	0.18	0.29	3.99	0.60	*0.32*	0.10	*3.8*	67.05	5.84	0.22

Table 6. Average values of parameters of Chl a fluorescence measured in youngest fully developed leaves of the same maize plants as shown in Table 5. T – Treatments. P – Parameters. Bold: Maximal values in a series. Italic: Minimal values in a series. r.u.: relative unit (According to Nikolić et al., 2014).

P/T	Fv/Fm (r.u.)	Fv/F_0 (r.u.)	Φ PS_2 (r.u.)	qP (r.u.)	NPQ (r.u.)	ETR (μmol elektrona m^{-2} s^{-1})	RFD_{730} (r.u.)
Start K 5→11	0.813	4.361	0.091	*0.278*	3.077	28.90	3.690
Start K 5	0.812	4.361	**0.206**	0.389	3.217	**49.06**	4.739
Start K 11	0.794	4.078	0.156	0.383	2.989	42.43	4.335
End K 5→11	0.786	3.756	0.100	0.305	3.144	21.55	3.925
End K 5	**0.839**	5.250	0.104	0.389	3.376	28.75	4.300
End K 11	0.793	3.836	0.107	0.389	2.944	33.56	3.711
End 24-EBL 5→11	0.836	5.117	0.180	**0.500**	3.876	45.53	5.228
End 24-EBL 5	0.837	**5.283**	0.151	0.333	**5.111**	39.35	**6.444**
End 24-EBL 11	0.792	3.822	0.088	0.389	3.126	22.77	3.788
End PCZ 5→11	0.805	4.137	0.091	0.444	3.182	27.17	4.067
End PCZ 5	*0.753*	*3.066*	0.153	0.472	3.194	38.55	4.183
End PCZ 11	0.785	3.667	*0.081*	0.389	2.799	18.47	3.485

by inhibition of BR synthesis (PCZ treatment). The parameter LMR I was also found to decrease throughout the trial, while RMR increased in "5" plants, regardless of the method of manipulation with BR contents in plants (Table 5). In "11" or "5→11" plants, LMR and SMR slightly increased, and RMR decreased, while PCZ treatment mildly intensified the trend (Table 5). In control plants and those treated with 24-EBL, the ΔG_{105} of leaves and stems of "5" and "5→11" plants were lower or equal to the values read in "11" plants, while the situation was reverse for the root parameter ΔG_{105}. When plants are treated with PCZ, the situation changes, which means that inhibition of BRs synthesis affects Gibbs free energy in some maize organs, and consequently their reaction to stress (Table 5).

What are the effects of treatments on maize photosynthesis, measured by Chl a fluorescence? Plants "5" achieved higher values of RC PS_2 activity indexes (Fv/Fm, Fv/F_0), except plants treated with PCZ, while the corresponding data for "11" plants were the same, regardless of treatment (Table 6). The situation is similar regarding the parameters of photochemical efficacy (ΦPS_2, qP). All NPQ data (parameter of photoprotective processes) were high, which indicates stress in plants during the experiment, irrespective of the type of treatment. Finally, changes in two independent parameters of total photosynthesis (ETR, RFD_{730}) also indicate that maize plants were stressed during trial (low temperature), and treatment with the BR phytohormone had negative effect on "11" plants.

A regression analysis of interaction between the described parameters (morphometric, thermodynamic and photosynthetic parameters) revealed the following significant relationships: thermodynamic parameter $\Delta G_{105\ tot}$ (J mol^{-1} K^{-1}) had a significantly positive association with the Chl a fluorescence parameters NPQ ($R^2 = 0.2193$) and RFD_{730} ($R^2 = 0.2262$) (Figure 1); the relative weight of root (RMR; g g^{-1}) was significantly positively associated with the thermodynamic parameters $\Delta G_{105\ root}$ ($R^2 = 0.8416$) and $\Delta G_{105\ tot}$ ($R^2 = 0.3708$) (Figure 2) and with the Chl a fluorescence parameters Φ PS_2 ($R^2 = 0.1877$), Fv/F_0 ($R^2 = 0.1617$), RFD_{730} ($R^2 = 0.3741$), NPQ ($R^2 = 0.4091$) and ETR ($R^2 = 0.2063$) (data not shown). Conversely, the accumulated total fresh weight (TFW; g) had a negative regression association with the thermodynamic parameters $\Delta G_{105\ root}$ ($R^2 = 0.2425$) and $\Delta G_{105\ tot}$ ($R^2 = 0.3864$) (Figure 3), and the Chl a fluorescence parameters Φ PS_2 ($R^2 = 0.4924$), Fv/F_0 ($R^2 = 0.0583$), RFD_{730} ($R^2 = 0.1807$) and ETR ($R^2 = 0.4472$) (results not shown).

