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# Úloha brassinosteroidů v odpovědi různých genotypů kukuřice na změny v zásobování vodou.

# Role of brassinosteroids in response of various maize genotypes to water supply changes.

Diplomová práce

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**PROHLÁŠENÍ:**

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Podpis

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**ABSTRAKT**

Brassinosteroidy (BR) zmírňují poškození způsobené vodním stresem a zlepšují toleranci rostlin k vodnímu deficitu. Máčení obilek kukuřice před výsevem v roztoku 24-epibrassinolidu o různých koncentracích (zejména $10^{-8}$ M) mělo před a v průběhu období stresu pozitivní vliv na růst rostlin, přestože neovlivnilo hodnoty relativního obsahu vody a fotosyntetické parametry. Po zotavení rostlin v optimálních podmínkách nebyly mezi ošetřeními pozorovány žádné rozdíly. Obecně nebyly prokázány žádné výhody máčení obilek ve srovnání s postřikem, ačkoli pozitivní vliv máčení obilek na klíčení rostlin je možný v podmínkách vodního deficitu. Na základě výsledků z druhé série pokusů není možné usuzovat na roli exogenní aplikace BR (postřik 24-epibrassinolidem) a endogenní biosyntézy BR (postřik inhibitorem biosyntézy – brassinazolem) v rostlinách vystavených vodnímu deficitu. Avšak pokles relativního obsahu vody v protikladu s nárůstem obsahu chlorofylů v dnech následujících po nástupu stresu suchem v obou kultivarech (lišících se citlivostí vůči suchu) vybízí k dalšímu zkoumání této problematiky. Pochopení role BR v toleranci a odolnosti rostlin vůči suchu je důležité nejenom pro základní výzkum, ale mohlo by se stát důležitým předpokladem pro šlechtění plodin tolerantních vůči suchu v určitých podmínkách prostředí.

**KLÍČOVÁ SLOVA:** brassinosteroidy, 24-epibrassinolid, brassinazol, genotypy *Zea mays* L., abiotický stres, vodní deficit, fotosyntéza, vývoj rostliny

**ABSTRACT**

Brassinosteroids (BRs) have been recognized to alleviate damages caused by drought stress and to enhance tolerance to water deficit. Soaking of maize kernels before the sowing in solution with different 24-epibrassinolide concentrations (especially the $10^{-8}$ M) had a positive effect on growth of plants before and after the stress period, although it did not affected relative water content and photosynthetic parameters. After the recovery under optimal conditions there were no differences among the treatments. When compared to spraying, soaking of maize kernels was not proved to have any advantages, though its positive effect on plants germination under water deficit conditions could be possible. Based on the results of the second season experiment it is not possible to deduce the role of exogenous BRs application (spraying with 24-epibrassinolide) or endogenous BRs biosynthesis (spraying with biosynthesis inhibitor – brassinazole) in maize plants subjected to water deficit. However, decrease in relative water content in contrast to chlorophylls content increase during the days following after drought stress onset in both cultivars (contrastive in drought sensitivity) challenges for further investigation of this problem. Understanding the role of BRs in plant drought tolerance and resistance is important not only for the basic research but could become an important prerequisite for breeding drought tolerant crops under specific environmental conditions.

**KEYWORDS:** brassinosteroids, 24-epibrassinolide, brassinazole, *Zea mays* L. genotypes, abiotic stress, water deficit, photosynthesis, plant development

# CONTENTS

## ABBREVIATIONS

| | |
|---|---|
| AA | ascorbic acid oxidase |
| ABA | abscisic acid |
| a.m. | *ante meridiem*; before midday |
| APX | ascorbate peroxidase |
| BL | brassinolide |
| BR | brassinosteroid |
| BRs | brassinosteroids |
| BRZ | brassinazole |
| BZR1 | brassinazole-resistant-1 |
| CAT | catalase |
| CK | cytokinin |
| DM | dry mass |
| DST | daylight saving time; summertime |
| DWF4 | DWARF4 |
| EBR | 24-epibrassinolide |
| $F_0$ | minimal fluorescence of Photosystem II |
| $F_m$ | maximal fluorescence of Photosystem II |
| $F_V/F_m$ | maximum quantum yield of Photosystem II |
| FM | fresh mass |
| GA | gibberellin |
| GAI | gibberellic acid-insensitive |
| GAs | gibberellins |
| GR | glutathione reductase |
| HBR | 28-homobrassinolide |
| IAA | indole-3-acetic acid |
| JA | jasmonic acid |
| NDVI | normalized difference vegetative index |
| PEG | polyethylene glycol |
| POD | peroxidase |
| PRI | photochemical reflectance index |
| ROS | reactive oxygen species |
| RuBisCo | ribulose-1,5-bisphosphate carboxylase oxygenase |
| RWC | relative water content |
| SOD | super oxide dismutase |
| TM | turgid mass |
| QY | quantum yield of Photosystem II |
| ZR | trans-zeatin riboside |

# 1. INTRODUCTION

Maize or corn, *Zea mays* L., is an annual arable crop. It is well adaptable and therefore widely world spread. It is a monoecious unisexual plant with number of haploid chromosomes n = 10. Usually it grows 1 up to 3 meters high and leafy stalk produces ears with kernels rich for starch. Due to C4 carbon fixation during the photosynthesis and good water management it produces large quantities of biomass. Maize is one of the most important crops in the world. It is grown to be used for a human consumption, as an animal feed or as a chemical feedstock. It is also a model organism for genetics and developmental biology. It is as well one of the most important genetically modified plants.

Decrease in yield of agricultural crops due to drought stress is a worldwide problem. Therefore drought tolerant or even drought resistant plants are demanded in still greater extent. Despite of many partial successes, fully drought resistant plant have not been reported up to this day. However, there have been plenty of reports of increased plant drought tolerance. One of the substances which help plants to increase their drought tolerance and overcome drought period are brassinosteroids (more in the first part of Literature Review).

Brassinosteroids (BRs) are steroid phytohormones occurring ubiquitously in lower and higher plants. They were found in every plant organ, particularly in pollen, seeds and young tissues. Since their recognition nearly 40 years ago, BRs have been proven to have various effects on plant growth and developmental processes. Their positive effects in plant defence against biotic and abiotic stresses (drought stress being one of them) was confirmed, as well. Although a considerable fragment of molecular mechanisms behind these processes have been revealed in the past years, experiments published in the recent time prove that there is still greater part which remains uncovered. Whether we talk about endogenously synthesized or exogenously applied BRs, the difference between tissues, organs, life stages or genetic background of plants, the way of application, and the amount and kind of brassinosteroid (BR) applied, or the type and duration of drought stress conditions as well as growth conditions of plants, we still do not understand physiological and biochemical changes induced in plants. To understand this complex actuation better,

studies on transcriptome, proteome and metabolome level changes are implemented, as well as studies focused on BRs crosstalk with other phytohormones or substances.

Experiments presented in this diploma work were done in connection with the longtime experiments focused on drought stress tolerance in different plant species and BRs effects evaluation experiments in the Laboratory of Plant Genetics at the Department of Genetics and Microbiology at Charles University in Prague, Faculty of science.

In these experiments, 24-epibrassinolide (EBR), one of the most stable BRs, was used to assess effects of bioactive BR exogenous application on drought tolerance of plants subjected to stress due to water deficit. As a complementary tool, brassinazole (BRZ), the inhibitor of BR biosynthesis, was used to confirm the role of endogenous BRs in plants subjected to drought stress conditions (more about BRZ in the second part of Literature Review). These experiments, focused on the role of BRs in maize drought tolerance, were investigating characteristics which had never been studied before in this plant material and under these conditions. Therefore it could turn into a valuable piece of this complicated puzzle one day.

With understanding the role of BRs in plants we can ameliorate yield losses of arable plants due to water deficit. Compared to the exogenous application in the agriculture crop production, transgenic plants seems to implement our current stage of knowledge of this topic much better. However, this would not be possible without experiments performed in the laboratory or greenhouse conditions, with exogenous application or endogenous levels evaluation, and without complementary tools as BR-insensitive and BR-deficient mutants as well as BR biosynthesis inhibitors.

## 2. AIMS OF THE THESIS

This work was written with a view to contribute to the knowledge of the role of BRs in maize plants of various genotypes subjected to water supply changes. To evaluate effects of BRs on maize drought resistance, two season of experiments were performed.

In the autumn season, it was evaluated:

a) if soaking of maize kernels in EBR prior to the sowing is a suitable way of BR application;
b) which of the EBR concentrations was the most effective in helping the plants to overcome the stress induced by water deficit and to recover after their return under optimal growth conditions.

In the spring season, there was investigated:

a) the role of exogenous BR application (spraying the maize plants with EBR);
b) the role of endogenous BR biosynthesis inhibition (spraying the maize plants with BRZ);

both before the plants were subjected to water deficit by withdrawing their water supply.

# 3. LITERATURE REVIEW

## 3.1 BRASSINOSTEROIDS AND DROUGHT STRESS

BRs have been recognized to have effect on plants growth and development as well as plant defense against various kinds of biotic and abiotic stress. Drought stress is considered to be the most aggravating factor of plant production in areas with rainfall deficiency. It induces variety of plant responses, from those at molecular to those at morphological level, which are evident at every phenological stage of plant development. BRs alleviate damages caused by drought stress and enhance tolerance to water deficit. To examine mechanism(s) of these effects, following experiments were performed.

### 3.1.1 BRASSINOSTEROIDS AND IMPROVED YIELD

In plants subjected to drought stress, oxidative damage, impaired water relations, which are influenced by relative water content (RWC), leaf water potential, stomatal resistance, rate of transpiration etc., and overall photosynthesis reduction is found. Subsequently, the growth and yield of crops is decreased.

As many experiments later, those first were focused on increasing the yield in plants subjected to drought stress by BR treatment. Besides, other parameters were investigated in an effort to understand the effects observed. SCHILLING *et al.* (1991) examined influence of BR treatment on sugar beet plants under the water stress relations. Greenhouse cultivated *Beta vulgaris* L., cultivar Ponemo plants were sprayed with 100 or 1,000 $mg.ha^{-1}$ of 28-homobrassinolide (HBR) after the first true leaves had appeared, at each level of water supply. These were referred to as “normal”, “mild stress” and “high stress”, correspondingly to 80 %, 40-50 % and 25-30 % of the maximal substrate water capacity. After 6-7 weeks under the both stress treatments HBR increased taproot dry mass as well as the sucrose content in plants subjected to high stress, while in plants subjected to mild stress the sucrose content did not change. Simultaneously with dry mass increase, increase in leaf length and leaf area was observed, consistently with an increase in acid invertase

activity in young leaves and taproots. This led to production of more assimilates and subsequently to the increased yield. Based on the observation that HBR affected yield only in the case of drought stress, it was concluded that this compound is able to reduce damage caused by drought stress. Sugar content and sugar yield were increased only in the case of high drought stress. Further, authors presumed that effects of mild drought stress were connected with a higher concentration of endogenous auxins. In their other experiments they found out that auxin (2,4-dichlorophenoxyacetic acid) increased the invertase activity in leaves of sugar beet plants likewise.

JANECZKO *et al.* (2010) examined the effect of exogenous EBR treatment on wheat production in the field experiment, which was performed when drought conditions occurred during the growing season. After the preliminary tests, 1 $mg.dm^{-3}$ concentration of EBR was chosen for 48 hour seed soaking and 0.25 $mg.dm^{-3}$ concentration for spraying plants at the heading stage. EBR application increased grain yield of *Triticum aestivum* L., cultivar Torka and Cytra plants by about 20 %. In wheat grains, endogenous brassinolide (BL), castasterone and 24-epicastasterone were found, while exogenously applied EBR was not accumulated in newly formed grains.

In 1994, SAIRAM (1994b) studied the influence of BR treatment on two contrasting cultivars of wheat under moisture stress/rainfed conditions. Plants of two *Triticum aestivum* L. cultivars, C306 (drought resistant) and HD2329 (drought susceptible), were grown in pots and in the field. 0.01 or 0.05 ppm HBR was applied by 6 hours lasting seed soaking or foliar spraying 25 days after sowing. In the pot experiment, plants were subjected to water stress by withholding the water supply for 7 days at the anthesis stage. In the field experiment, plants were cultivated under irrigated and rainfed conditions. HBR application increased relative water content (RWC), nitrate reductase activity, chlorophyll content and photosynthesis, and improved membrane stability in both stressed and control plants. These resulted in higher leaf area, biomass production, grain yield and yield-related parameters (e.g. ear number *per* meter row, grain number *per* ear, 1,000 grain weight, harvest index) in the treated plants. The 0.05 ppm treatment was more effective than the 0.01 ppm treatment and both were significantly better than the untreated control. Plants of C306 genotype

responded to HBR application under stress/rainfed conditions generally better, however, plants of HD2329 genotype showed higher nitrate reductase activity under stress/rainfed conditions and increased biomass under both conditions, on a percentage basis. The author presumed that HBR-induced drought tolerance was related to increased water uptake, membrane stability and higher carbon dioxide and nitrogen assimilation rates.

In another paper published the same year SAIRAM (1994a) experimented with *Triticum aestivum* L., cultivar C306 plants cultivated in pots. The HBR of 0.1 or 1.0 ppm concentration was sprayed on leaves 30 and 32 days after sowing. At the time of anthesis the water supply was withhold for one set of plants for 7 days, and then they were allowed to recover. Metabolic activities of irrigated plants and plants subjected to moisture stress were estimated at the anthesis stage, while yield and yield-related parameters (mentioned above) were recorded at the harvest. The HBR treatment increased RWC and transpiration in leaves and decreased diffusion resistance in water stress subjected and recovered plants. Further, nitrate reductase and glutamin synthase activities, photosynthesis, chlorophyll and total soluble protein content, yield and yield attributing parameters in stressed, irrigated and revived plants were increased in HBR-treated plants. The author concluded that the HBR-induced promotion in metabolic activity was mediated through increased enzyme protein synthesis and uptake of water, what resulted in enhanced RWC and better revival of moisture-stressed plants.

As also presented here, water stress leads to various changes in plants, including stomatal closure, changes in the composition of the cell or plasma membrane as well as activity of various enzymes and substances, and can result in impaired growth as turgor and photosynthesis decrease (JAGER *et al.* 2008, FAROOQ *et al.* 2009b). Among other abilities, BRs were proved to have stimulatory effect on photosynthesis, particularly on chlorophyll and total carotenoids content, maximum quantum yield of Photosystem II ($F_v/F_m$), ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCo) activity, and subsequently net photosynthetic rate and carbon dioxide assimilation (SAIRAM 1994a, 1994b, ZHANG *et al.* 2008, FARIDUDDIN *et al.* 2009, FAROOQ *et al.* 2009a, YUAN *et al.* 2010, LI *et al.* 2012, HU *et al.* 2013) in plants subjected to water stress, what resulted in their better survival and improved growth.

However, as experiments focused on increased yield under these conditions had a poor reproducibility of BR efficiency, definitive genetic and biochemical proof of BRs ability to modulate plant stress responses was demanded (KRISHNA 2003).

### 3.1.2 BRASSINOSTEROIDS AND ANTIOXIDANT SYSTEM ACTIVATION

BRs are involved in drought resistance mechanisms of plants at many levels. These mechanisms are connected with aquaporins, stress proteins and signalling at molecular level, drought stress escape, drought avoidance and phenotypic flexibility at morphological level, and osmoprotection, osmotic adjustment and antioxidant scavenging defense system at physiological level.

It is well known that drought stress is manifested as one of the oxidative stresses. Its harmful effect is mediated particularly by reactive oxygen species (ROS). Membrane damage and subsequently deterioration of plant water management are caused consequently. Plant defense leans mainly on changed expression of stress-related genes and in antioxidant system activation. Activity of antioxidant enzymes, which prevents cells from oxidative damage, is one component of this system. Super oxide dismutase (SOD) detoxifies free superoxid radical anions by production of peroxide without damaging chloroplasts, nucleic acids or proteins. The peroxide can be further detoxified by catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) activity. Another antioxidant enzyme, glutathione reductase (GR) and ascorbic acid oxidase (AA) are also involved in cell antioxidant defense system (Fig. 1). Another component of antioxidant system is the production of ROS scavenging molecules (ascorbate, glutathion, tocopherols, soluble phenolics, anthocyanins, proline), which also stabilize membranes, or production of molecules protecting chlorophylls (carotenoids). Both these components are managed *via* hormone signaling, with BRs being recognized as one type of hormones participated in this management.

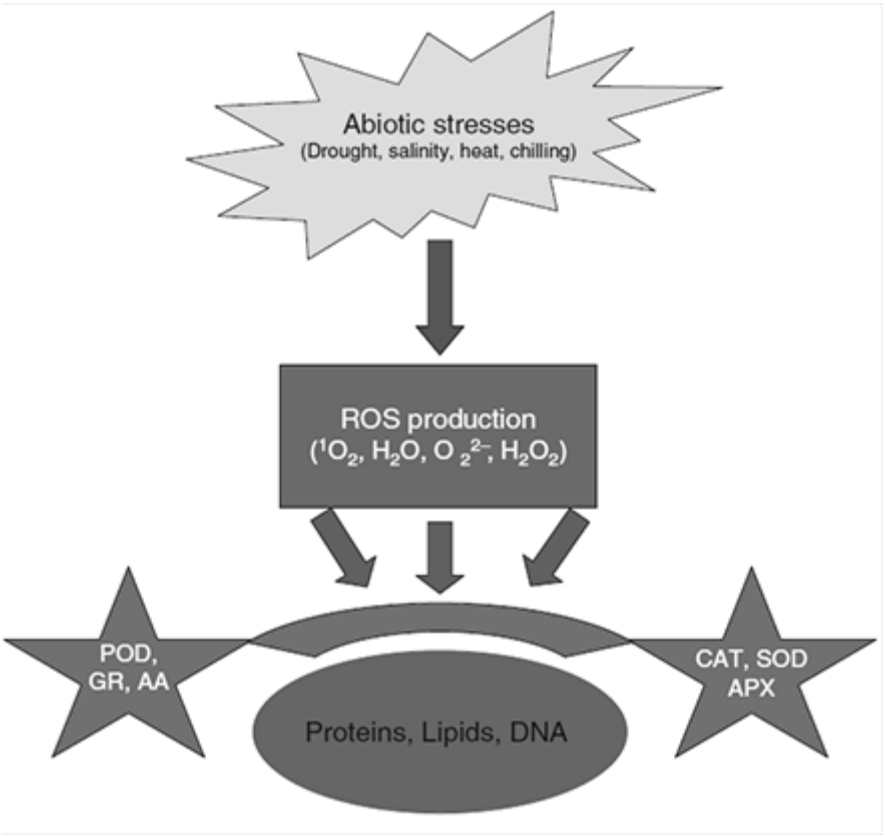


Fig. 1. Role of antioxidant enzymes in the reactive oxygen species (ROS) scavenging mechanism. Exposure to abiotic stresses (including drought) leads to the generation of ROS, which may react with proteins, lipids and DNA, causing oxidative damage and impairing the normal functions of cells. The antioxidant defense system in the plant cell includes both enzymatic and non-enzymatic constituents. Amongst the enzymatic components are superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), glutathione reductase (GR), and ascorbic acid oxidase (AA). When exposed to abiotic stresses, tolerant cells activate their enzymatic antioxidant system, which then starts quenching the ROS and protecting the cell. (Adjusted according to FAROOQ *et al.* 2009b.)

In 2001, PUSTOVOITOVA *et al.* Investigated the influence of EBR treatment on the drought resistance of *Cucumis sativus* L. plants grown in the greenhouse. Plants were sprayed with $10^{-6}$, $10^{-8}$, $10^{-9}$ or $10^{-11}$ M EBR solution at the stage of 3 developed leaves. The next day the watering was deceased for 11 days for the stressed plants. EBR treatment resulted in improved plant resistance to dessication and overheating, further it improved capacity for water retaining, and subsequently increased water content in leaves of cucumber plants subjected to drought. It was suggested that accumulation of osmotically active compounds (free aminoacids, in particular) contributed to cucumber plant osmoregulation.

LI *et al.* (1998a) examined the effects of BL on the antioxidant system in maize seedlings subjected to water stress. Two contrasting cultivars of *Zea mays* L., PAN 6043 (drought resistant) and SC 701 (drought senssitive), were used in this study. Kernels were soaked for 17 hours in 12.5 $mg.l^{-1}$ BL and after germination in dark, seedlings were grown under 14 hour photoperiod in growth chamber. Water stress in 7 day old seedlings was induced by soaking them in -1.0 MPa polyethylene glycol (PEG) 6000 solution and they were analysed 48 hours thereafter. BL treatment resulted in increased tolerance to drought stress due to increased antioxidant enzymes (SOD, CAT, APX) activities as well as increased antioxidant substances (ascorbic acid, total carotenoids) levels in resistant cultivar only.