A similar negative regression trend was revealed for the association of dry weight accumulation parameters ln TDW (g) and the thermodynamic parameters $\Delta G_{105\ root}$ ($R^2 = 0.0658$) and $\Delta G_{105\ tot}$ ($R^2 = 0.0866$) (Figure 4), as well as the Chl a fluorescence parameters Φ PS_2 ($R^2 = 0.4910$), Fv/F_0 ($R^2 = 0{,}0079$), RFD_{730} ($R^2 = 0{,}0430$) and ETR ($R^2 = 0.4364$) (data not shown).

Methodology details were reported by Nikolić et al. (2014).

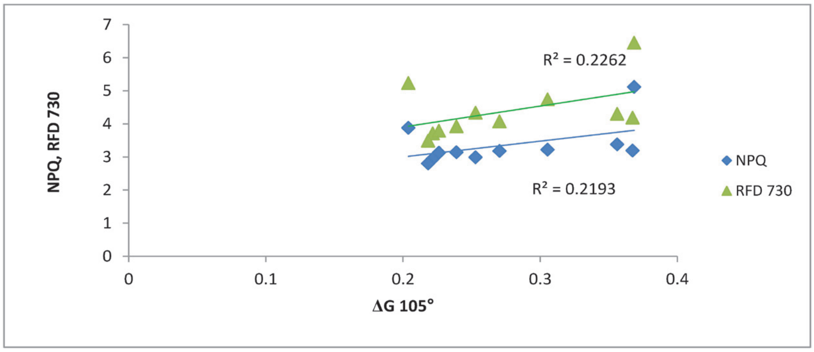

Figure 1. Regression between termodynamic parameter ΔG 105^0 and photosynthetic parameters NPQ and RFD 730 (According to Nikolić et al., 2014).

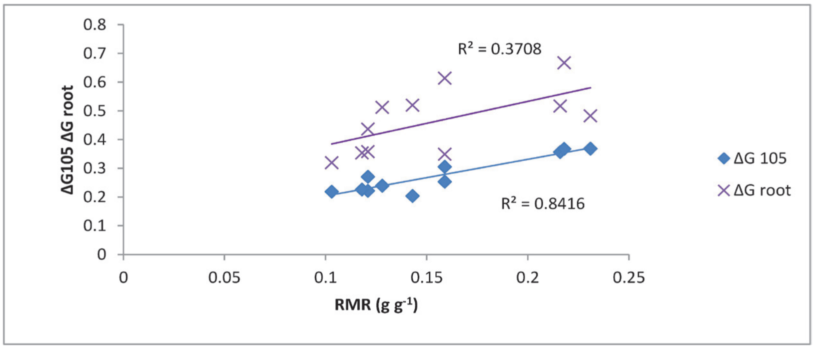

Figure 2. Regression between RMR parameter of plant weight allocation and termodynamic parameters ΔG 105^0 and ΔG root (According to Nikolić et al., 2014).

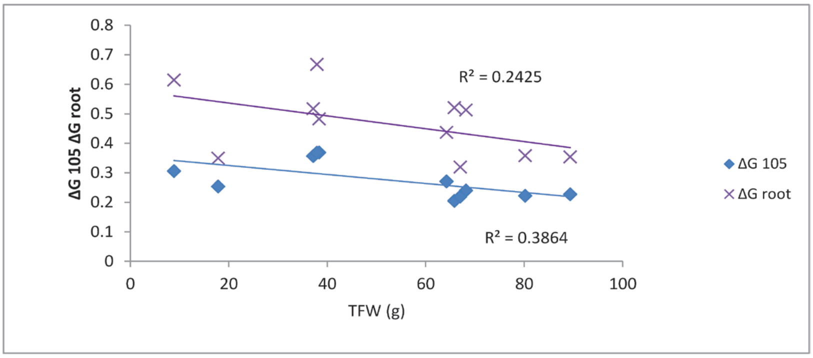

Figure 3. Regression between TFW parameter of plant weight accumulation and termodynamic parameters ΔG 105^0 and ΔG root (According to Nikolić et al., 2014).

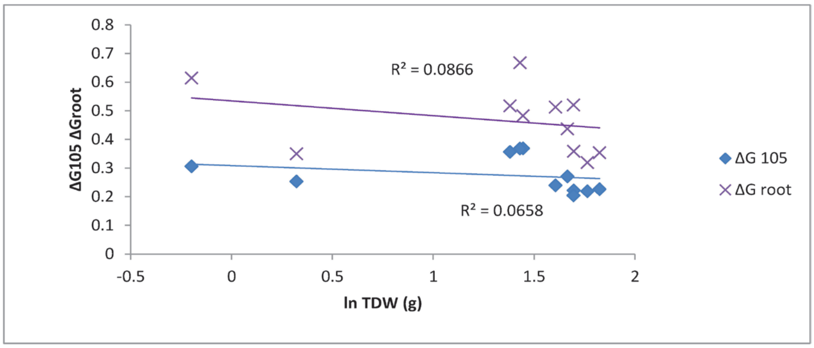

Figure 4. Regression between ln TDW parameter of plant weight accumulation and thermodynamic parameters ΔG 105^0 and ΔG root (According to Nikolić et al., 2014).

Field treatment of maize plants: effects of brassinosteroids on maize plants over entire vegetation season

In a field trial conducted in 2014, no significant difference was found between treatments regarding total yield (t/ha), except for a reduced yield (≈-30% against control) of maize plants treated with the highest 24-EBL concentration of $5.2 \cdot 10^{-7}$ M (Tables 7 and 8). Mean yield was 14.56 t/ha (18% grain moisture), and 13.977 t/ha (14% grain moisture), which is a very good result. However, looking at some yield components and grain chemical composition, including reserves (total proteins, starch), a different situation was observed. In both hybrids (Tables 7 and 8), only treatment with $5.2 \cdot 10^{-7}$ M 24-EBL was found to reduce the number of grain columns per cob (a highly hereditary property, genotype characteristic) from 14-16 to 12 (ZP341), and from 16 to 14 (ZP434), which indicates that this property may be determined also by BRs. Also, treatments of both genotypes with 24-EBL also affected the number of grains per column, reducing them from around 38 in control plants to 33 in the genotype ZP434 treated with the highest 24-EBL concentration of $5.2 \cdot 10^{-7}$ M, and 36 in the genotype ZP341, while the number of grains per column in both genotypes after all other BRs treatments was around 39-40 (Tables 7 and 8), which shows that BRs may affect that yield parameter to some extent.

Table 7. Average values of different yield characteristics of ZP434 hybrid in 2014 field trial. Bold: Maximal values in a series. Italic: Minimal values in a series (according to Waisi et al., 2015b).

Averaged values of yield and different yield components	Treatments during trial							
	Control	$5.2 \cdot 10^{-7}$ mol of 24-EBL	$5.2 \cdot 10^{-9}$ mol of 24-EBL	$5.2 \cdot 10^{-11}$ mol of 24-EBL	$5.2 \cdot 10^{-13}$ mol of 24-EBL	$5.2 \cdot 10^{-15}$ mol of 24-EBL	10^{-6} mol of PCZ	10^{-7} mol of PCZ
Yield (t/ ha) calculated at 14% grain moisture	19.44 ±0.88	*12.01* *±1.85*	19.58 ±2.04	19.97 ±1.22	17.23 ±0.40	**20.04** **±0.10**	18.22 ±0.13	18.67 ±1.04
Weight of cob (g)	63.73 ±3.40	*40.27* *±6.38*	63.87 ±4.55	66.27 ±4.09	56.13 ±2.34	65.6 ±2.43	**66.40** **±3.12**	62.67 ±2.27
Grain weight/cob weight ratio (%)	87.94 ±0.93	*85.92* *±0.34*	87.69 ±1.88	87.74 ±0.75	87.10 ±1.18	88.17 ±1.39	**88.28** **±1.47**	87.10 ±0.32
Number of grain columns in cob	15.33 ±1.63	*14.17* *±1.95*	15.58 ±1.56	15.83 ±1.55	15.75 ±1.48	15.17 ±1.01	**15.92** **±1.50**	15.58 ±1.56
Number of grains in grain column	37.62 ±4.34	*32.67* *±6.04*	**40.33** **±4.61**	40.17 ±4.62	39.12 ±4.80	40.21 ±4.02	39.79 ±3.40	39.67 ±4.22

Table 8. Average values of different yield characteristics of ZP341 hybrid in 2014 field trial. Bold: Maximal values in a series. Italic: Minimal values in a series (According to Waisi et al., 2015b).