Similar results were observed in the experiment of LI and VAN STADEN (1998b) when 8 day old calli of two maize cultivars mentioned in the previous experiment were soaked in 12.5 $mg.l^{-1}$ BL for 3 hours. Water stress was subsequently induced by incubating the calli in -0.5 MPa PEG 6000 solution for 24 hours. For another 30 days the calli were grown in illuminated incubator under 16 hour photoperiod. Activities of antioxidant enzymes (SOD, CAT, APX, POD and GR) remained higher in the callus of drought resistant cultivar and it was suggested that this may be due to an up-regulation of the genes controlling the synthesis of these enzymes or an increased activation of constitutive enzyme pools. Drought resistant callus had also higher survival percentage and less apparent damage than the callus of drought senssitive cultivar.

In another paper from that year LI and VAN STADEN (1998a) published the experiment in which the identical plant material, hormonal treatment and experimental design as mentioned above (LI *et al.* 1998) were used. In drought resistant cultivar seedlings BL treatment increased RWC and diffusive resistance, and decreased the relative conductivity and transpiration rate, while in the drought senssitive cultivar seedlings it decreased seedlings height and RWC, despite increasing relative conductivity, transpiration rate and diffusive resistance. They suggested that pre-treatment of maize kernels of individual cultivars with BL can lower crop loss due to drought.

Different group of scientists (LI *et al.* 2008) investigated the effect of BL treatment on *Robinia pseudoacacia* L. seedlings. In the field experiment performed in a dryland area roots of 1 year old seedlings were soaked for 5 minutes in solution with 0.1, 0.2, 0.3 or 0.4 $mg.l^{-1}$ BL, and planted. Their growth and survival rate were evaluated almost 8 months after. In the pot experiment roots of 1 year old seedlings were soaked for 5 minutes in BL solution of the same concentration as for the field experiment. Plants were further grown in a rain-out shelter which was normally opened but covered with plastic when it was raining. In less than two weeks BL treatment was applied again at the same concentrations, this time by spraying the seedlings after they leafed out. After more than two months, three water regimes were imposed: normal watering, mild water stress and severe water stress, respectively 75 %, 55 % and 35 % of field water holding capacity reached. Ten days later, physiological parameters of leaves from the middle part of each seedling were analysed. Pre-treatment with 0.2 and 0.3 $mg.l^{-1}$ concentration of BL increased growth and survival rate of seedlings in the field experiment. In the pot experiment pre-treatment with BL increased leaf water content, RWC, water potential, soluble sugar and free proline content, and activities of antioxidant enzymes SOD, POD, and CAT. Conversely, it decreased transpiration rate, stomatal conductance and malone dialdehyde (MDA) content. MDA is a product and indicator of lipid peroxidation. The concentration of 0.2 $mg.l^{-1}$ BL was the most effective. Authors suggested that BL treatment enhanced drought resistance of *Robinia pseudoacacia* seedlings can be utilized in afforestation projects in arid and semiarid areas.

Also ZHANG *et al.* (2008) studied the effect of BL application, this time on soybean plants subjected to water deficit. *Glycine max* L., cultivar Ken 5 plants were grown in pots in a field sheltered from rain by a removable polyethylene shelter. The BL was applied at the beginning of flowering by spraying the plant leaves with 0.1 $mg.l^{-1}$ concentration. By regulating the watering 7 days after, two levels of soil moisture started to be applied on soybean plants, 80 % and 35 % of soil field capacity respectively. Another 7 days after plants were analysed. Pre-treatment with BL before inducing the water stress enhanced assimilated 14C translocation from leaves to other parts, improved biomass and seed yield, leaf photosynthesis and chlorophyll content, and $F_v/F_m$. It also increased SOD and POD activity and promoted osmoprotectants accumulation (soluble sugars, free proline), while MDA

accumulation and electrical conductivity decreased under water stress conditions. Consequently, pre-treatment with BL partially alleviated the detrimental effect of water stress and minimized the yield loss caused by water deficits in soybean plants.

FARIDUDDIN *et al.* (2009) investigated the effect of HBR on drought stress-induced changes in photosynthesis and antioxidant system of Indian mustard. The 8 and 15 day old *Brassica juncea* L. seedlings grown in net house under 14 hour photoperiod were subjected to 7 days lasting water stress and then returned to normal conditions of growth. When 30 day old, seedlings were sprayed with 0.01 μM HBR, and when 60 day old, they were analysed. Inhibitory effect of water stress on photosynthesis and growth was more apparent in plants subjected to water stress at the earlier stage. HBR treatment increased antioxidant enzymes (CAT, SOD, POD) activity and proline content in both stressed and control plants. This helped to detoxify ROS generated by drought stress what resulted in improved and altered physiological performance of plants subjected to water stress. Eventually, HBR treatment ameliorated drought stress *via* modification of their antioxidant system.

VARDHINI and RAO (2003) studied the effect of HBR and EBR treatment on germination and growth of seedlings of three *Sorghum bicolor* L. cultivars, CSH-14 and ICSV-745 (susceptible to water stress) and M-35-1 (resistant to water stress), under osmotic stress conditions. Seeds were soaked in 20 % PEG 6000 with 2 μM or 3 μM HBR or EBR. The percentage of germination of BR-treated seeds under osmotic stress nearly reached (CSH-14) that of control seeds or even exceeded it (ICSV-745 and M-35-1). These treatments also enhanced growth of seedlings and restored protein levels, or even improved it when high content of soluble proteins and increased free proline levels were took into account. BR treatment resulted in increased CAT and AA activity and reduced peroxidase activity in all three cultivars.

KAGALE at al. (2007) examined the effect of EBR application on *Arabidopsis* and rape seedlings subjected to different abiotic stresses, including the drought stress. *Arabidopsis thaliana* (L.) Heynh., cultivar Columbia seeds and *Brassica napus* L.,

cultivar Westar seeds were grown in the culture room. 21 day old *Arabidopsis* seedlings were transferred into pots filled with sand and allowed to reestablish growth for 5 days after which the watering was withhold for them for up to 96 hours. 14 day old rape seedlings were transferred likewise, then allowed to reestablish growth for 3 days and subsequently subjected to drought stress by withholding the water supply for up to 60 hours. After the stress period seedlings of both plant species were allowed to recover for the next two days and those which survived and continued to grow were counted. EBR treatment ameliorated morphological changes induced by drought stress and increased survival rate and tolerance to drought stress in seedlings of *Arabidopsis* and rape. Transcriptional changes in drought stress marker genes were more evident in *Arabidopsis* seedlings during the earlier time points of stress, implicating that EBR-treated seedlings are likely positioned to tolerate stress better right from the beginning. Higher drought tolerance in the rape seedlings correlated with higher expression of genes enabling better distribution of water and efficient defense against the large number of proteases produced during stress conditions, while one aquaporin gene and a dehydrin homologue accumulated to higher levels in untreated seedlings. Possible explanation is that untreated seedlings were not so robust and so fast in raising stress response as EBR-treated seedlings, and therefore they possibly accumulated products of stress-responsive genes (induced *via* some other pathway) to higher levels what resulted in higher degree of stress in these seedlings.

In consistency with previous studies (PUSTOVOITOVA *et al.* 2001, VARDHINI and RAO 2003, KAGALE *et al.* 2007), EBR treatment ameliorated drought stress and improved growth also in tomato seedlings (BEHNAMNIA *et al.* 2009a).
After the germination, *Lycopersicon esculentum* L., cultivar Tomba (BB204) plants were grown in the greenhouse. When at stage of 3 fully expanded leaves, plants were transferred to growth chamber with 16 hour photoperiod for 5 days. Next, their leaves were sprayed with 0.01 or 1 μM EBR for 3 days, once a day. Subsequently, three levels of water stress were applied when withholding the watering for 0, 3 or 5 days. Pre-treatment with EBR increased shoot mass, content of antioxidants (ascorbate and carotenoids) and free proline, and SOD, CAT, POD and APX activities, while it decreased MDA and peroxide content in comparison with untreated control, especially in plants under severe drought stress. More effective was the 1 μM

EBR treatment. To sum up, EBR treatment definitely ameliorated damage caused by water stress in tomato plants.

In the same year, BEHNAMNIA *et al.* (2009b) published a similar study. Plant material, EBR treatment and experimental design were the same as in the previous study, with plants at stage of 4 fully expanded leaves used in the experiment being the only difference. EBR pre-treatment increased activity of antioxidant enzymes (POD, CAT, APX, GR and SOD), proline and protein content, and decreased peroxide content as well as it reduced peroxidation of lipids. In conclusion, pre-treatment with EBR alleviated unfavourable effect off water stress in tomato plants through decreasing the oxidative damage of plant membranes, possibly *via* induction of compatible solutes for osmotic adjustment and induction of antioxidant defense system.

In experiments listed by now, exogenous application of HBR or EBR was proved to ameliorate oxidative stress and consequent damage in plants. Therefore comparision of effectiveness of these two BRs was logically forthcoming. FAROOQ *et al.* (2009b) examined the effect of BR treatment on water relations and gas exchange in rice plants under drought stress. *Oryza sativa* L., cultivar Super-basmati plants were cultivated in phytotron under 14 hour photoperiod. The HBR and EBR treatment were applied as a seed treatment or foliar spraying. The seeds were soaked in 0.01 μM aerated solution of HBR or EBR for 48 hours. Foliar spraying with exactly the same concentration of both BRs was applied on plants at 5 leaf stage, 4 weeks after the sowing respectively. At 4 leaf stage, respectively 3 weeks after the sowing, plants were subjected to drought stress when 50 % field capacity water content was maintained. One week after BR spraying, seedlings were analysed in parameters excepting their fresh and dry weight, which were measured after three weeks when the experiment was ended due to the fact that 50 % of leaves of drought-stressed plants were wilting. Exogenous application of BRs enabled rice to withstand the drought period *via* improved net carbon dioxide assimilation and leaf water economy (water use efficiency, leaf water status and membrane properties) as well as production of free proline, anthocyanins and soluble phenolics, while MDA and peroxide production decreased. Of the two applications, foliar spraying had

better effect under drought stress conditions, while EBR proved to be more effective than HBR.

One year later, FAROOQ *et al.* (2010) published a study where they investigated effect of treatment with different plant growth regulators on improving drought resistance of rice. Plants of *Oryza sativa* L., cultivar Super-basmati were grown in the phytotron under 13 hour photoperiod. At 4 leaf stage, 4 weeks after the sowing, water deficit conditions were applied by maintaining the soil moisture at 50 % of the field capacity. One week later, when plants were at the 5 leaf stage, they were treated with 0.01 μM EBR among the other substances. All analyses except that of seedlings weight were done at day 4, 8, 12 and 16 after drought stress imposition, at day 21 the experiment was terminated due to wilting of stressed plants. The EBR pre-treatment significantly improved leaf gas exchange properties and water relation attributes, and consequently rice growth and photosynthesis, what resulted in higher dry mass accumulation in plants subjected to drought stress. It also increased effectivity of carbon assimilation by stabilizing membrane structures, and enhanced biosynthesis of free proline, soluble phenolics and anthocyanins, and activity of SOD, POD, CAT and APX, while it reduced MDA and peroxide production in plants under stress conditions. EBR application was proved to induce multiple pathways of protection from oxidative damage and it was proposed that these findings can be used for sustained rice production in relatively water scarce areas and at critical stages of rice growth.

Also LI *et al.* (2012) examined the influence of EBR pre-treatment on growth, photosynthesis and antioxidant system of *Chorispora bungeana* plants under drought stress*. Chorispora bungeana* Fisch. & C.A. Mey plantlets were regenerated from cell cultures and when approximately 5 cm tall, 0.1 μM solution of EBR was sprayed three times on their leaves every 2 hours. One day after the treatment, 20 % PEG 6000 was applied by watering the plants to induce drought stress. Leaves were collected 72 hours after the stress was induced. When compared to control, EBR pre-treatment significantly improved RWC, chlorophyll content and $F_v/F_m$, increased SOD, POD, CAT, APX, GR and AA activities, while MDA content, membrane permeability and proline content were less increased when compared

to non-pretreated plants. Thus, exogenous treatment with EBR improved *Chorispora bungeana* growth under water stress conditions.

Similarly, HU *et al.* 2013 studied the effect of EBR treatment on drought tolerance, leaf gas exchange and chlorophyll fluorescence parameters in pepper. *Capsicum annuum* L., cultivar Longkouz (drought senssitive) plants were germinated in a greenhouse and then transferred to a growth chamber under 12 hour photoperiod. When 2 month old (at 20-25 leaf stage), plants were divided into two groups, well-watered (80 % relative soil water content) and drought-stressed (45 % relative water soil content). After 10 days, the drought-stressed plants were allowed to recover for 5 days. The 0.01 $mg.l^{-1}$ EBR treatment was applied one day before and 5 days after the drought stress treatment started. EBR treatment significantly alleviated drought-induced inhibition of photosynthesis, which was particularly attributed to the increased capacity of carbon dioxide assimilation and the efficiency of light utilization as well as drought-induced photoinhibition, what was possible due to the dissipation of excitation energy in a form of heat from the light harvesting complex of Photosystem II. It was concluded that exogenously applied EBR could ameliorate drought-induced photoinhibition, mainly by increasing the efficiency of light utilization and dissipation of excitation energy in the Photosystem II antennae.

As presented above, most of the works published on the topic of BRs effects on plants subjected to drought stress is focused on the antioxidant system, however, recently an importance of photosynthetic characteristics for yield attributes was recognised.

### 3.1.3 BRASSINOSTEROIDS CROSSTALK

Stress responses in plants are regulated not through the linear pathways, but *via* the complex molecular networks (YUAN *et al.* 2010). Based on the molecular studies, crosstalk of BRs with other phytohormones was established as reviewed by KRISHNA (2003), CHOUDHARY *et al.* (2012) and HAO *et al.* (2013). KRISHNA *et al.* (2003) also pointed out that when determining molecular changes associated with BR-induced drought tolerance, not all changes in genes expression represent

the BR primary response, and conclusively that some of these changes are the result of BR crosstalk with other hormones.

UPRETI and MURTI (2004) studied the effects of HBR and EBR treatment on root nodulation and yield of French beans under water stress. *Phasoelus vulgaris* L., cultivar Arka Suvidha seedlings were grown in a polyhouse under natural day length conditions. At flowering stage they were sprayed with 1 or 5 μM EBR or HBR. Five days later, water supply was withheld for 4 and 8 days. Exogenous pre-treatment with BRs ameliorated decrease in the nodule number and pod yield, and increased dry mass of nodulated roots under water stress. BR treatment did not alter the abscisic acid (ABA) content, however it increased trans-zeatin riboside (ZR), a cytokinin, content and nitrogenase activity in nodulated roots of both stressed and control plants. ZR increase was proposed to facilitate nodulation. The 5 μM EBR treatment was the most effective treatment of all.

PELEG *et al.* (2011) examined the possibility of enhancing the drought tolerance in rice plants by delaying the stress-induced senescence through the stress-induced synthesis of cytokinines in rice. Wild type and transgenic plants expressing the isopentenyltransferase gene driven by the for senescence-associated receptor kinase, a stress- and maturation-induced promotor in *Oryza sativa* L., cultivar kitaake plants. Isopentenyltransferase catalyses cytokinin biosynthesis. In transgenic plants and in wild type plants treated with exogenous cytokinin (CK), there was an up-regulation of BR biosynthesis and BR regulation and signalling genes, possibly due to interaction between CK and BR. However, it was not established whether the positive interaction between CK and BR in transgenic plants was a direct consequence of the crosstalk between CK, ABA and BR, or indirectly mediated by ABA. Changes in hormone homeostasis, including the jasmonic acid (JA) and indole-3-acetic acid (IAA), were associated with resource mobilization during the stress period and resulted in higher grain yield and improved grain quality in transgenic rice plants.

XU *et al.* (1994a, 1994b) investigated the relation of EBR and/or ABA treatment on sorghum plants resistance under water deficit. *Sorghum bicolor* (L.) Moench, cultivar Lucky plants were grown in a greenhouse under natural light conditions. They

were subjected to different levels of soil water deficit under five different water regimes. Spraying of water, 0.1 ppm EBR, 50 ppm ABA, or 0.1 ppm EBR and 50 ppm ABA was performed four times. EBR treatment slightly increased length and dry mass of both the top and root of sorghum plants under various water stress conditions. In contrast with previously mentioned study (UPRETI and MURTI 2004) when endogenous levels of ABA were investigated, in combination with exogenous ABA treatment EBR inhibited growth even more than ABA treatment of control plants, while it increased the growth rate even more than ABA treatment alone under water stress conditions. Thus, it seems that EBR strengthened both the inhibitive and promotive effects of ABA. Based on these results, authors concluded that EBR increased the effect of ABA in well-watered as well as in water stress conditions. The synergetic effect of EBR and ABA treatment was confirmed also by increased survival ability of plants and leaves under severe drought. In combination, EBR and ABA treatment increased *in situ* RWC in plants even more than EBR or ABA treatment alone. Further, EBR treatment did not show any effect on cuticular transpiration alone, but it enhanced its decrease due to the ABA treatment. Consequently, authors suggested that ABA or EBR and ABA treatment promoted wax deposition on the leaf surface what resulted in decrease in water loss through the cuticular transpiration. Based on the value of RWC when stomata were closed they also suggested that the treatment with EBR and/or ABA not only increased water retention but also enhanced the physiological tolerance to low water status. While EBR-induced water maintenance was mainly a physiological consequence, this treatment strengthened the effect of ABA treatment on both physiological and morphological bases.

YUAN *et al.* (2010) studied the effect of exogenously applied EBR on drought resistance and ABA concentration in tomato plants under water stress. *Lycopersicon esculentum* (L.) Mill, cultivar Ailsa Craing plants were grown in the greenhouse under 12 hour photoperiod. At 5 true leaves stage, the 1 μM EBR was sprayed over the seedlings and water cessation was applied. EBR application significantly increased ABA concentration and SOD, CAT, and APX activities as well as RWC and net photosynthetic rate, and decreased peroxide and MDA content, stomatal conductance and intracellular carbon dioxide concentration in plants under drought stress. It was suggested that EBR treatment induced biosynthesis of endogenous

ABA, which subsequently induced up-regulation of antioxidant system in tomato plants subjected water drought stress.

ZHANG *et al.* (2011) investigated the nitric oxide-mediated BL-induced ABA synthesis involved in the oxidative stress tolerance in maize leaves. Kernels of *Zea mays* L., cultivar Nongda plants were grown in light chamber under 14 hour photoperiod. At stage of 2 fully expanded leaves plants were detached and treated by wrapping in aluminium foil with 5 μM brassinazole (BRZ) and 100 μM ABA or 10 nM BL for 4 hours. Subsequently, plants were exposed to 10 % PEG treatment for 8 hours and their second leaves were analyzed afterwards. BRZ application aggravated the oxidative damage induced by PEG treatment and this effect was alleviated by the application of either BL or ABA. Enhanced production of nitric oxide in mesophyll cells of maize leaves induced by BL mediated ABA biosynthesis, which resulted in enhanced tolerance to oxidative damage caused by water stress.
The authors suggested that ABA is involved in BR-induced water stress tolerance, but BR is not involved in in ABA-induced water stress tolerance. Furthermore, based on observations made through BRZ use, they concluded that both exogenous and endogenous BRs can enhance the stress tolerance to oxidative damage during water stress in maize leaves, what is in the contrast with following study.

In previous studies it was demonstrated that exogenous application of bioactive BRs improved various aspects of plant growth and increased water stress tolerance in several plant species. To examine whether the changes in levels of endogenous BRs are involved in mediating response of plants to water stress, JAGER *et al.* (2008) performed an experiment with *Pisum sativum* L., cultivar Hobart Line 107 plants, and BR-deficient as well as BR-perception mutants. Pea plants were grown in a heated glasshouse with natural photoperiod prolonged to 18 hours. When 21 to 22 day old, they were subjected to water stress by withholding the water supply up to 14 days, until the leaves began to wilt. The period during which plants were not watered varied from 8 to 14 days as a result of environmental changes occurred throughout the seasons. Water stress significantly increased the level of endogenous ABA but not of bioactive BR (level of castasterone, in particular) in apical, internode and leaf tissue. The authors suggested that the ABA levels might increase as a result of plants response to water stress, while mild increase in castasterone levels might

had been a response to other factors (e.g. growth inhibition or senescence). Based on the observation that elevated level of ABA had no effect on endogenous BR levels they concluded that BRs are not required for normal response of pea plants to water stress. However, they did not exclude the possibility that exogenous application of BRs can increase the drought tolerance in many plant species.