Yield and different yield components, average	Treatments during trial							
	Control	$5.2 \cdot 10^{-7}$ mol of 24-EBL	$5.2 \cdot 10^{-9}$ mol of 24-EBL	$5.2 \cdot 10^{-11}$ mol of 24-EBL	$5.2 \cdot 10^{-13}$ mol of 24-EBL	$5.2 \cdot 10^{-15}$ mol of 24-EBL	10^{-6} mol of PCZ	10^{-7} mol of PCZ
Yield (t/ ha) calculated at 14% grain moisture	17.28 ±1.59	*11.46* *±1.46*	16.84 ±2.04	18.03 ±1.41	17.77 ±0.83	17.44 ±1.91	**19.20** **±1.62**	18.03 ±1.37
Weight of cob (g)	60.80 ±4.85	*41.67* *±6.00*	59.47 ±7.42	61.67 ±4.47	62.00 ±0.80	59.93 ±4.92	**65.20** **±3.20**	63.33 ±2.95
Grain weight/cob weight ratio (%)	87.06 ±0.93	*85.58* *±1.59*	86.73 ±1.42	87.38 ±0.48	**88.01** **±1.72**	87.30 ±0.35	86.54 ±1.07	86.23 ±0.99
Number of grain columns per cob	14.38 ±0.53	*12.75* *±1.66*	15.08 ±1.56	14.75 ±1.29	14.83 ±1.17	14.75 ±1.65	**15.17** **±1.66**	14.67 ±1.63
Number of grains in grain column	38.25 ±1.06	*36.38* *±1.59*	39.17 ±3.80	**41.42** **±3.89**	42.17 ±3.67	39.54 ±3.93	40.71 ±3.63	38.17 ±4.52

Higher 24-EBL concentrations in ZP434 hybrid were found to mainly increase the content of biochemical parameters, while the application of PCZ, as an inhibitor of BRs biosynthesis, had non-conclusive effects on their contents (Table 9). In hybrid ZP341 (Table 10), higher 24-EBL concentrations were found to mainly increase the content of biochemical parameters, while the application of PCZ, as an inhibitor of BRs biosynthesis, mainly decreased their contents. These findings were consistent with literature data (Hola et al, 2010).

Table 9. Average values of relative content (% against control) of different chemical and biochemical parameters in crude extract of ZP434 maize grain from 2014 field trial. Absolute control values of different parameters: 1. Starch: 74.60%; 2. Total phenols: 260.05 μg/g; 3. Moisture: 9.95%; 4. Total proteins: 7.16%; 5. Total oil: 3.45%; 6. Pphy: 3.22 mg/g; 7. Pi: 0.36 mg/g; 8. GSH: 1053.63 nmol/g; 9. K: 3185.12 mg/g; 10. Ca: 36.38 mg/g; 11. Mg: 384.64 mg/g; 12. Fe: 5.08 μg/g; 13. Zn: 6.10 μg/g; 14: Si: 23.88 μg/g. Bold: Maximal values in a series. Italic: Minimal values in a series (According to Waisi et al., 2015b).

Relative content different compounds (% against 100% of control)	Treatments during trial							
	K	$5.2 \cdot 10^{-7}$ of 24-EBL	$5.2 \cdot 10^{-9}$ of 24-EBL	$5.2 \cdot 10^{-11}$ of 24-EBL	$5.2 \cdot 10^{-13}$ of 24-EBL	$5.2 \cdot 10^{-15}$ of 24-EBL	10^{-6} of PCZ	10^{-7} of PCZ
Starch	**100**	98.19	99.60	98.86	95.51	98.39	*95.17*	98.86
Total phenols	100	99.73	94.51	**148.63**	95.88	114.01	*92.03*	96.98
Moisture	100	**111.06**	*96.48*	104.52	108.04	108.04	110.05	105.02
Total proteins	*100*	108.72	101.19	105.58	**118.42**	102.51	115.42	107.47
Total oils	100	101.45	95.65	97.10	**105.80**	102.90	98.55	*94.20*
Pphy	100	100.73	95.62	*95.25*	99.03	102.31	103.16	**108.03**
Pi	100	**111.59**	100.29	96.01	107.98	98.10	97.44	*77.01*
GSH	100	122.21	*87.11*	110.69	**130.92**	107.73	104.02	117.43
K	100	99.33	95.76	98.25	96.19	**100.67**	97.99	*93.82*
Ca	100	79.90	122.53	145.37	478.45	89.92	*68.50*	**2.755.82**
Mg	100	95.62	*78.81*	100.80	93.66	96.95	108.98	**112.02**
Fe	*100*	103.57	111.33	156.34	208.87	322.84	319.21	**384.17**
Zn	100	73.04	*49.26*	55.97	49.31	91.75	62.74	**118.40**
Si	100	118.65	88.89	80.20	88.01	99.16	*77.66*	**127.72**