Plants display variety of physiological and biochemical responses towards prevailing the drought stress at cellular and whole-organism levels, making it a complex phenomenon consequently (FAROOQ *et al.* 2009b). Proton pumping, nucleic acid activation and protein synthesis, and regulation of gene expression were proposed to be responsible for plant growth, survival and increased yield under drought stress conditions as reviewed by FAROOQ *et al.* (2009a) and BEHNAMNIA *et al.* (2009b). However, variable effects of BR application in growth and development depend not only on dosage, but also on the method of BR application, plant cultivar, treated plant organ (JANECZKO and SWACZYNOVÁ 2010) and stage of plant development as well as on interactions with other influences, e.g. other hormones and macromolecular signals, internal conditions at the cellular and organ level such as pH, and ion and metabolite concentrations, as well as external conditions (SASSE 2003). Therefore although the molecular mechanism of BR signaling is almost revealed, many questions regarding biochemical and physiological processes in plants connected to endogenous or exogenously applied BRs remain indefinite.

## 3.2 BRASSINAZOLE

To investigate the role of BRs in plants, BR-deficient mutants analyses were implemented in their research at the end of the last century. However, as these BR-deficient mutants have been known only in *Arabidopsis*, tomato and pea (ASAMI *et al.* 2000), other experimental strategies were required to identify the specific physiological activities at the tissue and cell level and at various developmental stages (ASAMI and YOSHIDA 1999). Generally, the specific inhibitors of biosynthesis have been considered to be effective for determining the physiological functions of endogenous substances, as it was previously

demonstrated in studies of the mode of action of gibberellin (GA). Analogously, there was an effort to find a specific inhibitor of BR biosynthesis which could provide a new complementary approach to understanding the functions of BRs (MIN *et al.* 1999, ASAMI *et al.* 2000). Furthermore, a specific inhibitor of BR metabolism (BAJGUZ and ASAMI 2004) and signal transduction (ASAMI and YOSHIDA 1999) were proposed to be good probes for clarifying the role of BRs and to find mutants in which genes involved in plant-hormone signal transduction were altered (ASAMI *et al.* 2003).

In 1999, there was reported a new compound, brassinazole (ASAMI and YOSHIDA 1999, MIN *et al.* 1999), which induced morphological changes in plants by interfering with the biosynthesis of BRs (ASAMI *et al.* 2000, ASAMI *et al.* 2001, NAGATA *et al.* 2000). BRZ (Fig. 2) is a triazole derivative in which tert-butyl group of uniconazole, a specific inhibitor of GA biosynthesis, was replaced by a phenyl group (KWON and CHOE 2005). Difference in these groups change the character of triazole derivatives from GA biosynthesis inhibitors to BR biosynthesis inhibitors (ASAMI *et al.* 2003). Furthermore, BRZ is unique in that it has a tertiary hydroxy group on the carbon adjacent to the carbon where a triazole ring is attached, whereas other known triazole plant growth regulators have a secondary hydroxyl group at this position (ASAMI *et al.* 2000).

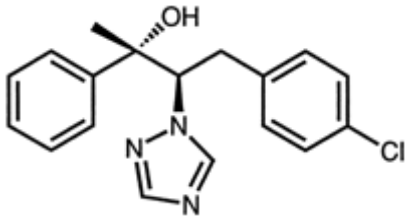


Fig. 2. Structure of brassinazole.
(Adjusted according to ASAMI *et al.* 2000.)

In the search for even more potent BR biosynthesis inhibitors, besides other substances there were also tested BRZ derivatives, for instance Brz2001 (ASAMI *et al.* 2003, SEKIMATA *et al.* 2002a, BAJGUZ and ASAMI 2005), Brz220

(SEKIMATA *et al.* 2002b) or Brz117 (ASAMI *et al.* 2001). Among other advantages of the treatment with BRZ or its derivatives are that it can control endogenous BR levels more freely than BR-deficient mutations, it can be applied to different growth stages and to different organs, tissues and cells (ASAMI and YOSHIDA 1999, ASAMI *et al.* 2000) at any time point and with any dose of choice, and that it enables studies in other plant species (ASAMI *et al.* 2000, KASCHANI and VAN DER HOORN 2007). BRZ was also applied for microarray analyses to comprehensively identify BR-regulated genes and to examine their expression in *Arabidopsis* (GODA *et al.* 2002). When ASAMI *et al.* (2000) varied the concentration of BRs in plants by varying the concentration of BRZ, they suggested that by comparing the concentration of BRs in BRZ-treated and non-treated plants it may be possible to titrate the minimal concentration of BRs for normal plants growth. The same approach was used in the experimental systems of SHIGETA *et al.* (2011) where contrary to the use of BR-deficient mutants, BRZ treatment showed up to be very effective for focusing on the rapid protein fluctuations occurring at an early phase of BR deficiency in plant cells, considering that BR-deficient mutants potentially have complex protein changes resulting from sustained growth under BR-deficient conditions since their germination (SHIGETA *et al.* 2011).

Consecutively, many observations and conclusions were made through the use of BRZ or its derivatives over the next years up to the present time. In Table 1 there are listed relevant significant experiments to demonstrate it. These experiments differ in the plant material, growth conditions, hormonal treatment (all shown in Table 1) as well as in the age of plant material, the way of hormone application or the whole experimental design. Therefore differences in observations arise and it is not easy to compare conclusions made upon individual experiments. For better view of the development progress of this issue, citations are ordered chronologically.

Table 1: Chronological list of the most significant experiments where brassinazole or its derivatives were used. If specified, plant species, cultivar and/or ecotype, growth conditions, brassinosteroid and brassinazole or its derivatives used in the experiment are cited in the table along with the observations and conclusions made thanks to the use of brassinazole or its derivatives.
(BR – brassinosteroid; BRs – brassinosteroids; BRZ – brassinazole; BL – brassinolide; EBR – 24-epibrassinolide; BZR1 – brassinazole-resistant-1; GA – gibberellin; GAs – gibberellins; GAI – gibberellic acid-insensitive; IAA – indole-3-acetic acid; ABA – abscisic acid; PEG – polyethylene glycol; RuBisCo – ribulose-1,5-bisphosphate carboxylase oxygenase; cv. – cultivar; h – hour)

| **Plant species, cultivar, ecotype** | **Growth conditions** | **Brassinosteroid / brassinazole or its derivatives used in the experiment** | **Observations and conclusions made through the use of brassinazole or its derivatives** | **Citation** |
|---|---|---|---|---|
| *Oryza sativa* L., *Arabidopsis thaliana* (L.) Heynh., *Lepidium sativum* L. | growth chamber, continuous dark or different photoperiods | 0.1 μM; 0.5 μM; 1 μM; 5 μM; 10 μM BRZ and/or 10 nM BL | BRZ-treated *Arabidopsis* seedlings phenotypically resembled BR-deficient mutants;<br>in the rice stem elongation test there was verified that BRZ is not a GA biosynthesis inhibitor;<br>*Arabidopsis* and cress seedlings were recovered by the co-application of BRZ with BL;<br>22-hydroxylation step of BR biosynthesis was proposed to be a target site of BRZ;<br>many possible utilizations of BRZ experimental applications were propounded. | ASAMI and YOSHIDA 1999 |
| *Arabidopsis thaliana* (L.) Heynh. ecotype Columbia, *Lepidium sativum* L. | growth chamber, 16 h photoperiod or dark | 0.1 μM; 0.5 μM; 1 μM; 5 μM; 10 μM BRZ and/or 10 nM BL | When *Arabidopsis* plants were grown in dark, BRZ-induced morphological changes were very similar to those of BR-deficient mutants and these plant characteristics were nearly restored to those of wild type by treatment with BL;<br>when effect of BRZ on retarding the hypocotyl elongation of cress seedlings was studied, these did not show recovery after the application of GA but showed good recovery after the addition of BL (implying that BRZ is a specific BR biosynthesis inhibitor);<br>the dwarfism in BRZ-treated *Arabidopsis* plants was due to the reduction in cell length growth and thickness of cell walls;<br>BRZ induces morphological changes by interfering with the biosynthesis of BRs. | ASAMI *et al.* 2000 |

| Plant species, cultivar, ecotype | Growth conditions | Brassinosteroid / brassinazole or its derivatives used in the experiment | Observations and conclusions made through the use of brassinazole or its derivatives | Citation |
|---|---|---|---|---|
| *Arabidopsis thaliana* (L.) Heynh. ecotype Columbia | growth chamber, continuous light or dark | 0.1 μM; 0.2 μM; 0.5 μM; 1 μM and 2 μM BRZ | BRZ (0,1 to 2 μM) treated *Arabidopsis* seedlings grown in the dark exhibited morphological features of light-grown plants in a dose-dependent manner; BRZ treatment induces development of true leaves and in dark it induces the initial step of plastid differentiation, which occurs prior to the development of thylakoid membranes. | NAGATA *et al.* 2000 |
| *Lepidium sativum* L. | culture room, continuous light | 5 μM BRZ and/or 0,1 μM BL | BRZ in the medium caused a slight predominance of phloem differentiation at the expense of xylem differentiation and remarkable inhibition of the development of secondary xylem in cress seedlings; BRZ treatment affects secondary wall formation, such as lignification of either the xylem or phloem; BRs may be involved in deciding which differentiated cells (phloem or xylem cells) are formed from cambium cells; BRs function in xylem development and vascular differentiation of cress *in vivo*. | NAGATA *et al.* 2001 |
| *Lepidium sativum* L., *Nicotiana tabacum* L. cv. Samsun NN, *Arabidopsis thaliana* (L.) Heynh. ecotype Columbia, *Oryza sativa* L. cv. Koshihikari | growth chamber, different photoperiods or dark | 0.1 μM; 0.5 μM; 1 μM; 5 μM; 10 μM BRZ and/or 10 nM BL | Brz2001 induced similar morphological changes to those seen in BR-treated plants, including *Arabidopsis*, tobacco, and cress and these changes were reversed by addition of BL; Brz2001-treated rice did not show any morphological changes; Brz2001 induces morphological changes in dicotyledonous plants by interfering with the biosynthesis of BRs and it is a more specific BR biosynthesis inhibitor than BRZ. | SEKIMATA *et al.* 2001a |

| Plant species, cultivar, ecotype | Growth conditions | Brassinosteroid / brassinazole or its derivatives used in the experiment | Observations and conclusions made through the use of brassinazole or its derivatives | Citation |
|---|---|---|---|---|
| *Arabidopsis thaliana* (L.) Heynh. ecotype Columbia | growth chamber, continuous light | 3 µM BRZ, 10nM BL | Exposure of plants to BL and BRZ treatment elicited opposite effects on gene expression of the identified genes, including transcription factor genes, auxin-related genes, P450 genes, and genes implicated in cell elongation and cell wall organization. | GODA *et al.* 2002 |
| *Nicotiana tabacum* L. cv. Xanthi | growth chamber, 16 h photoperiod | 30 µM or 150 µM Brz 2001 or 20 µM; 40 µM; 200 µM BL | BL-induced resistance does not require salicylic acid biosynthesis and is distinct from systemic acquired resistance; Brz 2001 treatment and measurement of BRs in tobacco mosaic virus-infected leaves indicate, that steroid hormone-mediated disease resistance plays part in defense response in tobacco, additively to systemic acquired resistance. | NAKASHITA *et al.* 2003 |
| *Arabidopsis thaliana* (L.) Heynh. ecotype Wasilewskija | growth chamber, continuous light or dark | $10^{-5}$-$10^{-9}$ M BRZ and/or $10^{-6}$; $10^{-8}$; $10^{-10}$ M BL | BRZ was used to elucidate the significance of endogenous BRs; it inhibited growth of roots, hypocotyls and cotyledonous leaf blades dose-dependently and independent of light conditions; BL-induced hypocotyl elongation was achieved through cell enlargement rather than cell division; BRs play an important role in the juvenile growth of *Arabidopsis*; moreover, BRs act on light-grown hypocotyl elongation independent of, but cooperatively with, GAs and auxin. | TANAKA *et al.* 2003 |

| Plant species, cultivar, ecotype | Growth conditions | Brassinosteroid / brassinazole or its derivatives used in the experiment | Observations and conclusions made through the use of brassinazole or its derivatives | Citation |
|---|---|---|---|---|
| *Chlorella vulgaris* Beijerinck | growth cabinet, 16 h photoperiod | 0.1 to 10 µM Brz2001 and/or 10 nM BL | Brz2001 treatment inhibited growth of the *Chlorella vulgaris* culture during the first 48 h of cultivation in the light; this inhibition is prevented by the co-application of BL;<br>Brz2001 has a dual effect on the metabolism of *Chlorella vulgaris*: early inhibitory effect on the content of RNA, protein, chlorophylls, carotenoids and sugar, later this effect is stimulatory; this effect is more significant for photosynthetic pigments;<br>when grown in the dark, treatment with Brz2001 alone, or in mixture of 10 nM BL and 0.1-10 µM Brz2001, also stimulates their growth;<br>other results suggest that the non-mevalonate pathway is used in *Chlorella vulgaris* to synthesise BRs. | BAJGUZ, ASAMI 2004 |
| *Wolffia arrhiza* (L.) Hork. ex Wimmer | culture room, 16 h photoperiod | $10^{-4}$-$10^{-6}$ M Brz2001, $10^{-13}$-$10^{-6}$ M EBR | EBR stimulated the growth and increased the content of photosynthetic pigments, sugar and protein in *Wolffia arrhiza*;<br>addition of Brz2001 to cultures inhibited their growth after 7 days of cultivation and this could have been reversed by addition of EBR;<br>in *Wolffia arrhiza* biosynthesis of BRs through the non-mevalonate pathway could be possible. | BAJGUZ, ASAMI 2005 |

| Plant species, cultivar, ecotype | Growth conditions | Brassinosteroid / brassinazole or its derivatives used in the experiment | Observations and conclusions made through the use of brassinazole or its derivatives | Citation |
|---|---|---|---|---|
| *Solenogyne mikadoi* L., *Solenogyne bellioides* L. | growth chamber, 16 h photoperiod | 10 µM Brz220, 0.1 µM BL | In *Solenogyne bellioides* Brz220 suppressed elongation and expansion of leaves, in *Solenogyne mikadoi* Brz220 inhibited leaf elongation; one-directional leaf elongation caused by the reduced sensitivity to BL in *Solenogyne mikadoi* and BL-dependent two-dimensional leaf expansion in *Solenogyne bellioides* both appear to be adaptations to their respective habitats; adaptive dwarfism of *Solenogyne mikadoi* could be caused by different sensitivity to GAs and BRs compared to *Solenogyne bellioides*. | ITOH *et al.* 2005 |
| *Gossypium hirsutum* L. cv. Coker 312 | greenhouse | 2.5 µM BRZ and/or 0.1 µM BL | Treatment of cotton floral buds with BRZ resulted in the complete absence of fiber differentiation; BRZ inhibits fiber elongation and this effect could be reversed by addition of BL; expression of BR-responsive genes in ovules correlated with early differentiation of fiber cells and fiber elongation; confirmed correlation between BR-regulated gene expression and fiber elongation in cotton ovule culture. | SUN *et al.* 2005 |
| *Arabidopsis thaliana* (L.) Heynh. ecotype Wasilewskija | culture room | 1 or 5 µM BRZ, 0.1 µM BL | Five BR-specific synthesis genes and two sterol biosynthesis genes were up-regulated in *Arabidopsis* plants grown under BRZ; four of these synthesis genes and one sterol synthesis gene were down-regulated and BR inactivation gene was up-regulated in plants fed with BL; BR homeostasis is finely modulated by the feedback expression of multiple BR metabolic genes, each of which is involved not only in BR-specific biosynthesis and inactivation, but also in sterol biosynthesis. | TANAKA *et al.* 2005 |

| Plant species, cultivar, ecotype | Growth conditions | Brassinosteroid / brassinazole or its derivatives used in the experiment | Observations and conclusions made through the use of brassinazole or its derivatives | Citation |
|---|---|---|---|---|
| *Glycine max* L. cv. Enrei and En6500 | growth chamber, 16 h photoperiod | 25 nM; 50 nM; 100 nM BRZ and/or 1nM; 10 nM; 15 nM; 100 nM BL | Application of BL on the leaves or direct injection of BL into the root base inhibited nodule formation and root development in the supernodulating mutant En6500, but not in the parental line Enrei; foliar application of BL induced internodal growth, while the foliar treatment of mature leaves with BRZ increased the nodule number along with a significant reduction of stem elongation in wild type Enrei and this effect was faster than when BRZ was added into culture media; BRs may regulate the nodule number in soybean plants. | TERAKADO *et al.* 2005 |
| *Vitis vinifera* L. cv. Cabernet Sauvignon | field experiment | BRZ 5 µL/10 µg/each berry, EBR 5 µL/200 ng/each berry | Increase in endogenous BR (castasterone) levels are associated with ripening in grapes; application of EBR to grape berries significantly promoted, while application of BRZ significantly delayed nonclimacteric fruit ripening, both evident in appearance of the skin coloration and the final sugar levels in ripe grape berries. | SYMONS *et al.* 2006 |

| Plant species, cultivar, ecotype | Growth conditions | Brassinosteroid / brassinazole or its derivatives used in the experiment | Observations and conclusions made through the use of brassinazole or its derivatives | Citation |
|---|---|---|---|---|
| *Arabidopsis thaliana* (L.) Heynh. ecotype Columbia-0 or Wassilewskija-0 | growth chamber, 16 h photoperiod | $5x10^{-7}$ M BRZ and/or $10^{-7}$-$10^{-10}$ M BL | BL increased both gravitropic curvature and length of primary roots of *Arabidopsis* plants at low concentration ($10^{-10}$ M), whereas at higher concentration BL further increased gravitropic curvature while inhibiting primary root growth; BRZ (primarily) and BL treatments decreased lengths of roots and in presence of either of these treatments $10^{-8}$ M IAA decreased the length of roots, less in a presence of BRZ; IAA treatment to the roots of BR-insensitive mutants or of plants pretreated with BRZ increased their sensitivity to gravity, while these treatments for the BL-hypersensitive transgenic plants were less effective; BRs interact negatively with IAA in the regulation of *Arabidopsis* plant gravitropic response and root growth, and its regulation is achieved partly by modulating the biosynthetic pathways of the counterpart hormone. | KIM *et al.* 2007 |
| *Cucumis sativus* L. cv. Jinchun No. 2 | greenhouse | 0.4 μM BRZ; 0.4 μM BRZ + 0.2 μM EBR; 4 μM BRZ; 4 μM BRZ + 0.2 μM EBR; 40 μM BRZ; 40 μM BRZ + 0.2 μM EBR | In Jinuchan No. 2 (cultivar with natural parthenocarpic capacity) BRZ treatment inhibited fruit set and growth, which could have been rescued by the application of EBR; BRs play an important role during an early fruit development in cucumber. | FU *et al.* 2008 |
| *Malus prunifolia* (Willd.) Borkh. cv. Marubakaido | culture room, 16 h photoperiod | 0.2-5 μg/shoot BRZ220 and/or 0.25-1.25 $\mu g/shoot^{-1}$ BL | BL differentially affected elongation and formation of the main and primary lateral shoots in explants from nodal segments of *Malus prunifolia*, resulting in reduced apical dominance; increasing doses of Brz220 led to a progressive inhibition of main shoot elongation, while stimulation of shooting and fresh mass accumulation were observed. | PEREIRA-NETTO *et al.* 2009 |

| Plant species, cultivar, ecotype | Growth conditions | Brassinosteroid / brassinazole or its derivatives used in the experiment | Observations and conclusions made through the use of brassinazole or its derivatives | Citation |
|---|---|---|---|---|
| *Cucumis sativus* L. cv. Jinyan No. 4 | growth chamber, 12 h photoperiod | 4 μM BRZ and/or 0.1 μM EBR | Differently, based on investigating the role of BRs in growth or regulation of photosynthesis, when EBR repeatedly sprayed it increased the carbon dioxide assimilation and quantum yield of Photosystem II, while BRZ reduced plant growth, decreased carbon dioxide assimilation and quantum yield of Photosystem II; BRs positively regulate synthesis and activation of a variety of photosynthetic enzymes, including RuBisCo, and so promote photosynthesis and growth in cucumber. | XIA *et al.* 2009 |
| *Brassica napus* L. cv. Topaz DH4079 | culture room | $5x10^{-7}$-$4x10^{-6}$ M BRZ, $10^{-8}$-$10^{-5}$ M BL | BL plays a key role during *Brassica napus* microspore-derived embryogenesis by improving embryo yield and quality and it affects glutathione and ascorbate pools by increasing the contributions of the oxidized forms, which by the proper expression and localization of meristem genes favour normal embryo development and apical meristem formation; BRZ application caused a reduced redox state, abnormal meristem development and poor post-embryonic performance; maintenance of cellular BL levels is required to modulate the ascorbate and glutathione redox status during embryogenesis to ensure the proper development of embryos and formation of functional apical meristems. | BELMONTE *et al.* 2010 |

| Plant species, cultivar, ecotype | Growth conditions | Brassinosteroid / brassinazole or its derivatives used in the experiment | Observations and conclusions made through the use of brassinazole or its derivatives | Citation |
|---|---|---|---|---|
| *Cucurbita pepo* L. cv. Vegetable spaghetti, Bolognese and Cora | climate controlled chamber, 16 h photoperiod | 10 µM BRZ | The application of BRZ slightly changed the production of ethylene in the three analysed genotypes (two inbred lines of squash contrasting in the sensitivity to ethylene and their hybrid variety), but those changes had little effect on their sexual phenotypes, and they did not alter the development of the unisexual flower;<br>BRs may regulate the induction of the female flower phase of development in *Cucurbita pepo*, although this regulation is genotype-dependent;<br>BRs appear to be dependent on ethylene response and their differential effect on the sexual expression of the different genotypes could therefore depend on the sensitivity to ethylene of the different genotypes;<br>ethylene had much greater effect on the sexual expression and flower development in *Cucurbita pepo* than BRs. | MANZANO *et al.* 2011 |
| *Zea mays* L. cv. Nongda | light chamber, 14 h photoperiod | 5 µM BRZ, then 10 nM EBR | Pre-treatment with BRZ aggravated the oxidative damage induced by PEG treatment, which was alleviated by the application of EBR or ABA;<br>BR-induced nitric oxide production mediates ABA biosynthesis, which results in the enhancement of tolerance to the oxidative stress damage caused by water stress in maize leaves. | ZHANG *et al.* 2011 |

| Plant species, cultivar, ecotype | Growth conditions | Brassinosteroid / brassinazole or its derivatives used in the experiment | Observations and conclusions made through the use of brassinazole or its derivatives | Citation |
|---|---|---|---|---|
| *Arabidopsis thaliana* (L.) Heynh. ecotype Columbia-0 | growth chamber, continuous dark | 0.3 µM; 1 µM; 3 µM; 10 µM BRZ 1 µM EBR or 3 µM BRZ + 1 µM EBR | Molecular mechanism for the integration of GA and BR signaling pathways in the control of cell expansion during photomorphogenesis is based on the inactivation of Brassinazole-Resistant-1 (BZR1) upon physical interaction with DELLA protein Gibberellic acid-Insensitive (GAI); GAI (major negative regulator of GA signaling pathway) inactivates transcriptional activity of BZR1 (which is the important transcription factor of BR signaling pathway) by inhibiting the ability of BZR1 to bind to target promoters; this model helps to understand how these two important hormone pathways regulate common developmental processes in plants during the entire life cycle. | GALLEGO-BARTOLOMÉ *et al.* 2012 |
| *Fragaria* x *ananassa* Duchense cv. Akihime | not specified | 200 mM BRZ, 400 mM EBR | Injected BL significantly promoted strawberry fruit ripening, while injected BRZ significantly inhibited it; BRs play a role in strawberry fruit ripening and possibly also in early strawberry fruit development. | CHAI *et al.* 2013 |

In *Arabidopsis* BRZ and its derivatives phenocopy BR-deficient mutants, showing the strong dwarfism with curly dark-green leaves when light-grown, and de-etiolated phenotype with short hypocotyls and expanded cotyledons, characteristics of light-grown plants, when grown in the dark (ASAMI and YOSHIDA 1999, ASAMI *et al.* 2000, NAGATA *et al.* 2000, SEKIMATA *et al.* 2001). TANAKA *et al.* (2003) pointed out the conflict in their results, when traits of dark-grown *Arabidopsis* plants as accumulation of anthocyanins and expanded cotyledons were not explicit and

BRZ treatment induced remarkable growth reduction of cotyledonous leaf blades along their longitudinal axes. They propounded that different results might reflect different experimental conditions with respect to the media constituents, photoperiod, temperature, procedure for chemical application, or timing of size measurements among distinct studies or that the difference might eventually result from different plant materials, for example *Arabidopsis* ecotypes.

Moreover, BRZ treatment inhibited in *Arabidopsis* plants growth of roots, hypocotyls, and cotyledonous leaf blades dose-dependently and independent of light conditions. BRZ-induced inhibited growth in *Arabidopsis* was due to reduced longitudinal growth of hypocotyl cells among the increased thickness of cell walls, while no differences were detected in the number of cells (ASAMI *et al.* 2000, TANAKA *et al.* 2003). Both antagonistic substances, BRZ and brassinolide (BL) inhibited root elongation in a dose-dependent manner. Arrested growth of roots under 10 μM BRZ treatment was probably caused by a deficiency of GAs in addition to BRs, as this concentration affects not only BR but also GA biosynthesis (SEKIMATA *et al.* 2001, TANAKA *et al.* 2003). In light-grown plants the hypocotyl elongation was independent of, but cooperative with, GAs and auxin, while the inhibition of root growth was mediated through the action of ethylene, the production of which depends on enhanced levels of auxin in the presence of BRs (TANAKA *et al.* 2003). This is supported by the observation of GODA *et al.* (2002) when BL and BRZ treatment of *Arabidopsis* plants had an opposite effects on the auxin-related genes expression. Contrary to these findings, KIM *et al.* (2007), based on the experiment with BL, BRZ- and IAA-treated *Arabidopsis* plants, postulated that BRs interact negatively with IAA in the regulation of gravitropic response and growth of the root, and that modulation of biosynthetic pathways of the counterpart hormone contributes to the regulation of this interaction. Different explanation was offered by NAKAMOTO *et al.* (2006). Due to the restoration of auxin sensitivity by a decrease in the BR level they suggested that it is not the absolute level of auxin and BR signals, but the ratio of the auxin-to-BR signal that determines the tropic responses of hypocotyls. While elongation of hypocotyls in which the auxin-BR interdependency has been observed is a one-dimensional response, tropic responses are two dimensional and therefore the response in each dimension could be regulated by auxin and BR independently. There have been also another observations of BRs interactions with other

phytohormones thanks to the use of BRZ. For example, BRZ slightly changed the production of ethylene, which is considered to be the principal regulator of sexual expression in Cucurbita pepo L. (MANZANO *et al.* 2011). Pre-treatment with BRZ significantly decreased the ABA content induced by water stress. BR-induced nitric oxide production and nitric oxide-activated ABA biosynthesis are important mechanisms for BR-enhanced water stress tolerance in leaves of maize plants after the PEG treatment (ZHANG *et al.* 2011). The most recent work of GALLEGO-BARTOLOMÉ *et al.* (2012) investigated the molecular mechanism of the concurrence of BR and GA signaling in the control of cell expansion during photomorphogenesis in *Arabidopsis*. They brought an evidence of physical interaction of components of these signal pathways, specifically that inactivation of BRASSINAZOLE RESISTANT1 (BRZ1), significant transcription factor in responses to BRs, is based upon interaction with DELLA proteins which mediate the response to multiple environmental signals. This is in the agreement with the inference of KRISHNA (2003) that there is a crosstalk between BRs and other plant hormones, in addition to the parallel hormone signaling pathways regulating the expression of common gene targets.

BRZ has been found to induce dwarfism and curly, darkgreen leaves in cress (ASAMI and YOSHIDA 1999, MIN *et al.* 1999, SEKIMATA *et al.* 2001), soybean (MAZORRA *et al.* 2004), cucumber and tobacco (ASAMI and YOSHIDA 1999) under the light. In the dark, BRZ induces photomorphogenetic changes in young seedlings of these plants. For example, cress developed short hypocotyls, open cotyledons and true leaves, while cucumber treated with BRZ demonstrated photomorphogenetic changes and rapid greening of cotyledons after 3 hour irradiation, cotyledons of control plants retained yellow color under the same conditions. Brz2001-treated rice did not show any morphological changes, implicating different roles of BRs in monocots and dicots (SEKIMATA *et al.* 2001) This was previously assumed by ASAMI and YOSHIDA (1999). Transgenic rice plants expressing maize, rice or *Arabidopsis* genes encoding C-22 hydroxylase that control BR hormone levels using a promoter that is active only in the stems, leaves and roots had about 15 to 44 % increases in grain yield *per* plant compared to wild type plants in greenhouse and field trials (WU *et al.* 2008). The authors suggested that BRs stimulate the flow of assimilates in rice.

To evaluate the exclusivity of BRZ and its derivatives they were bioassayed with rice, *Arabidopsis* and cress seedlings. Rice stem elongation test was used to eliminate GA biosynthesis inhibitors (MIN *et al.* 1999, ASAMI and YOSHIDA 1999, SEKIMATA *et al.* 2001). Recovery of retardation of the rice stem elongation after BRZ treatment was due to the addition of GA, but not BRs, implying that the reason was inhibition of GA biosynthesis. However, Brz2001 had no effect on the retardation of rice stem elongation (SEKIMATA *et al.* 2001). In the reversion test in BRZ-treated (ASAMI and YOSHIDA 1999, ASAMI *et al.* 2000, ASAMI *et al.* 2003) or Brz2001-treated (SEKIMATA *et al.* 2001a, SEKIMATA *et al.* 2002) *Arabidopsis* plants normal phenotype was reported to be rescued by application of BRs. Application of GA did not restore the normal phenotype. BAJGUZ, ASAMI (2005) reported that the inhibition of growth of BRZ-treated *Wolffia arrhiza* plants was reversed by the addition of EBR, but there was not complete recovery to the level of the control, especially at $5x10^{-5}$–$10^{-4}$ M Brz2001 treatment. SEKIMATA *et al.* (2001) reported that growth recovery of cress seedlings after the high concentration Brz2001 treatment was better than of BRZ-treated plants and implied that Brz2001 is a more specific inhibitor of BR biosynthesis than BRZ. Cress plants recovered after the BR application in reversion test (NAGATA *et al.* 2001, ASAMI *et al.* 2000), but tended to be sensitive to growth conditions (ASAMI *et al.* 2000). As a possible reason it were proposed the slow uptake and transport of BL within cress and/or a secondary effects of BRZ on other aspects of plant metabolism (ASAMI and YOSHIDA 1999). Findings of ASAMI *et al.* (2001) and TANAKA *et al.* (2003) support the concept that BRZ as a triazole derivative targets heme irons of cytochrome P450 monooxygenases, which exist commonly in the biosynthetic pathways of BRs and GAs. Based on this assumption, TANAKA *et al.* (2003) suggested that hypocotyl elongation of *Arabidopsis* seedlings caused by BL-application was, in fact, achieved by cooperative action of exogenous BL with endogenous GAs, and likewise in the case of exogenous GA with endogenous BRs.

BRZ and its derivates are triazole compounds. Although the specificity and versatility of the triazole compounds is very similar to each other, they specifically modify levels of three different phytohormones (brassinosteroids, gibberellins and abscisic acid) by inhibiting different cytochrome P450s (KASCHANI and VAN DER HOORN 2007).

BRZ is a specific inhibitor of BR biosynthesis which can bind through its triazole base directly to the DWARF4 (DWF4) enzyme, a cytochrome P450 monooxygenase that catalyzes the 22-hydroxylation of BR side chains. Thus, BRZ treatment induce BR deficiency in plant cells (ASAMI *et al.* 2001). Primarily, the target sites of BRZ were investigated by chemical analyses of endogenous BRs in *Catharanthus roseus* cells after 5 μM BRZ treatment. Afterwards, direct analyses of the interaction between BRZ and its derivatives and DWF4 protein expressed in *Escherichia coli* has revealed that BRZ and its derivates inhibits the hydroxylation of the C-22 position of the side chain in BRs by direct binding to DWF4 and that DWF4 catalyzes this hydroxylation reaction (ASAMI *et al.* 2001). *Arabidopsis* DWF4 has been previously proposed to be a key enzyme determining the flux in BR biosynthesis (CHOE *et al.* 1998). KIM *et al.* (2006) observed that DWF4 transcripts accumulate in the actively growing tissues (roots, shoot apices with floral clusters, joint tissues of shoot and root, dark-grown seedlings) and assumed that by DWF4 expression, tissue specificity of BR biosynthesis and accumulation, and localized response of BRs are likely to be imparted. They also established that the expression patterns of BRZ1 and DWF4 are mutually exclusive and concluded that DWF4 expression is regulated by a strong inhibitory mechanism, with BRZ1 being a repressor. Therefore they concluded that it is likely that in the specific tissues of *Arabidopsis* DWF4 promoter serves as a focal point in maintaining the homeostasis of endogenous bioactive BRs pools (Fig. 3).

In addition to BR-deficient mutants, specific BR biosynthesis inhibitors play an essential role in the elucidation of BR function in plants. ASAMI *et al.* (2000) presumed that next to its use in the basic science, BRZ can be developed as a new commercial plant growth regulator. However, limited availability and high costs of BRZ and its derivatives constrain their key advantage as a species-independent tool for commercial use (HARTWIG *et al.* 2012).

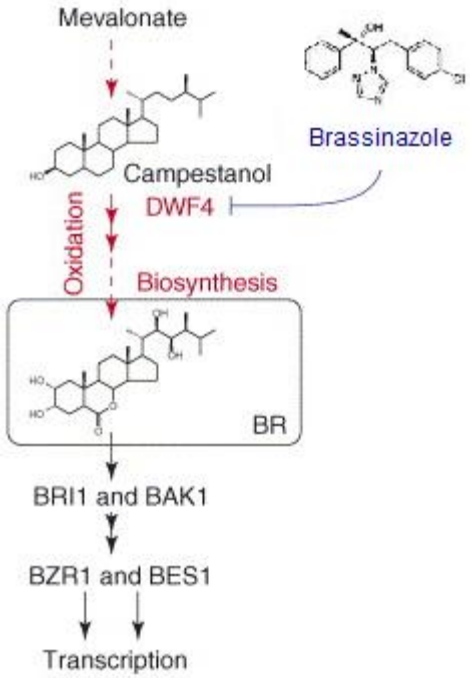


Fig. 3. Specific interference of brassinazole with brassinosteroids. Brassinazole inhibits DWARF4 (DWF4), a cytochrome P450 that confers the initial oxidation of campestanol, leading to the brassinosteroid (BR) synthesis. BRs acts through its receptors brassinosteroid-insensitive-1 (BRI1) and BRI1-associated receptor kinase-1 (BAK1), leading to the activation of transcription factors brassinazole-resistant-1 (BZR1) and BRI1-EMS-suppressor-1 protein (BES1), resulting in transcription of BR-responsive genes. (Adjusted according to KASCHANI and VAN DER HOORN 2007.)

## 3.3 PERSPECTIVES

In 1990s, BR-deficient and BR-insensitive mutants investigation confirmed the status of BRs as phytohormones. However, BR-deficient mutants of only *Arabidopsis*, pea and tomato were reported by this day. In the following decade, BR-biosynthesis inhibitor, brassinazole, was used to reveal mechanisms of actuation of endogenous or exogenously applied BRs. In the past few years, studies concerning transgenic plants expressing BR response-related genes were implemented. Currently, all these tools are complementary used in the experiments focused on revealing the mechanism of BR action. KRISHNA (2003) propounded that once it is understood, new opportunities for agricultural biotechnology may become evident.

Currently, there are no economically viable technological means to facilitate the crop production under drought (FAROOQ *et al.* 2009b). DIVI and KRISHNA (2009) reported an extensive testing of EBR in China, Japan and Russia. However, high cost of this synthetic BR and variability in results discouraged its use in agriculture. With understanding the mechanism of BRs interactions and actuation on plant survival and increased yield under water stress conditions, change in endogenous BR activity and/or modification of their signaling pathways might promote them to a world-wide routinely used natural prevention against the drought stress consequences (TRUBANOVÁ 2011).

BRs have been reported to have low toxicity and mutagenicity (SASSE 2003). This was confirmed by a couple of studies (ESPOSITO *et al.* 2011a, 2012) investigating the rat muscle cell growth. BRs triggered a selective anabolic response with minimal or no androgenic side effects. It also triggered a selective anabolic response that was associated with lower blood glucose and their antidiabetic effects were confirmed in another study by ESPOSITO *et al.* (2012b) on obese mice. Even more interesting are the findings of MALÍKOVÁ *et al.* (2008) and STEIGEROVÁ *et al.* (2010, 2012). BRs inhibited growth of several human cancer cell lines without affecting the growth of normal cells. Thus, their favourable utilization in human medicine arises.

# 4 MATERIAL AND METHODS

All experiments were performed with the plants grown in greenhouses in Brožek's genetic garden of the Department of Genetics and Microbiology, Faculty of Science, Charles University in Prague, Czech Republic (50° 04' 08.09" N, 14° 25' 34.81" E, altitude ~238 m) under natural light conditions, in which air temperature and relative humidity were partially controlled with the difference between day and night.

## 4.1 PLANT MATERIAL

Kernels of two maize inbred lines (CE704 and 2023) and their reciprocal hybrids (CE704x2023 and 2023xCE704) were purchased from CEZEA Breeding Station (Čejč, Czech Republic). These two contrastive parent lines (regarding sensibility to water deficit) have been used in the Laboratory of Plant Genetics for many years and were proved to be suitable for this type of experiments (BENEŠOVÁ *et al.* 2012).

CE704 – this line is characterized with faster growth and development (in comparison with 2023 line), its leaves are narrow and dark green. This genotype is supposed to overcome the water deficit more efficiently and to recover more quickly than the 2023 one, and is referred as a drought resistant cultivar.

2023 – this line is characterized with slower growth and development (in comparison with CE704 line), its leaves are wide, sinuated at the edges and rich green. This genotype is considered to be sensitive to water deprivation and drought, and is referred as a drought senssitive cultivar.

## 4.2 CHEMICALS

For the experiments following chemicals were used:

- Tween 20 (Sigma-Aldrich, St. Louise, Missouri, USA),
- Brassinazole (Tokyo Chemical Industry, Tokyo, Japan),

- 24-epibrassinolide (synthesized as described by KOHOUT (1994) at the Department of Steroid Chemistry at Institute of Organic Chemistry and Biochemistry AS ČR, Prague, Czech Republic, and kindly provided for these experiments).

## 4.3 EXPERIMENTAL DESIGN

The first season of experiments took place in the autumn. Maize kernels were soaked for 24 hours in a tap water solution of EBR (concentration $10^{-8}$ M, $10^{-10}$ M, $10^{-12}$ M, $10^{-14}$ M or 0 M). Right after this treatment the kernels were sown (one kernel *per* pot) in the plastic pots (d = 12 cm) filled with approximately 500 $cm^3$ of compost soil from the local garden and grown as shown in Figure 4.