Table 10. Average values of relative content (% against control) of different chemical and biochemical parameters in crude extract of ZP341 maize grain from 2014 field trial. Absolute control values of different parameters: 1. Starch: 70.95%; 2. Total phenols: 243.62 μg/g; 3. Moisture: 10.80%; 4. Total proteins: 8.20%; 5. Total oil: 3.80%; 6. Pphy: 3.45 mg/g; 7. Pi: 0.28 mg/g; 8. GSH: 1908.14 nmol/g; 9. K: 2895.06 mg/g; 10. Ca: 138.36 mg/g; 11. Mg: 436.60 mg/g; 12. Fe: 8.47 μg/g; 13. Zn: 3.98 μg/g; 14: Si: 23.63 μg/g. Bold: Maximal values in a series. Italic: Minimal values in a series (According to Waisi et al., 2015b).

Relative content different compounds (% against 100% of control)	Treatments during trial							
	K	$5.2 \cdot 10^{-7}$ of 24-EBL	$5.2 \cdot 10^{-9}$ of 24-EBL	$5.2 \cdot 10^{-11}$ of 24-EBL	$5.2 \cdot 10^{-13}$ of 24-EBL	$5.2 \cdot 10^{-15}$ of 24-EBL	10^{-6} of PCZ	10^{-7} of PCZ
Starch	100	*99.37*	101.55	101.69	99.58	102.04	**102.61**	101.55
Total phenols	**100**	**100**	94.13	90.62	91.50	93.55	94.72	*82.40*
Moisture	100	102.78	101.39	**104.17**	101.39	98.15	*98.61*	*98.61*
Total proteins	100	**105.61**	102.07	97.32	108.11	98.90	*91.34*	101.95
Total oils	100	93.42	89.47	**101.32**	90.79	89.47	*86.84*	93.42
Pphy	100	**101.25**	96.48	100.34	98.86	94.09	96.70	*95.23*
Pi	100	**122.82**	*84.46*	84.95	87.42	99.26	117.02	110.49
GSH	**100**	87.44	82.88	79.66	73.26	84.89	*53.79*	82.38
K	100	105.17	98.89	86.36	*76.64*	89.60	**105.47**	88.30
Ca	100	*30.88*	43.33	86.28	**118.35**	43.63	32.08	32.43
Mg	**100**	*79.83*	90.87	84.34	96.90	88.53	82.63	89.75
Fe	100	*53.71*	67.29	**155.44**	142.75	71.87	60.35	101.22
Zn	100	*81.15*	97.39	**159.54**	-	-	-	92.54
Si	100	**109.27**	97.91	79.49	*64.95*	69.44	76.68	85.90

All data discussed here infer that the brassinosteroid phytohormone product (24-EBL) used in our experiments acted on maize plants at their different growth stages: a) during germination and early stages of seedling growth; b) during vegetative stages; c) over the entire growth season in the field. The highest impact on physiological processes, and consequently on stress tolerance, including better yield, is possible to achieve in the early stages of germination and vegetative growth. The available methodology has confirmed the influence of a moderate concentration of 24-EBL towards changes in metabolic pathways, which may increase seedling vigour in the critical early stages of growth. Differences in genotypic response to the applied 24-EBL concentrations were also noted. Treatments with the BRs product at the later stages of vegetative growth have shown some ineffectiveness under stress episodes of different intensity (low temperature), so that applications of triazole-based inhibitors of BR biosynthesis should perhaps be given priority in order to modify the endogenous process of BR synthesis, while the former should not be necessarily be excluded. Finally, all these findings were tested in the field and preliminary results show that foliar treatments of maize plants at the recommended stages have no significant effect on yield, but may influence specific components of yield and chemical composition of maize grain, which may be important in some practical situations.