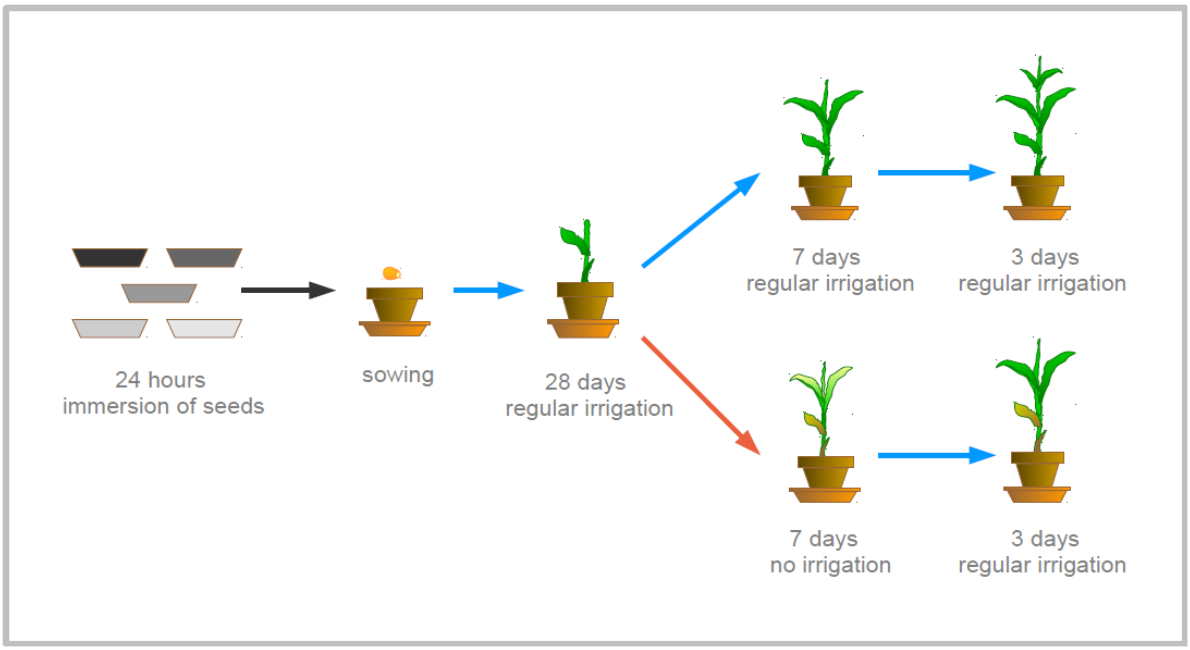


Fig. 4. Scheme of the experimental design during the autumn season. Kernels of two maize inbred lines (CE704 and 2023) and their reciprocal hybrids (CE704x2023 and 2023xCE704) were soaked for 24 hours in the solution of tap water with $10^{-8}$ M, $10^{-10}$ M, $10^{-12}$ M, $10^{-14}$ M or 0 M concentration of 24-epibrassinolide, and subsequently sown and irrigated as shown. There were twelve replications of each variant. (Adjusted according to Rothová *et al.* 2011.)

For 28 days all the plants were irrigated daily and their developmental characteristics were observed. Then they were divided into two groups, denominated “drought” and “recovery”. In each group the water supply was withdrawn for one half of the plants for 7 days, while the other half was kept irrigated. At the end of this period “drought” group of plants was analysed regarding the morphological parameters, relative water content and photosynthetic characteristics as well as the photosynthetic pigments content, as it will be described below.

Next, one half of the “recovery” group of plants, previously subjected to water deficit, was irrigated again for 3 days, while the second half of this group was supplied by the water for the duration of the whole experiment (38 days). At the end of this period, morphological parameters, relative water content and photosynthetic characteristics and content of photosynthetic pigments were again analysed in plants. All analysis were performed with twelve replicates, using a randomized block design where blocks were dates, as sequential sowing was used during this season. In total, 528 plants were analyzed in this experimental season.

The second season of experiments took place in the spring. Kernels were sown (one kernel *per* pot) in the plastic pots (d = 12 cm) filled with approximately 500 $cm^3$ of mixture consisting of fifteen parts compost soil from the local garden and one part commercial potting Baltisches Tray Substrate (Hawita Gruppe, Vechta, Germany). Plants were grown for 22 days with daily irrigation, and their development was observed.

Subsequently, the plants were divided into three groups in konsistence with the hormonal treatment (EBR, BRZ, no supplement). Three types of the solution were sprayed over the maize whorls and this day was designated as day 0 of the analysis. The volume of 3 ml was sprayed on the top leaves of each plant, ensuring that the surface of the whorl would be covered with it completely. In every solution there was a tap water with addition of Tween 20 as a surfactant. This was a composition of the first solution, in the second one there was added EBR in concentration of $10^{-8}$ M (KUKLÍKOVÁ 2011), in the third one BRZ in concentration of $10^{-5}$ M, which was chosen on the basis of experiments of ZHANG *et al.* (2010), SEKIMATA *et al.* (2001), MANZANO *et al.* (2011).

Next, the water supply was withdrawn for one half of each group for the period of 10 days, while the second half was kept irrigated as earlier (Fig. 5). Morphological parameters, relative water content, and photosynthetic characteristics, together with photosynthetic pigments content of plants were analysed at day 0, 2, 4, 6, 8 and 10 of this period, as described later in this chapter. Eight pots were kept for each combination of treatment, genotype and cultivation, using a completely randomized design. In total, 528 plants were analyzed in this experimental season.

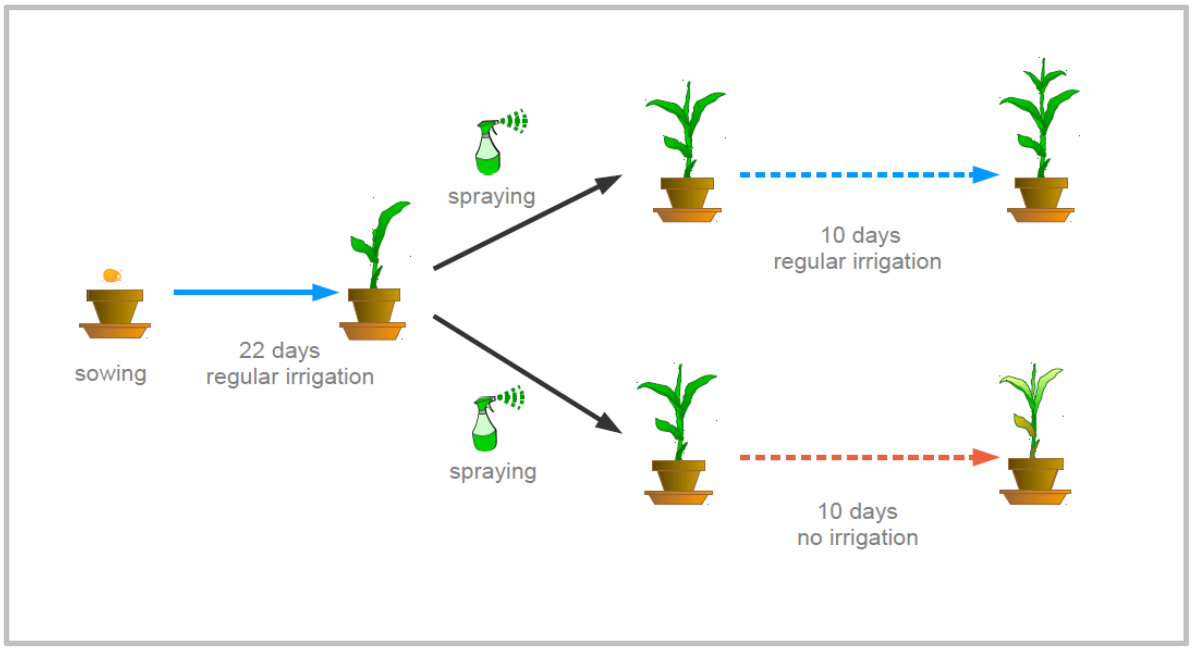


Fig. 5. Scheme of the experimental design during the spring season. Kernels of two maize inbred lines (CE704 and 2023) were sown and grown for 22 days. After, they were sprayed with tap water with Tween 20 and addition of $10^{-8}$ M concentration of 24-epibrassinolide, $10^{-5}$ M concentration of brassinazole or with no supplement. Plants were irrigated as shown. There were eight replications of each variant.

## 4.4 GROWTH CONDITIONS

Irradiation was measured randomly several times each day at about 9 a.m. at the level of upper leaves, approximately at the time when fluorescence

of chlorophyll *a* was measured. In the autumn season, luxmeter LX 107 (Merci, Brno, Czech Republic) was used to determine the average irradiation which means varied during the experiments from 1374 to 4438 lux. In spring experimental season, the irradiation was measured by Testo 435-4 device (Testo, Prague, Czech Republic) and its means varied from 42 to 94 PAR (from 1129 to 6675 lux). During both experimental seasons conditions for all the plants were kept uniform at a time.

Soil temperature in 5 cm depth was measured during the autumn experimental season randomly each day in four pots of “stressed” or “recovered” plants and four pots of corresponding “control” plants. Thrust thermometer Multi-D (Thermo Fisher Scientific, Waltham, Massachusetts, USA) was used to measure this characteristic before the fluorescence of chlorophyll *a* was measured. Its means varied from 18 to 25°C. During the spring season, soil temperature (Fig. 6) and relative moisture (Fig. 7) were recorded one day before and at the time when fluorescence of chlorophyll *a* was measured for the same plants, approximately in 5 cm depth by the Moisture Meter type HH2 (Delta - Devices, Cambridge, United Kingdom).

## 4.5 EXPERIMENTAL METHODS

In the autumn experimental season, at the end of “drought” period (35 days after sowing) as well as at the end of “recovery” period (38 days after sowing) different characteristics were measured. In the spring experimental season, different characteristics were measured at day 0, 2, 4, 6, 8 and 10 after spraying the plants.

### 4.5.1 DEVELOPMENT AND MORPHOLOGICAL CHARACTERISTICS

From the day of the sprouting until the day plants were analysed and cut up for samples and measurements, their development was observed. Each day a number of fully expanded and visible true leaves were noted down. Their physiological characteristics, colour and dryness/freshness in particular, were observed as well. The percentage of sprouting and rate of development were established from this data.

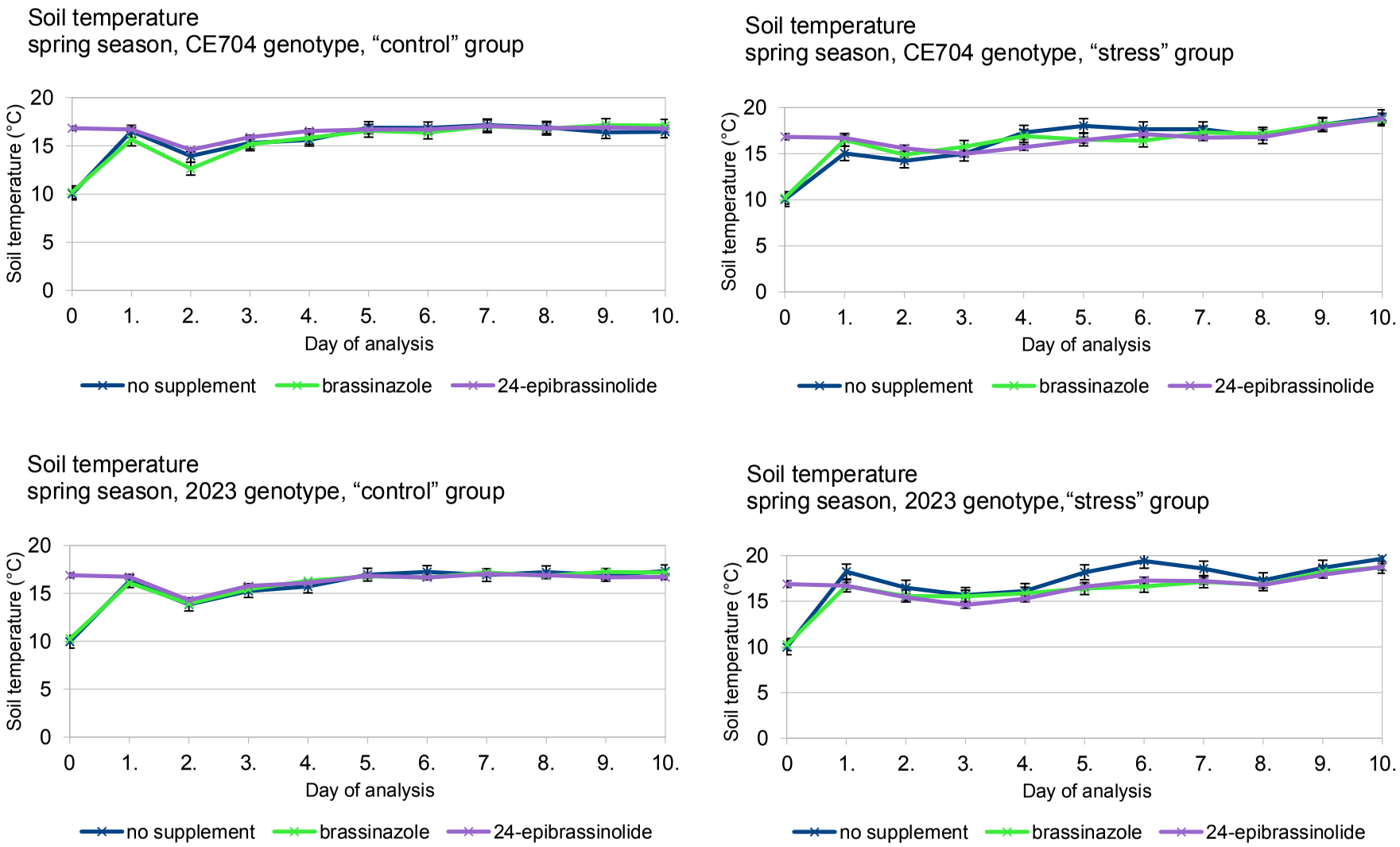


Fig. 6. Soil temperature measured each day of the experimental period during the spring season in 5 cm depth. For each day mean and standard error of the mean (n = 8) is shown individually for two genotypes (CE704 and 2023) and two types of the soil moisture conditions (“control” and “stress” group).

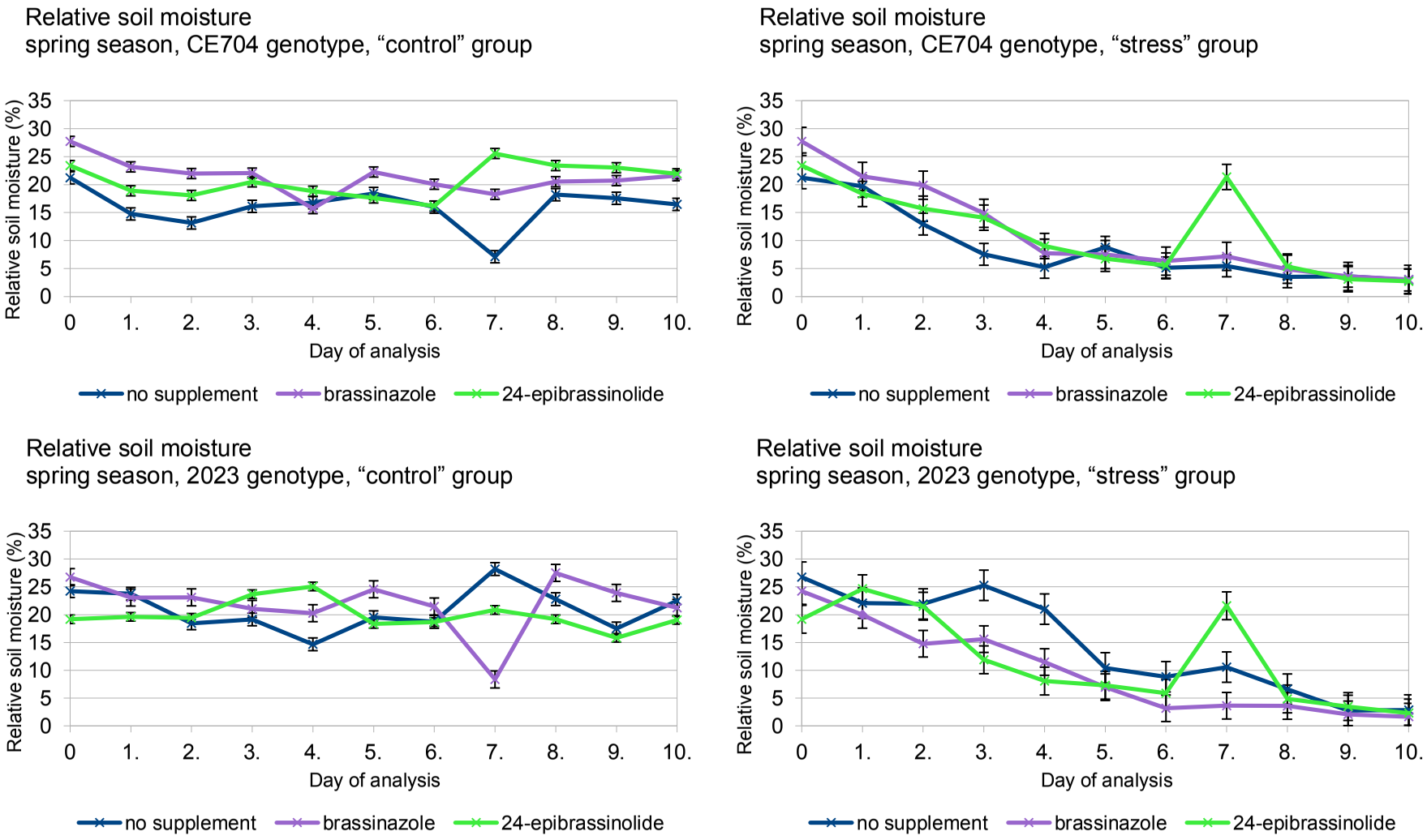


Fig. 7. Relative soil moisture measured each day of the experimental period during the spring season in 5 cm depth. For each day mean and standard error of the mean (n = 8) is shown individually for two genotypes (CE704 and 2023) and two types of the soil moisture conditions (“control” and “stress” group).

At the day of analyses, plant height was measured from the soil surface where the stalk starts to the whorl on the top of the plant (as long as all other length characteristics) within a scale of millimeters. Simultaneously, the position of nodes was measured from the bottom to the top, providing data for internode lengths calculation.

All fully expanded leaves growing from nodes were cut off with the use of scissors and both their length (from the leaf collar to the tip) and width (in the widest part of the leave) were measured. Next, each leaf separately was pinned to the polystyrene plate and put in a dryer for 5 days at 80°C. Then their individual mass was weighted within the scale of milligrams. This data were later used to determine contents of pigments.

When all fully expanded leaves were separated, plants were cautiously extracted from the pots and residues of soil were carefully and properly rinsed away from roots with water. After the stalk and roots were cut apart, the maximal length of main root was measured and stalks with not fully expanded leaves and roots (separately for each plant) were put in dryer for 7 days at 80°C. When dehydrated, their mass was weighted. Shoot-root ratio, shoot-whole plant ratio and root-whole plant ratio was determined for each plant.

### 4.5.2 DETERMINATION OF RELATIVE WATER CONTENT

To asses the water status of plants, twelve discs in the autumn experimental season (d = 8 mm), respectively four discs in the spring experimental season (d = 6 mm) were cut from the middle of the 4$^{th}$ leaves, excluding the midrib. Fresh mass (FM) of discs *per* plant was weighted quickly by analytical balances (ABJ 200-4M, Kern, Balingen, Germany or XT 120 A, Precisa, Dietikon, Switzerland, both with accuracy 0.1 mg and used for all the other weighing named bellow) and then discs were immediately put in the small Petri dishes on a piece of sterile gauze (stopping discs from moving and sticking together) and poured over by tap water from a washing bottle. After 5 hours when placed at dark (this time is sufficient for our plant material, BENEŠOVÁ *et al.* 2012) to saturate with water, discs were carefully taken out of Petri

dishes using a pin, firmly blotted with a dry piece of sterile gauze and their turgid mass (TM) was quickly weighted. Afterwards, the discs were put for 48 hours to a dryer Memmert UM 500 or Memmert UNE 200 (both Memmert GmbH + Co. KG, Achwabach, Germany) set to 80°C. When dehydrated, dry mass (DM) of discs was weighted for each plant. RWC was calculated from these values according the following formula (JONES and TURNER 1978):

$$RWC\ (\%) = [(FM - DM)/ (TM - DM)] \times 100$$

### 4.5.3 PHOTOSYNTHETIC CHARACTERISTICS

Fluorescence of chlorophyll *a* and related characteristics were measured always between 8 and 10 a.m. DST (daylight saving time or summertime) when there is the highest activity of photosynthetically active light.

During the autumn experimental season leaves (Photosystem II) were adapted to the dark by using the non-destructive clips, which were placed for 20 minutes in the middle part of the 4$^{th}$ (mostly not fully expanded) leave (excluding the midrib) of each plant intended to be analysed that day. Next, $F_0$ (minimal fluorescence, thought to represent emission by excited chlorophyll *a* molecules in the antennae structure of Photosystem II) and $F_m$ (maximal fluorescence value obtained for a continuous light intensity) characteristics were measured by OS-30p device (ADC BioScientific, Hoddesdon, United Kingdom), a portable pulse excitation instrument optimised for the rapid screening of plant stress. When the leaf clip shutter was withdrawn, the dark adapted site (78,5 mm$^2$) was exposed to 1 second lasting pulse of excitation 660 nm LED light with the intensity 3,000 µmol m$^{-2}$ s$^{-1}$. Induced fluorescence was then measured by a PIN photodiode at >700 nm. Based on the $F_0$ and $F_m$ values, $F_V/F_m$ value (which indicates the maximum quantum efficiency of Photosystem II and is widely considered to be a sensitive indicator of plant photosynthetic performance) was calculated using the following formula:

$$F_V/F_m = (F_m - F_0)/F_m$$

During the spring experimental season NDVI (normalized difference vegetative index), PRI (photochemical reflectance index) and chlorophyll *a* fluorescence parameters were measured on the plants intended to be analysed the next day and the very same day, also on the 4$^{th}$ (mostly not fully expanded) leave. During this season we performed transfers of plants to the dark room for 20 minutes instead of placing the clips on the leaves. After this period, to measure following characteristics, clip on the side of each machine was depressed to expose the optical window and the leave was placed against this window, while the clip held the leaf in place. After reading the values the leaf was released undamaged.

NDVI was measured by PlantPen NDVI 300 (Photon Systems Instruments, Drasov, Czech Republic). This parameter is considered to be an important indicator of chlorophyll content. The device with internal dual wavelength light source (VIS = 660 nm and NIR = 740 nm) compares reflected light as two distinct wavelengths.

PRI was measured by PlantPen PRI 200 (Photon Systems Instruments, Drasov, Czech Republic). This parameter can be used to measure photosynthetic light use efficiency and as a reliable water stress index. The device measures leaf reflectance in two narrow wavelength bands centered close to 531 and 570 nm. PRI is calculated placing the values in this formula:

$$PRI = (R531 – R570)/(R531 + R570)$$

Chlorophyll fluorescence primary photosynthesis parameters as photosynthetically active radiation measured as photosynthetic photon flux density, continuous fluorescence yield in non-actinic light, which is an equivalent to $F_0$ in dark-adapted leaves, QY (Photosystem II quantum yield), which is an equivalent to $F_V/F_m$ in the dark-adapted leaves, OJIP analysis (chlorophyll fluorescence fast-transient analysis which is a simple and non-invasive tool to monitor chloroplast function and can be used as a sensitive and reliable fast test for the functionality and vitality of photosynthetic system), non-photochemical quenching, which is typically used

for quantification of photochemical and non-photochemical quenching in dark-adapted samples were measured by PAR-FluorPen FP 100 device (Photon Systems Instruments, Drasov, Czech Republic).

Values of the fluorescence parameters from two experimental seasons (measured by different devices) are not comparable to each other, but relatively as differences within the each experimental season.

The content of photosynthetic pigments (chlorophyll *a*, chlorophyll *b* and total carotenoids) was established by the method of WELLBURN (1994).
After the fluorescence measurement, there were cut six discs in the autumn season, respectively four discs in the spring season (d = 8 mm, resp. d = 6 mm) from the middle part of the $4^{th}$ leave excluding the midrib, and separately for each plant put into a test tube and poured with 10 ml, respectively 5 ml of N,N-dimethylformamide. Test tubes covered with Parafilm (Pechiney Plastic Packaging Company, Chicago, Illinois, USA) and aluminium foil were put for 7 days in the fridge. During this period each test tube was vortexed three times. After this period samples were kept out of the fridge until they were the same temperature as the room and then their absorbance was measured at 480 nm (A480), 647 nm (A647), 664 nm (A664) and 710 nm (A710) by UV/VIS spectrophotometer Anthelie Advanced 2 (Secomam, Ales, France). Chlorophyll *a*, chlorophyll *b* and total carotenoids content was computed placing this values in following equations:

$$chl_a = 11{,}65 \times (A664 - A710) - 2{,}69 \times (A647 - A710)$$
$$chl_b = 20{,}81 \times (A647 - A710) - 4{,}53 \times (A664 - A710)$$
$$car = (1{,}000 \times (A480 - A710) - 0{,}89 \times chl_a - 52{,}02 \times chl_b) / 245$$

Different photosynthetic pigments content *per* unit leaf area or *per* unit leaf dry mass was then specified. The ratio of chlorophyll *a* to chlorophyll *b* and total carotenoids to total chlorophylls ratio was determined, as well.

## 4.6 STATISTICS

Data obtained by different analyses, measurements and calculations were statistically analysed using a CoStat software (version 6.204, CoHort Software, Monterey, California, USA) to reveal confirmatory differences between genotypes, hormonal treatments and different water supply.

Data from the autumn experimental season were analysed using the one-way, two-way (with interactions) and three-way (with interactions) randomized blocks ANOVA, where blocks were dates, as the sowing was done gradually. Differences were then tested by Tukey's HSD (honestly significant difference, when there were equal sample sizes in two groups being compared) or by Tukey-Kramer (when there were unequal sample sizes in two groups being compared) *post hoc* test for multiple comparisons and considered significant at level of $P \leq 0.05$.

Data from the spring experimental season were analysed using the one-way, two-way (with interactions) and three-way (with interactions) completely randomized ANOVA, followed by Tukey's HSD or Tukey-Kramer test (as described above).

# 5. RESULTS

Results of the two experimental seasons are presented in separated chapters.

## 5.1 THE AUTUMN SEASON

During the autumn season we tested the effect of soaking the maize kernels in solution with different concentrations of EBR. Two inbred maize lines with contrastive reaction to water deficit (2023 and CE704) and their reciprocal crosses (2023xCE704 and CE704x2023) were tested in this experiment. After the soaking, kernels were sown and grown for 28 days with the regular water supply. Then the water supply was withdrawn for 7 days during the period referred as the “stress period” (according to the way of cultivation plants were divided into “drought” group, which was without water supply, and “control1” group, which was kept irrigated during this period). Afterwards, plants were let to recover for the period of 3 days, referred as the “recovery period” (plants were divided into “recovery” group, which was re-irrigated after overcoming the water supply deficit, and “control2” group, which was kept irrigated during the whole experiment). It was tested, if this type of EBR application is suitable for the further investigation and which concentration ($10^{-8}$ M, $10^{-10}$ M, $10^{-12}$ M, $10^{-14}$ M or 0 M, respectively E8, E10, E12, E14 or E0) of EBR has the most positive effect on maize plants of tested genotypes and under the conditions mentioned above. Characteristics were analysed using the one-way randomized blocks ANOVA, where blocks were dates, as the sowing was done gradually. They were statistically significant for most of the characteristics. Differences in means were tested by Tukey's HSD or by Tukey-Kramer *post hoc* test for multiple comparisons and considered significant at level of $P \leq 0.05$. Results of the statistical analysis can be found in the Section I of the S1 Table in the Supplement (CD enclosed).

### 5.1.1 GENOTYPES

Compared to the CE704 genotype, plants of the 2023 genotype germinated sooner, grew bigger and produced more biomass. In the velocity of development

of the second and the third leaf plants of the CE704 genotype surpassed the 2023 plants. Generally, the hybrid plants had similar characteristics as their maternal lines, and their performance was even better. For particular results of the statistical analysis see Table S1, Section I.

### 5.1.2. CULTIVATION

The drought period resulted in the retarded development, decreased RWC and shoot-root dry mass ratio, and changed fluorescence characteristics in plants subjected to water deficit. However, biomass accumulation was not affected.
At the end of the recovery period there was no significant difference in the most of the characteristics of plants recovered after water deficit and control2 group plants. For particular results of the statistical analysis see Table S1, Section I.

### 5.1.3 TREATMENT

#### 5.1.3.1 DEVELOPMENT AND GROWTH

Soaking of the maize kernels in different concentrations of EBR previously to sowing had no effect on germinability of plants. In plants of 2023, CE704 and CE704x2023 genotype was the development of the third leaf accelerated by $10^{-8}$ M EBR treatment, while soaking in 0 M EBR solution resulted in slower development of this leaf.
In plants of 2023 genotype $10^{-8}$ M EBR treatment had positive effect also on the second leaf development in comparison with $10^{-14}$ M EBR treatment. See the overview of statistical analysis in Table 2.

Treatment with $10^{-8}$ M EBR resulted in faster growth of 2023, CE704 and CE704x2023 plants when compared to 0 M or $10^{-14}$ M EBR treatment in maize plants before the beginning of the stress period. For details see the overview of statistical analysis in Table 3.

Table 2. Overview of the statistical analysis of differences (α = 0.05) in developmental characteristics of maize plants treated with 0 M, $10^{-8}$ M, $10^{-10}$ M, $10^{-12}$ M, and $10^{-14}$ M (E0, E8, E10, E12 and E14) 24-epibrassinolide. Genotypes 2023, CE704, 2023xCE704, CE704x2023 were used and difference in the number of days until germination or visibility of leaves were compared among the treatments. For particular results of statistical analysis see Table S1, Section I. When compared to each other:

values are significantly lower  values are significantly higher

| Parameter | 2023 | | | | | CE704 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E0 | E8 | E10 | E12 | E14 | E0 | E8 | E10 | E12 | E14 |
| Germinability | | | | | | | | | | |
| Days until the shoot visible | | | | | | | | | | |
| Days until the 1st leaf visible | | | | | | | | | | |
| Days until the 2nd leaf visible | | | | | | | | | | |
| Days until the 3rd leaf visible | | | | | | | | | | |

| Parameter | 2023xCE704 | | | | | CE704x2023 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E0 | E8 | E10 | E12 | E14 | E0 | E8 | E10 | E12 | E14 |
| Germinability | | | | | | | | | | |
| Days until the shoot visible | | | | | | | | | | |
| Days until the 1st leaf visible | | | | | | | | | | |
| Days until the 2nd leaf visible | | | | | | | | | | |
| Days until the 3rd leaf visible | | | | | | | | | | |

In plants of the CE704x2023 genotype subjected to water deficit $10^{-8}$ M EBR treatment increased the length of the second internode, height of the plant and number of visible leaves when compared to $10^{-14}$ M EBR treatment. Height of CE704 plants also increased after $10^{-8}$ M EBR treatment, while plants treated with 0 M EBR were smaller. The $10^{-10}$ M EBR treatment affected the number of fully expanded leaves of 2023 plants in comparison with 0 M treatment. The same effect of these EBR concentrations was exhibited in the number of visible leaves of 2023 control plants, while height of these plants was increased by $10^{-8}$ M EBR treatment, both when compared to 0 M EBR treatment. There was also a positive effect of $10^{-10}$ M EBR treatment on the internode growth of both hybrids from control1 group when compared to $10^{-14}$ M treatment. For details see the overview of statistical analysis in Table 4 at the end of the chapter 5.1.3.

Table 3. Overview of the statistical analysis of differences (α = 0.05) in growth characteristics of maize plants before the stress period (28 days after sowing). Kernels of 2023, CE704, 2023xCE704 and CE704x2023 genotype were soaked in 0 M, $10^{-8}$ M, $10^{-10}$ M, $10^{-12}$ M, and $10^{-14}$ M (E0, E8, E10, E12 and E14) 24-epibrassinolide solution previously to sowing. For particular results of statistical analysis see Table S1, Section I. When compared to each other:

values are significantly lower  values are significantly higher

| Parameter | 2023 | | | | | CE704 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E0 | E8 | E10 | E12 | E14 | E0 | E8 | E10 | E12 | E14 |
| Lengths of the internodes | | | | | | | | | | |
| Length of the 1st internode | | | | | | | | | | |
| Length of the 2nd internode | | | | | | | | | | |
| Height of the plants | | | | | | | | | | |
| Height of the plant | | | | | | | | | | |
| Lengths of the leaves | | | | | | | | | | |
| Length of the 1st leaf | | | | | | | | | | |
| Length of the 2nd leaf | | | | | | | | | | |
| Length of the 3rd leaf | | | | | | | | | | |
| Number of visible leaves | | | | | | | | | | |
| Visible leaves | | | | | | | | | | |
| Number of fully expanded leaves | | | | | | | | | | |
| Fully expanded leaves | | | | | | | | | | |

| Parameter | 2023xCE704 | | | | | CE704x2023 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E0 | E8 | E10 | E12 | E14 | E0 | E8 | E10 | E12 | E14 |
| Lengths of the internodes | | | | | | | | | | |
| Length of the 1st internode | | | | | | | | | | |
| Length of the 2nd internode | | | | | | | | | | |
| Height of the plants | | | | | | | | | | |
| Height of the plant | | | | | | | | | | |
| Lengths of the leaves | | | | | | | | | | |
| Length of the 1st leaf | | | | | | | | | | |
| Length of the 2nd leaf | | | | | | | | | | |
| Length of the 3rd leaf | | | | | | | | | | |
| Number of visible leaves | | | | | | | | | | |
| Visible leaves | | | | | | | | | | |
| Number of fully expanded leaves | | | | | | | | | | |
| Fully expanded leaves | | | | | | | | | | |

After the recovery period, there was a difference in treatments only in the length of the second internodes and the height of the plants (see Table 5 at the end of the chapter 5.1.3). To demonstrate the effect of EBR treatment on the plant growth, heights of plants after the stress period and after the recovery period were compared (Fig. 8). The $10^{-8}$ M EBR treatment increased heights of CE704x2023 plants subjected to water deficit when compared to $10^{-14}$ M treatment, and heights of CE704 drought group plants and 2023 control1 plants in comparison with $10^{-8}$ M EBR treatment. After the recovery period, there was a significant difference in plants height only in CE704 recovered plants.

### 5.1.3.2 DRY MASS ACCUMULATION

The treatment with $10^{-12}$ M EBR increased dry mass accumulation of the shoot and subsequently the whole plant in 2023 plants subjected to water deficit when compared to 0 M and $10^{-14}$ M EBR-treated plants, while in CE704 plants $10^{-8}$ M and $10^{-10}$ M EBR treatment increased this accumulation more than in 0 M EBR-treated plants. The treatment also influenced dry mass of the first leaf and dry mass of the shoot residue of CE704x2023 plants subjected to water deficit diversely, however, no significant difference was evident in the whole plant dry mass accumulation. See the overview of statistical analysis in Table 4 at the end of the chapter 5.1.3.

After the recovery, the positive effect of $10^{-8}$ M EBR treatment was evident in 2023 plants from the control group and CE704x2023 recovered plants in shoot dry mass accumulation and subsequently accumulation of dry mass of the whole plants when compared to $10^{-14}$ M EBR treatment. The same effect of these concentrations was observed also in accumulation of dry mass of the fourth leave and root dry mass. Dry mass accumulation of the fourth leaves of CE704x2023 control plants treated with $10^{-8}$ M EBR was higher than in 0 M EBR-treated plants, while dry mass accumulation of the first leaves of CE704x2023 recovered plants treated with 0 M EBR was higher than of $10^{-10}$ M EBR-treated plants. See the overview of statistical analysis in Table 5 at the end of the chapter 5.1.3.

To demonstrate the effect of EBR treatment on the plant growth, dry masses of the whole plants after the stress period and after the recovery period were compared (Fig. 9). In plants subjected to water deficit, the accumulation of dry mass was higher in $10^{-8}$ M EBR-treated 2023 plants when compared to 0 M and $10^{-14}$ M EBR-treated, and in CE704 plants $10^{-8}$ M and $10^{-10}$ M EBR treatment increased it more than 0 M one. In recovered plants of CE704x2023 genotype was the dry mass accumulation higher after the $10^{-8}$ M EBR treatment in comparison with $10^{-14}$ M treatment.

#### 5.1.3.3 RELATIVE WATER CONTENT

As shown in Figure 10 and in Table 5, the only difference in treatments with EBR manifested in higher RWC of CE704 control2 group plants when treated with $10^{-14}$ M EBR in comparison with 0 M EBR-treated plants.

#### 5.1.3.4 PIGMENTS CONTENT

In plants subjected to water deficit there was a positive influence of $10^{-14}$ M EBR treatment when in 2023xCE704 plants the contents of chlorophyll *a* and also of chlorophyll *b per* unit leaf dry mass were higher in comparison with $10^{-10}$ M EBR- treated plants (Fig. 11). In control1 group CE704x2023 plants, chlorophyll *b* content *per* unit leaf dry mass was higher in $10^{-10}$ M EBR-treated in comparison to $10^{-12}$ M EBR-treated plants, and total carotenoids content *per* unit leaf dry mass was higher in $10^{-10}$ M EBR treated plants than in $10^{-8}$ M EBR treated. For details see the overview of statistical analysis in Table 4 at the end of the chapter 5.1.3.

After the recovery period, the only significant difference was in the content of total carotenoids *per* unit leaf area in CE704 recovered plants. It was higher in $10^{-12}$ M EBR-treated plants than in $10^{-14}$ M EBR-treated. See the overview of statistical analysis in Table 5 at the end of the chapter 5.1.3.

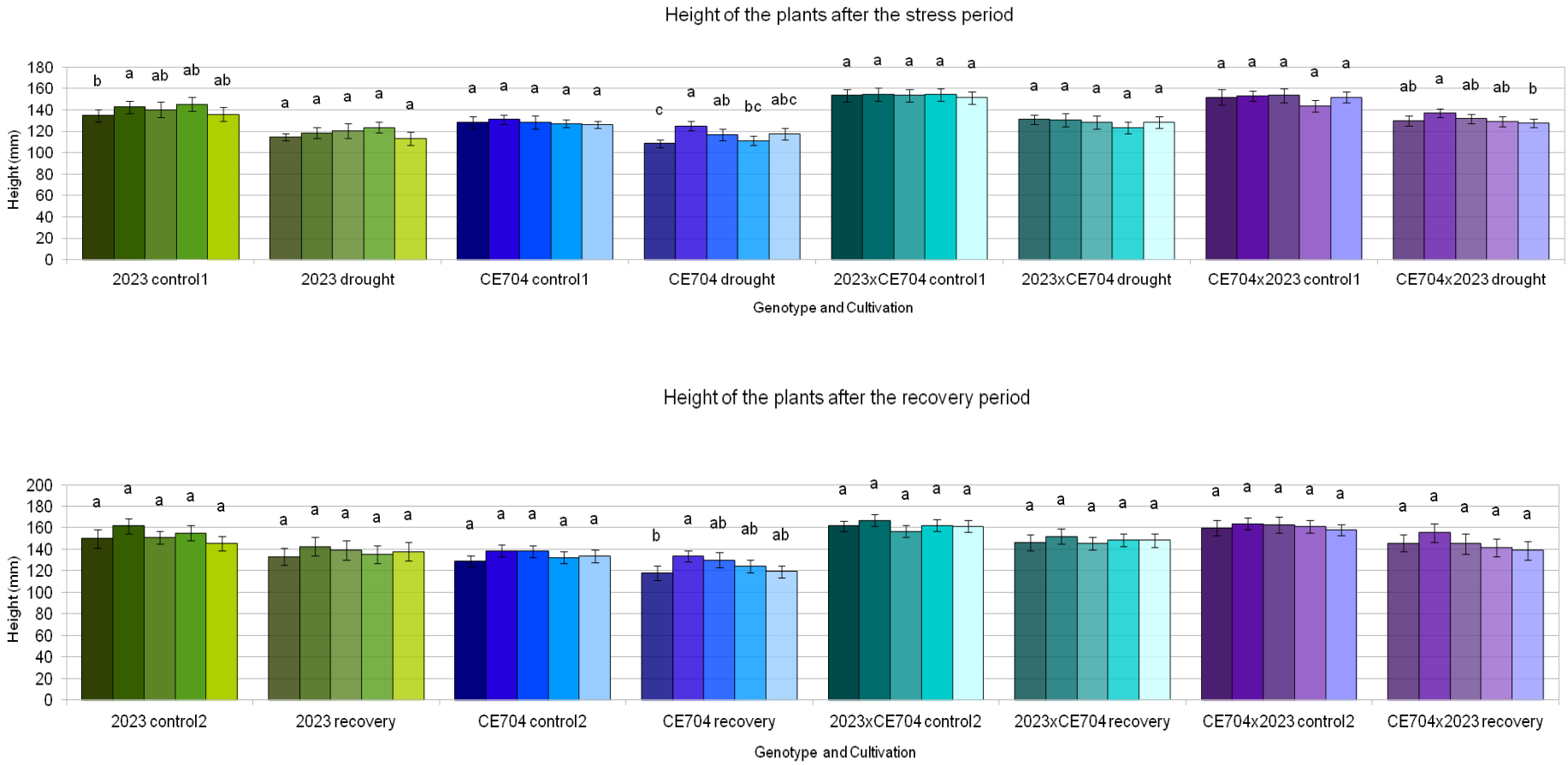


Fig. 8. Heights of plants after the stress period and after the recovery period. For each combination of genotype (2023, CE704, 2023xCE704, CE704x2023) and cultivation (drought and control1 or recovery and control2, respectively) the concentrations of 24-epibrassinolide used for kernels soaking follows as 0 M, $10^{-8}$ M, $10^{-10}$ M, $10^{-12}$ M and $10^{-14}$ M (from the darkest to the lightest colour). Each treatment is represented by its mean and the standard error of mean (n = 12). Different letters indicate significant differences between the treatments (one-way ANOVA, Tukey's HSD or Tukey-Kramer test, $P \leq 0.05$).