Methods applied in these trials were explained in detail by Waisi et al. (2015b).

Field treatment of other crops with brasinosteroids: effects on other crops over entire vegetation season

Finally, we present some partial results of micro-trials conducted in different other crops. The effects of brassinosteroids on yield and yield components (Nikolić & Waisi, 2012), and on plant protection (Stevanović et al., 2012) of apples, were tested. Given that cytochrome P450 oxidase is one of the key factors in detoxification of pesticides, the effects of BRs on yield and components of apple yield (Nikolić & Waisi, 2012), and also on plant protection (Stevanović et al., 2012), were tested at optimal and reduced doses of fungicides simultaneously applied in two apple orchards (cv. „Idared"). In the first orchard, the evaluated yield/ha of 24-EBL-treated apples is the same as in control plots, and the pomological and fruit quality parameters of apples were comparable. In the second orchard, the evaluated yield/ha of 24-EBL-treated apples was higher by almost a quarter than the apple yield from control plots (treated with half and full doses of fungicides) and other treatments, also with comparable pomological and fruit quality parameters of apple fruits. Considering the aspect of plant protection, these procedures were also satisfactory with 78.71% and 77.69% plant protection efficacy using 24-EBL+half fungicide doses for treatment of leaves and fruits (compared to 84.17% and 87.90% efficacy when using full fungicide doses for treatment) in the first orchard, which is a satisfactory result. These results are very similar to findings reported by other researchers (Clouse & Sasse 1998; Khripach et al. 2000).

We also examined the influence of the BRs-based preparation on yield and yield components in soybean and barley (Dragicevic & Stojkovic, 2016; Dragičević et al. 2016a, b).

Three soybean genotypes were treated (ZP-015, "Nena", and "Laura") with 24-EBL-based, and with other non-standard fertilizers (based on plant extracts), as a type of biofortification. This approach was found to be less affected by alterations in Pphy (content of phytic phosphorus), an important factor which restrains the availability of mineral nutrients. It was only regarding Zn that this dependence was significant, where lowering Pphy at the same time increased Zn concentration in grain. Moreover, the influence of β-carotene is significant for the availability of mineral nutrients, but more important is the fact that its increase is linked with a parallel Fe increase, mainly in grains of higher weight, as part of a better yielding potential. It is important to stress that the ratios between Pphy, β-carotene and the mineral nutrients could be modified to some degree by applying foliar fertilizers to potentially increase the availability of mineral nutrients, but this also depends on soybean variety. The 24-EBL-based preparation and a plant extract (Zircon) were efficient in decreasing the mentioned ratio in ZP-015 and "Nena" grains, while some plant extracts (Zlatno inje and Zircon) were efficient for "Laura". Also, the correlation between 1,000 grain weight (as a significant yield component) and β-carotene and Zn contents in soybean grain is very significant (Dragičević et al. 2016b).

In late winter of two different years, we sowed hull-less barley (*Hordeum vulgare* L. var. *nudum*; cv. "Apolon"), and after that, in the following spring, we treated the crop with the 24-EBL-based preparation and with other non-standard fertilizers (based mainly on plant extracts and other phytohormones). After the summer harvest, we assessed crop yield (at 14% grain moisture; kg ha^{-1}) and determined different chemical ingredients in barley grain. The results (Dragičević et al., 2016a) indicate that the timing of treatment (year) affected barley grain yield and chemical composition, and the highest impact was found for Si under unfavourable conditions. The applied treatments were most effective regarding grain yield and increase in grain quality, mainly by reducing the Pphy/β-carotene ratio and increasing GSH content, thus increasing the potential bioavailability of the examined mineral elements. What is more, the stress resulting from high amounts of precipitation could be mitigated by applications of fertilizers to increase the potential bioavailability of P, Mg, Ca and Fe. Generally, the 24-EBL preparation influenced the contents of P_i, Zn and Fe, and other fertilizers mainly affected potential availability of some other nutritive factors (Ca, Mn, Si and GSH).

Based on previous field trials on one fruit (apple) and two field crops (soybean and barley), we concluded that, when compared with other non-standard fertilizers, the preparation based on 24-EBL has effect on the quality and chemical composition of crops, rather than on their yield (Nikolić & Waisi 2012; Dragičević et al. 2016a, b), and it acts to protect crops under stressful conditions (Stevanović et al. 2012).