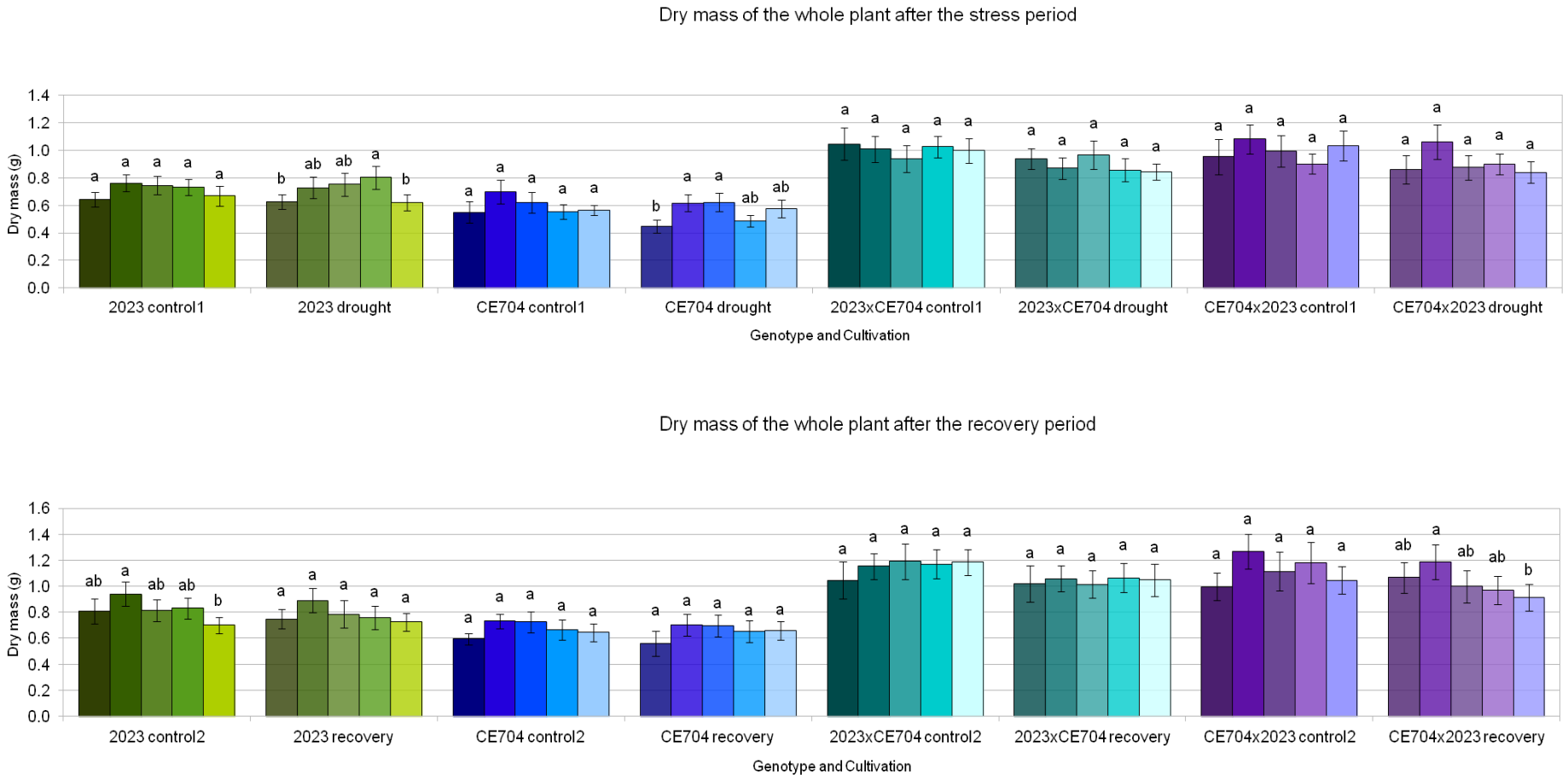


Fig. 9. Dry masses of the whole plants after the stress period and after the recovery period. For each combination of genotype (2023, CE704, 2023xCE704, CE704x2023) and cultivation (drought and control1 or recovery and control2, respectively) the concentrations of 24-epibrassinolide used for kernels soaking follows as 0 M, $10^{-8}$ M, $10^{-10}$ M, $10^{-12}$ M and $10^{-14}$ M (from the darkest to the lightest colour). Each treatment is represented by its mean and the standard error of mean (n = 12). Different letters indicate significant differences between the treatments (one-way ANOVA, Tukey's HSD or Tukey-Kramer test, $P \leq 0.05$).

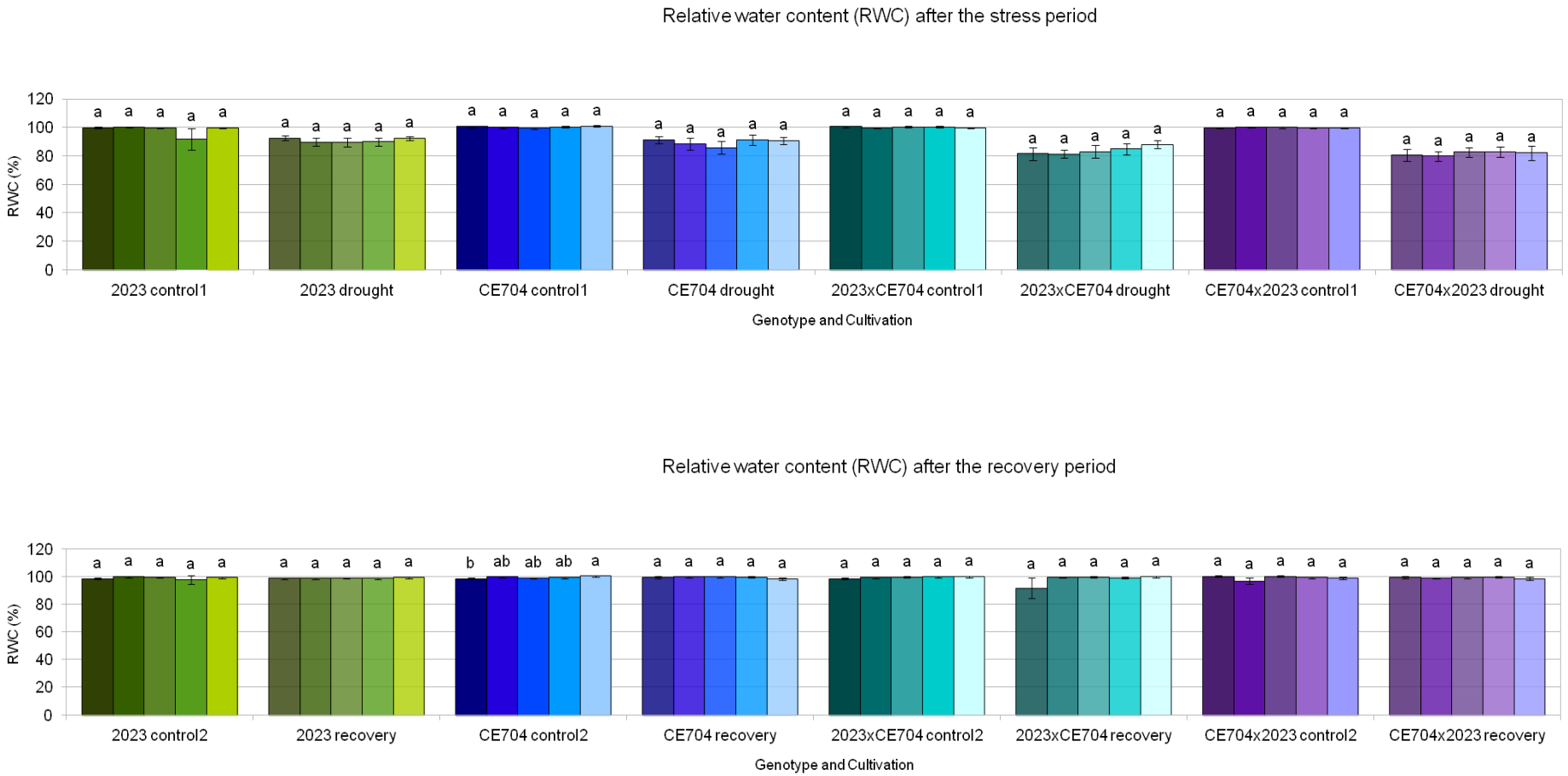


Fig. 10. Relative water content of plants after the stress period and after the recovery period. For each combination of genotype (2023, CE704, 2023xCE704, CE704x2023) and cultivation (drought and control1 or recovery and control2, respectively) the concentrations of 24-epibrassinolide used for kernels soaking follows as 0 M, $10^{-8}$ M, $10^{-10}$ M, $10^{-12}$ M and $10^{-14}$ M (from the darkest to the lightest colour). Each treatment is represented by its mean and the standard error of mean (n = 12). Different letters indicate significant differences between the treatments (one-way ANOVA, Tukey's HSD or Tukey-Kramer test, $P \leq 0.05$).

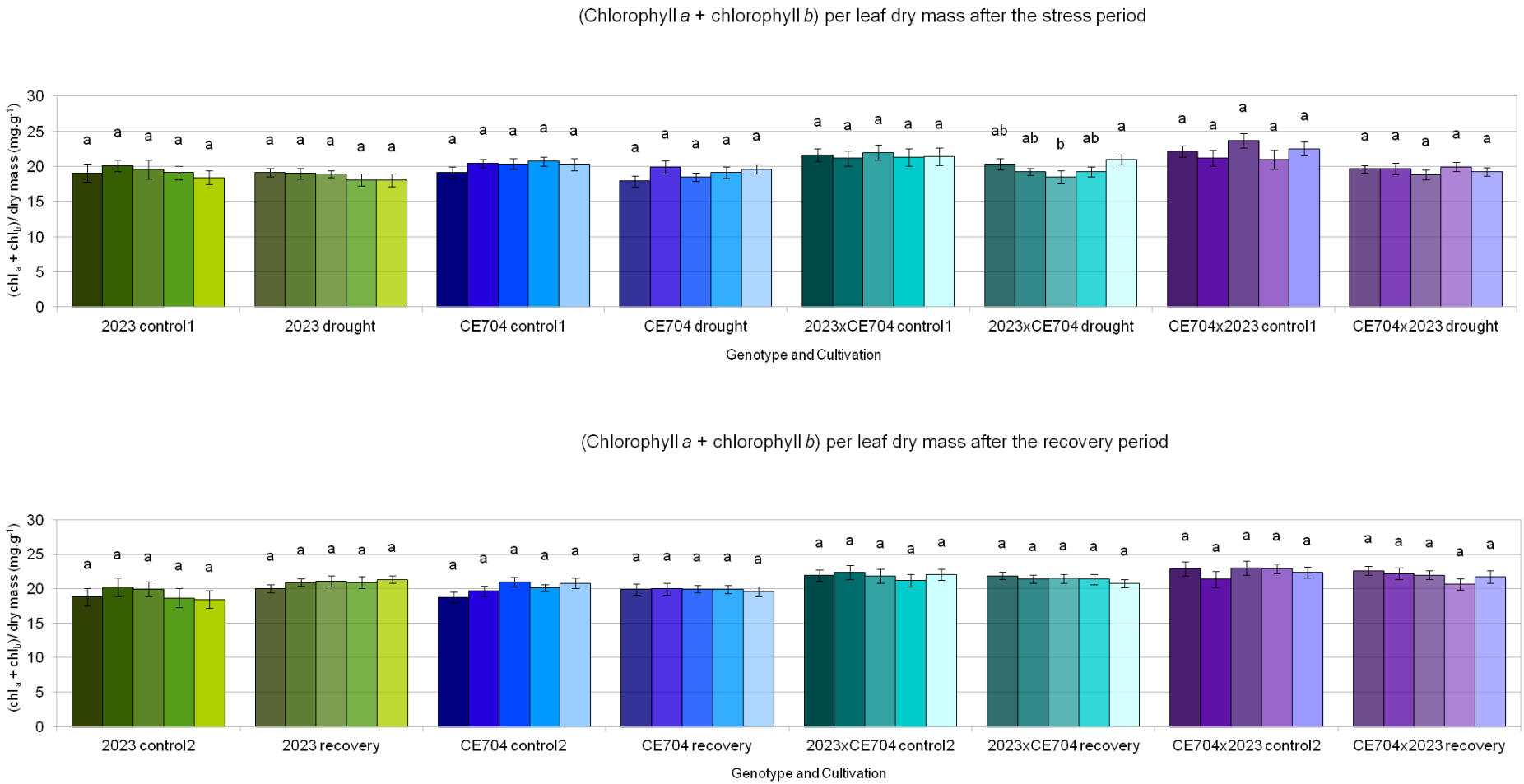


Fig. 11. The sum of chlorophyll *a* and chlorophyll *b per* unit leaf dry mass of plants after the stress period and after the recovery period. For each combination of genotype (2023, CE704, 2023xCE704, CE704x2023) and cultivation (drought and control1 or recovery and control2, respectively) the concentrations of 24-epibrassinolide used for kernels soaking follows as 0 M, $10^{-8}$ M, $10^{-10}$ M, $10^{-12}$ M and $10^{-14}$ M (from the darkest to the lightest colour). Each treatment is represented by its mean and the standard error of mean (n = 12). Different letters indicate significant differences between the treatments (one-way ANOVA, Tukey's HSD or Tukey-Kramer test, $P \leq 0.05$).

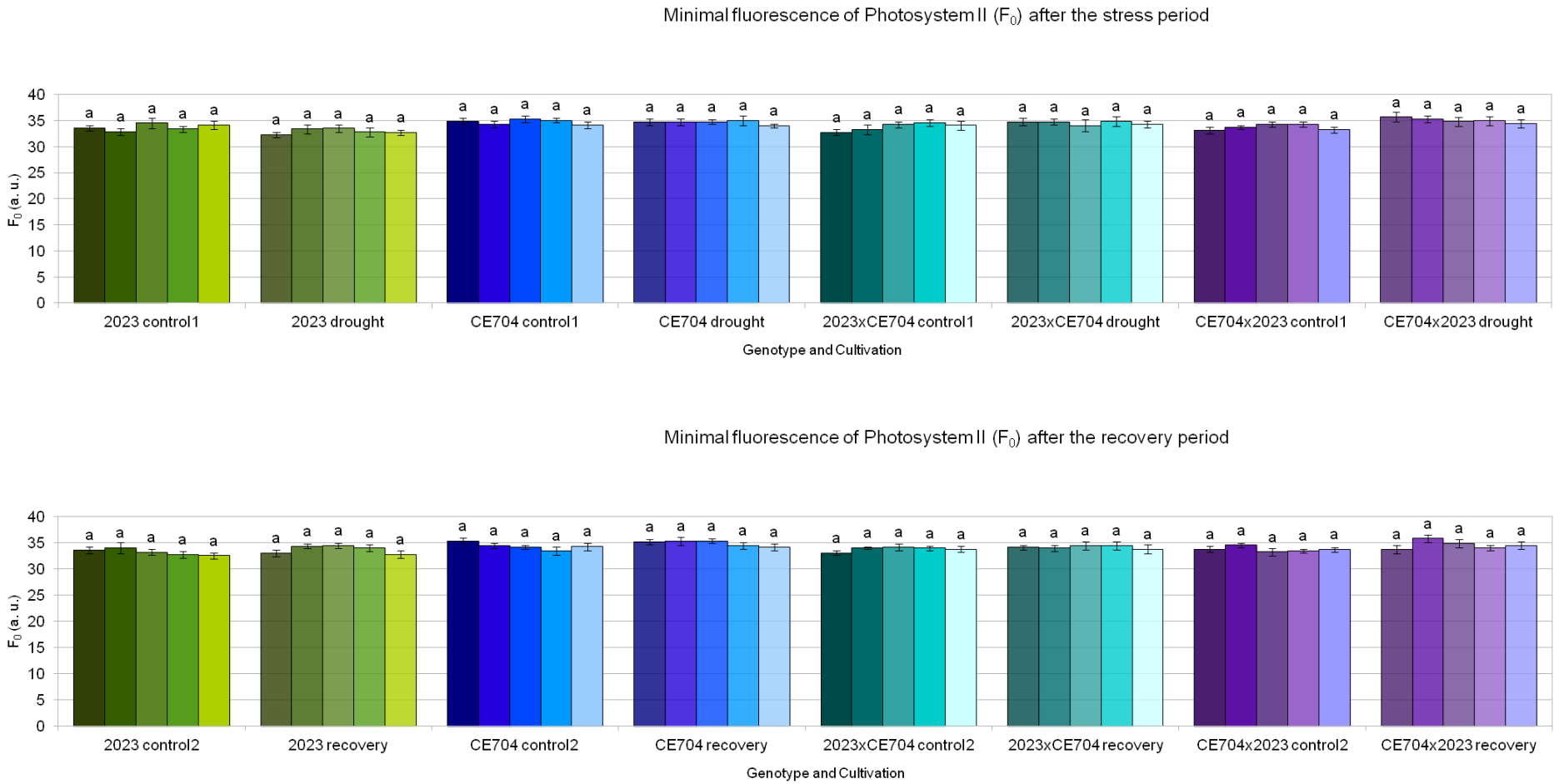


Fig. 12. Minimal fluorescence of Photosystem II ($F_0$) of plants after the stress period and after the recovery period. For each combination of genotype (2023, CE704, 2023xCE704, CE704x2023) and cultivation (drought and control1 or recovery and control2, respectively) the concentrations of 24-epibrassinolide used for kernels soaking follows as 0 M, $10^{-8}$ M, $10^{-10}$ M, $10^{-12}$ M and $10^{-14}$ M (from the darkest to the lightest colour). Each treatment is represented by its mean and the standard error of mean (n = 12). Different letters indicate significant differences between the treatments (one-way ANOVA, Tukey's HSD or Tukey-Kramer test, $P \leq 0.05$).