Comparison of our findings with reports from other studies examining BRs effects on crops - Guidelines for the future

How does this research relate to other studies and modern agricultural practices? Contrary to a molecular paradigm (the usual present day method of testing BRs) (Vriet et al. 2013), aiming to optimize crop traits for better yield (Vriet et al. 2012) and crop resistance to ambiental stresses (Bajguz & Hayat, 2009), we approached the problem from a different point of view. Firstly, terrestrial plants (which include all crops) are thermodynamically open systems (like all other living creatures) which, for reasons of survival, growth and reproduction, exchange matter and energy with the environment. But unlike animals, higher plants with their sesile life style and poikilothermal metabolism had to develop a completely different strategy in order to obtain resources for survival and reproduction. This allows approaching the problem from a cybernetic point of view (Ashby, 1957), examining the energy, as well as matter entry and exit in plant systems without extensive examinations of plant structure, imposed by the molecular paradigm. Such an approach is also used in research of the effects of BRs, especially in the so-called crosstalks of BRs with other phytohormones (Sankar et al. 2011), similar to some earlier studies of cell metabolite fluxes. But insights into the processes occurring in the seed and seedling system, and developing under the influence of different 24-EBL constellations, which are defined as almost "perfect" a correlation enthalpy-entropy effect (Waisi et al., 2017b; Waisi 2016), point to a possibility that problems regarding plant development under the influence of brassinosteroids can be clarified purely by thermodynamic-cybernetic considerations. What is the point of this new approach in the context of requests coming from modern agriculture? In that context it is possible to compare the reactions of seedlings of various crops and their genotypes to environmental stresses without entering the methodologically demanding examination of molecular bases of plant resistance to stress, while retaining a significantly higher degree of reliability compared to classical biotests, particularly tests of the effects of agrochemicals such as a BRs-based preparation.

Analyzing plants at a higher level, as the system of whole individual plant, we note that regardless of various manipulations of the status of leaves and roots, and whether or not the plant is in a state of stress, the system of the whole plant is very dependent on an interplay of energy production and transformation of that energy into redistributed masses of plant organs, and invested in plant growth, which opens a possibility of monitoring energy transformations under the influence of agrochemicals that affect the level of BRs throughout the plant by proven methods such as Chl a fluorescence (Lichtenthaler & Miehe, 1997; Maxwell & Johnson, 2000).

Finally, at the level of crop agrophytocenoses, and besides BR effects on other crops (Nikolić & Waisi 2012; Stevanović et al. 2012; Dragičević et al. 2016a, b), we notice that along with small differences in bioproduction (Tables 7 and 8) of maize crops treated with different doses of 24-EBL and PCZ, a great diversity of changes occur in maize metabolic processes (synthesis of different compounds, such as phenols, proteins and oils and absorption of various elements) under the influence of different BR treatments (Tables 9 and 10). All this points to a "network" of signals (made by BRs, other hormones, and non-hormonal signal pathways) that are "hiding" behind this phenomenon, which point not to determinism (which implies a molecular paradigm) but to the stochasticity of these processes, based on the flow of energy and matter. The shohasticity of the process that influences the quality of yield indicates that more careful planning of the application of agrochemicals (in our case based on BRs phytohormones) is needed.

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Brasinosteroidi kao regulatori rasta biljaka i modulatori uticaja pesticida i đubriva

REZIME

Način delovanja agrohemikalija na biljke podrazumeva ukupan uticaj na metabolizam, rast i razvoj biljaka. U tom smislu u ovom radu je prikazan efekat 24-epibrasinolida (24-EBL), kao klase fitohormona brasinosteroida, na rast i druge fiziološke procese u biljkama kukuruza u različitim dozama i u različitim razvojnim fazama, kako bi se procenio uticaj na razne faktore koji određuju prinos ovog važnog poljoprivrednog useva. Pored toga, dato je nekoliko primera efekata ovih fitohormona na druge useve, voće i povrće, u smislu njihovog uticaja na prinos, kvalitet prinosa i povećanje otpornosti useva na neke vrste stresa. Rezultati su diskutovani u odnosu na druge podatke iz literature.

Ključne reči: Fitohormoni; Brasinosteroidi; Rast biljaka; Ishrana biljaka; Zaštita bilja