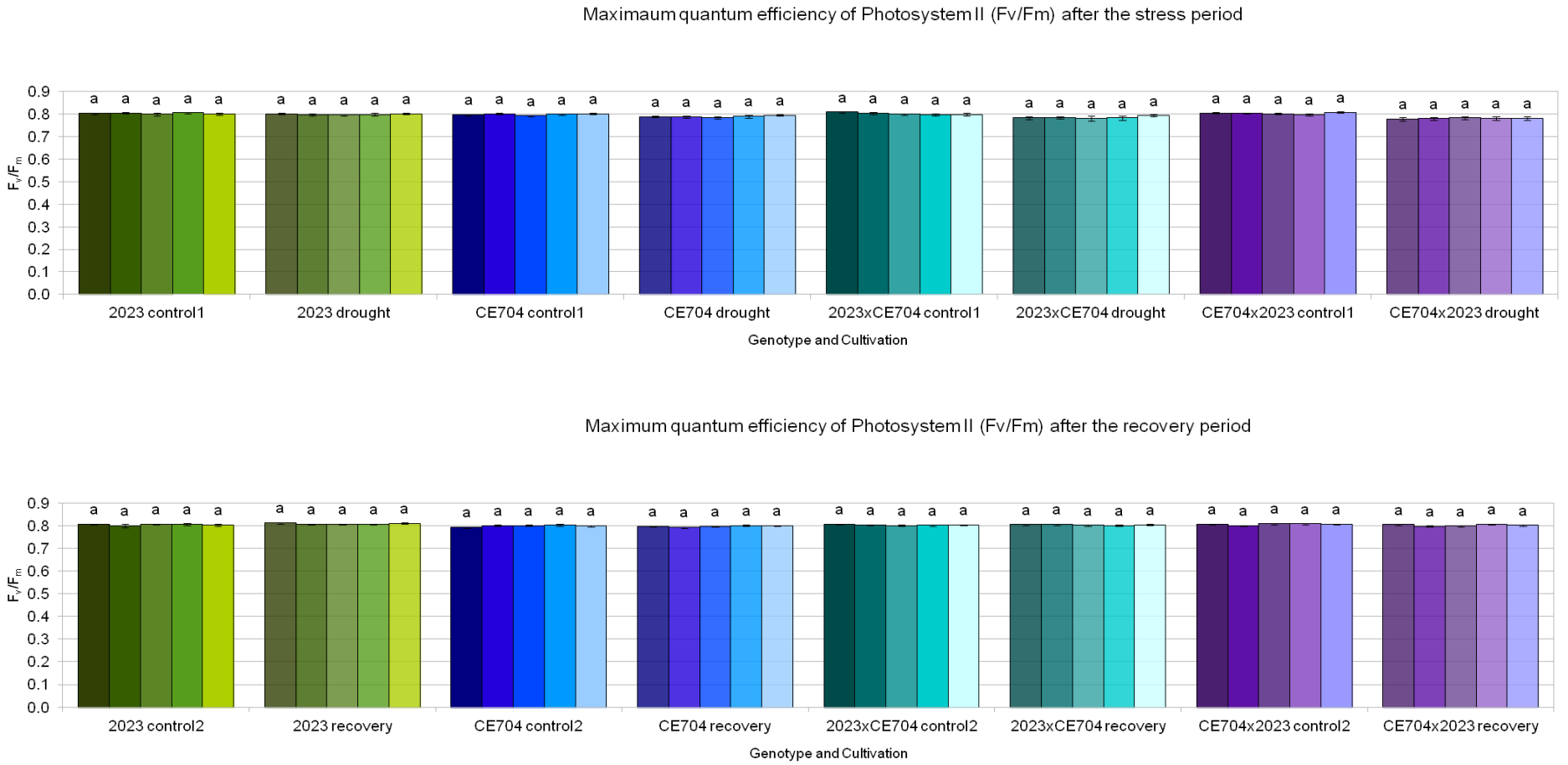


Fig. 13. Maximum quantum efficiency of Photosystem II ($F_v/F_m$) of plants after the stress period and after the recovery period. For each combination of genotype (2023, CE704, 2023xCE704, CE704x2023) and cultivation (drought and control1 or recovery and control2, respectively) the concentrations of 24-epibrassinolide used for kernels soaking follows as 0 M, $10^{-8}$ M, $10^{-10}$ M, $10^{-12}$ M and $10^{-14}$ M (from the darkest to the lightest colour). Each treatment is represented by its mean and the standard error of mean (n = 12). Different letters indicate significant differences between the treatments (one-way ANOVA, Tukey's HSD or Tukey-Kramer test, $P \leq 0.05$).

### 5.1.3.5 PHOTOSYNTHETIC CHARACTERISTICS

There was no significant difference in different concentrations of EBR treatments on fluorescent characteristics: $F_0$ and $F_m$ (minimal and maximal fluorescence of Photosystem II), and $F_V/F_m$ (maximum quantum efficiency of Photosystem II). The only exception was the $F_m$ value of 2023xCE704 plants after the stress period, when this value was higher for $10^{-14}$ M EBR-treated plants when compared to $10^{-10}$ M EBR-treated. See the overview of statistical analysis in Table 4 and Table 5 at the end of the chapter 5.1.3.

To demonstrate the effect of EBR treatment on chlorophyll *a* fluorescence characteristics, $F_0$ and $F_V/F_m$ values of the plants after the stress period and after the recovery period were compared (Fig. 12 and 13).

Table 4. Overview of the statistical analysis of differences (α = 0.05) in characteristics of maize plants after the 7 days lasting stress period (35 days after sowing). Kernels of 2023, CE704, 2023xCE704 and CE704x2023 genotype were soaked in 0 M, $10^{-8}$ M, $10^{-10}$ M, $10^{-12}$ M, and $10^{-14}$ M (E0, E8, E10, E12 and E14) 24-epibrassinolide solution previously to sowing. For particular results of statistical analysis see Table S1, Section I. When compared to each other:

values are significantly lower  values are significantly higher

| Parameter | control1/2023 | | | | | drought/2023 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E0 | E8 | E10 | E12 | E14 | E0 | E8 | E10 | E12 | E14 |
| Lengths of the internodes | | | | | | | | | | |
| Length of the 1st internode | | | | | | | | | | |
| Length of the 2nd internode | | | | | | | | | | |
| Lengths of the internodes - difference between the start and the end of the stress period | | | | | | | | | | |
| Δ (length of the 1st internode) | | | | | | | | | | |
| Δ (length of the 2nd internode) | | | | | | | | | | |
| Height of the plants | | | | | | | | | | |
| Height of the plant | | | | | | | | | | |
| Height of the plant - difference between the start and the end of the stress period | | | | | | | | | | |
| Δ (height of the plant) | | | | | | | | | | |

| Parameter | control1/2023 | | | | | drought/2023 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E0 | E8 | E10 | E12 | E14 | E0 | E8 | E10 | E12 | E14 |
| Lengths of the leaves | | | | | | | | | | |
| Length of the 1st leaf | | | | | | | | | | |
| Length of the 2nd leaf | | | | | | | | | | |
| Length of the 3rd leaf | | | | | | | | | | |
| Number of visible leaves | | | | | | | | | | |
| Visible leaves | | | | | | | | | | |
| Number of visible leaves - difference between the start and the end of the stress period | | | | | | | | | | |
| Δ (visible leaves) | | | | | | | | | | |
| Number of fully expanded leaves | | | | | | | | | | |
| Fully expanded leaves | | | | | | | | | | |
| Number of fully expanded leaves - difference between the start and the end of the stress period | | | | | | | | | | |
| Δ (fully expanded leaves) | | | | | | | | | | |
| Dry masses (DM) of the leaves | | | | | | | | | | |
| DM of the 1st leaf | | | | | | | | | | |
| DM of the 2nd leaf | | | | | | | | | | |
| DM of the 3rd leaf | | | | | | | | | | |
| Dry mass (DM) of the shoot residues | | | | | | | | | | |
| DM of the shoot residue | | | | | | | | | | |
| Dry mass (DM) of the shoots (including dry masses of leaves) | | | | | | | | | | |
| DM of the shoot (with leaves) | | | | | | | | | | |
| Dry mass (DM) of the roots | | | | | | | | | | |
| DM of the root | | | | | | | | | | |
| Dry mass (DM) of the whole plants | | | | | | | | | | |
| DM of the whole plant | | | | | | | | | | |
| Shoot to root dry mass (DM) ratios | | | | | | | | | | |
| Shoot-root DM ratio | | | | | | | | | | |
| Relative water content (RWC) | | | | | | | | | | |
| RWC | | | | | | | | | | |
| Specific leaf weight (SLW) | | | | | | | | | | |
| SLW | | | | | | | | | | |
| Pigments content *per* leaf unit area (LA) | | | | | | | | | | |
| Chl *a*/LA | | | | | | | | | | |
| Chl *b*/LA | | | | | | | | | | |
| (Chl *a* + chl *b*)/LA | | | | | | | | | | |
| Carotenoids/LA | | | | | | | | | | |
| Pigments content *per* leaf dry mass (DM) | | | | | | | | | | |
| Chl *a*/DM | | | | | | | | | | |
| Chl *b*/DM | | | | | | | | | | |
| (Chl *a* + chl *b*)/DM | | | | | | | | | | |
| Carotenoids/DM | | | | | | | | | | |

| Parameter | control1/2023 | | | | | drought/2023 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E0 | E8 | E10 | E12 | E14 | E0 | E8 | E10 | E12 | E14 |
| Chlorophyll *a* to chlorophyll *b* ratio | | | | | | | | | | |
| Chl *a*/chl *b* | | | | | | | | | | |
| Total carotenoids to chlorophyll *a* and chlorophyll *b* ratio | | | | | | | | | | |
| Carotenoids/(chl *a* + chl *b*) | | | | | | | | | | |
| Minimal fluorescence of Photosystem II ($F_0$) | | | | | | | | | | |
| $F_0$ | | | | | | | | | | |
| Maximal fluorescence of Photosystem II ($F_m$) | | | | | | | | | | |
| $F_m$ | | | | | | | | | | |
| Maximum quantum efficiency of Photosystem II ($F_v/F_m$) | | | | | | | | | | |
| $F_v/F_m$ | | | | | | | | | | |

| Parameter | control1/CE704 | | | | | drought/CE704 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E0 | E8 | E10 | E12 | E14 | E0 | E8 | E10 | E12 | E14 |
| Lengths of the internodes | | | | | | | | | | |
| Length of the 1st internode | | | | | | | | | | |
| Length of the 2nd internode | | | | | | | | | | |
| Lengths of the internodes - difference between the start and the end of the stress period | | | | | | | | | | |
| Δ (length of the 1st internode) | | | | | | | | | | |
| Δ (length of the 2nd internode) | | | | | | | | | | |
| Height of the plants | | | | | | | | | | |
| Height of the plant | | | | | | | | | | |
| Height of the plant - difference between the start and the end of the stress period | | | | | | | | | | |
| Δ (height of the plant) | | | | | | | | | | |
| Lengths of the leaves | | | | | | | | | | |
| Length of the 1st leaf | | | | | | | | | | |
| Length of the 2nd leaf | | | | | | | | | | |
| Length of the 3rd leaf | | | | | | | | | | |
| Number of visible leaves | | | | | | | | | | |
| Visible leaves | | | | | | | | | | |
| Number of visible leaves - difference between the start and the end of the stress period | | | | | | | | | | |
| Δ (visible leaves) | | | | | | | | | | |
| Number of fully expanded leaves | | | | | | | | | | |
| Fully expanded leaves | | | | | | | | | | |
| Number of fully expanded leaves - difference between the start and the end of the stress period | | | | | | | | | | |
| Δ (fully expanded leaves) | | | | | | | | | | |

| Parameter | control1/CE704 | | | | | drought/CE704 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E0 | E8 | E10 | E12 | E14 | E0 | E8 | E10 | E12 | E14 |
| Dry masses (DM) of the leaves | | | | | | | | | | |
| DM of the 1st leaf | | | | | | | | | | |
| DM of the 2nd leaf | | | | | | | | | | |
| DM of the 3rd leaf | | | | | | | | | | |
| Dry mass (DM) of the shoot residues | | | | | | | | | | |
| DM of the shoot residue | | | | | | | | | | |
| Dry mass (DM) of the shoots (including dry masses of leaves) | | | | | | | | | | |
| DM of the shoot (with leaves) | | | | | | | | | | |
| Dry mass (DM) of the roots | | | | | | | | | | |
| DM of the root | | | | | | | | | | |
| Dry mass (DM) of the whole plants | | | | | | | | | | |
| DM of the whole plant | | | | | | | | | | |
| Shoot to root dry mass (DM) ratios | | | | | | | | | | |
| Shoot-root DM ratio | | | | | | | | | | |
| Relative water content (RWC) | | | | | | | | | | |
| RWC | | | | | | | | | | |
| Specific leaf weight (SLW) | | | | | | | | | | |
| SLW | | | | | | | | | | |
| Pigments content *per* leaf unit area (LA) | | | | | | | | | | |
| Chl *a*/LA | | | | | | | | | | |
| Chl *b*/LA | | | | | | | | | | |
| (Chl *a* + chl *b*)/LA | | | | | | | | | | |
| Carotenoids/LA | | | | | | | | | | |
| Pigments content *per* leaf dry mass (DM) | | | | | | | | | | |
| Chl *a*/DM | | | | | | | | | | |
| Chl *b*/DM | | | | | | | | | | |
| (Chl *a* + chl *b*)/DM | | | | | | | | | | |
| Carotenoids/DM | | | | | | | | | | |
| Chlorophyll *a* to chlorophyll *b* ratio | | | | | | | | | | |
| Chl *a*/chl *b* | | | | | | | | | | |
| Total carotenoids to chlorophyll *a* and chlorophyll *b* ratio | | | | | | | | | | |
| Carotenoids/(chl *a* + chl *b*) | | | | | | | | | | |
| Minimal fluorescence of Photosystem II ($F_0$) | | | | | | | | | | |
| $F_0$ | | | | | | | | | | |
| Maximal fluorescence of Photosystem II ($F_m$) | | | | | | | | | | |
| $F_m$ | | | | | | | | | | |
| Maximum quantum efficiency of Photosystem II ($F_v/F_m$) | | | | | | | | | | |
| $F_v/F_m$ | | | | | | | | | | |

| Parameter | control1/2023xCE704 | | | | | drought/2023xCE704 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E0 | E8 | E10 | E12 | E14 | E0 | E8 | E10 | E12 | E14 |
| Lengths of the internodes | | | | | | | | | | |
| Length of the 1st internode | | | | | | | | | | |
| Length of the 2nd internode | | | | | | | | | | |
| Lengths of the internodes - difference between the start and the end of the stress period | | | | | | | | | | |
| Δ (length of the 1st internode) | | | | | | | | | | |
| Δ (length of the 2nd internode) | | | | | | | | | | |
| Height of the plants | | | | | | | | | | |
| Height of the plant | | | | | | | | | | |
| Height of the plant - difference between the start and the end of the stress period | | | | | | | | | | |
| Δ (height of the plant) | | | | | | | | | | |
| Lengths of the leaves | | | | | | | | | | |
| Length of the 1st leaf | | | | | | | | | | |
| Length of the 2nd leaf | | | | | | | | | | |
| Length of the 3rd leaf | | | | | | | | | | |
| Number of visible leaves | | | | | | | | | | |
| Visible leaves | | | | | | | | | | |
| Number of visible leaves - difference between the start and the end of the stress period | | | | | | | | | | |
| Δ (visible leaves) | | | | | | | | | | |
| Number of fully expanded leaves | | | | | | | | | | |
| Fully expanded leaves | | | | | | | | | | |
| Number of fully expanded leaves - difference between the start and the end of the stress period | | | | | | | | | | |
| Δ (fully expanded leaves) | | | | | | | | | | |
| Dry masses (DM) of the leaves | | | | | | | | | | |
| DM of the 1st leaf | | | | | | | | | | |
| DM of the 2nd leaf | | | | | | | | | | |
| DM of the 3rd leaf | | | | | | | | | | |
| Dry mass (DM) of the shoot residues | | | | | | | | | | |
| DM of the shoot residue | | | | | | | | | | |
| Dry mass (DM) of the shoots (including dry masses of leaves) | | | | | | | | | | |
| DM of the shoot (with leaves) | | | | | | | | | | |
| Dry mass (DM) of the roots | | | | | | | | | | |
| DM of the root | | | | | | | | | | |
| Dry mass (DM) of the whole plants | | | | | | | | | | |
| DM of the whole plant | | | | | | | | | | |
| Shoot to root dry mass (DM) ratios | | | | | | | | | | |
| Shoot-root DM ratio | | | | | | | | | | |
| Relative water content (RWC) | | | | | | | | | | |
| RWC | | | | | | | | | | |

| Parameter | control1/2023xCE704 | | | | | drought/2023xCE704 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E0 | E8 | E10 | E12 | E14 | E0 | E8 | E10 | E12 | E14 |
| Specific leaf weight (SLW) | | | | | | | | | | |
| SLW | | | | | | | | | | |
| Pigments content *per* leaf unit area (LA) | | | | | | | | | | |
| Chl *a*/LA | | | | | | | | | | |
| Chl *b*/LA | | | | | | | | | | |
| (Chl *a* + chl *b*)/LA | | | | | | | | | | |
| Carotenoids/LA | | | | | | | | | | |
| Pigments content *per* leaf dry mass (DM) | | | | | | | | | | |
| Chl *a*/DM | | | | | | | | | | |
| Chl *b*/DM | | | | | | | | | | |
| (Chl *a* + chl *b*)/DM | | | | | | | | | | |
| Carotenoids/DM | | | | | | | | | | |
| Chlorophyll *a* to chlorophyll *b* ratio | | | | | | | | | | |
| Chl *a*/chl *b* | | | | | | | | | | |
| Total carotenoids to chlorophyll *a* and chlorophyll *b* ratio | | | | | | | | | | |
| Carotenoids/(chl *a* + chl *b*) | | | | | | | | | | |
| Minimal fluorescence of Photosystem II ($F_0$) | | | | | | | | | | |
| $F_0$ | | | | | | | | | | |
| Maximal fluorescence of Photosystem II ($F_m$) | | | | | | | | | | |
| $F_m$ | | | | | | | | | | |
| Maximum quantum efficiency of Photosystem II ($F_v/F_m$) | | | | | | | | | | |
| $F_v/F_m$ | | | | | | | | | | |

| Parameter | control1/CE704x2023 | | | | | drought/CE704x2023 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E0 | E8 | E10 | E12 | E14 | E0 | E8 | E10 | E12 | E14 |
| Lengths of the internodes | | | | | | | | | | |
| Length of the 1st internode | | | | | | | | | | |
| Length of the 2nd internode | | | | | | | | | | |
| Lengths of the internodes - difference between the start and the end of the stress period | | | | | | | | | | |
| Δ (length of the 1st internode) | | | | | | | | | | |
| Δ (length of the 2nd internode) | | | | | | | | | | |
| Height of the plants | | | | | | | | | | |
| Height of the plant | | | | | | | | | | |
| Height of the plant - difference between the start and the end of the stress period | | | | | | | | | | |
| Δ (height of the plant) | | | | | | | | | | |
| Lengths of the leaves | | | | | | | | | | |
| Length of the 1st leaf | | | | | | | | | | |
| Length of the 2nd leaf | | | | | | | | | | |
| Length of the 3rd leaf | | | | | | | | | | |

| Parameter | control1/CE704x2023 | | | | | drought/CE704x2023 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E0 | E8 | E10 | E12 | E14 | E0 | E8 | E10 | E12 | E14 |
| Number of visible leaves | | | | | | | | | | |
| Visible leaves | | | | | | | | | | |
| Number of visible leaves - difference between the start and the end of the stress period | | | | | | | | | | |
| Δ (visible leaves) | | | | | | | | | | |
| Number of fully expanded leaves | | | | | | | | | | |
| Fully expanded leaves | | | | | | | | | | |
| Number of fully expanded leaves - difference between the start and the end of the stress period | | | | | | | | | | |
| Δ (fully expanded leaves) | | | | | | | | | | |
| Dry masses (DM) of the leaves | | | | | | | | | | |
| DM of the 1st leaf | | | | | | | | | | |
| DM of the 2nd leaf | | | | | | | | | | |
| DM of the 3rd leaf | | | | | | | | | | |
| Dry mass (DM) of the shoot residues | | | | | | | | | | |
| DM of the shoot residue | | | | | | | | | | |
| Dry mass (DM) of the shoots (including dry masses of leaves) | | | | | | | | | | |
| DM of the shoot (with leaves) | | | | | | | | | | |
| Dry mass (DM) of the roots | | | | | | | | | | |
| DM of the root | | | | | | | | | | |
| Dry mass (DM) of the whole plants | | | | | | | | | | |
| DM of the whole plant | | | | | | | | | | |
| Shoot to root dry mass (DM) ratios | | | | | | | | | | |
| Shoot-root DM ratio | | | | | | | | | | |
| Relative water content (RWC) | | | | | | | | | | |
| RWC | | | | | | | | | | |
| Specific leaf weight (SLW) | | | | | | | | | | |
| SLW | | | | | | | | | | |
| Pigments content *per* leaf unit area (LA) | | | | | | | | | | |
| Chl *a*/LA | | | | | | | | | | |
| Chl *b*/LA | | | | | | | | | | |
| (Chl *a* + chl *b*)/LA | | | | | | | | | | |
| Carotenoids/LA | | | | | | | | | | |
| Pigments content *per* leaf dry mass (DM) | | | | | | | | | | |
| Chl *a*/DM | | | | | | | | | | |
| Chl *b*/DM | | | | | | | | | | |
| (Chl *a* + chl *b*)/DM | | | | | | | | | | |
| Carotenoids/DM | | | | | | | | | | |
| Chlorophyll *a* to chlorophyll *b* ratio | | | | | | | | | | |
| Chl *a*/chl *b* | | | | | | | | | | |

| Parameter | control1/CE704x2023 | | | | | drought/CE704x2023 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E0 | E8 | E10 | E12 | E14 | E0 | E8 | E10 | E12 | E14 |
| Total carotenoids to chlorophyll *a* and chlorophyll *b* ratio | | | | | | | | | | |
| Carotenoids/(chl *a* + chl *b*) | | | | | | | | | | |
| Minimal fluorescence of Photosystem II ($F_0$) | | | | | | | | | | |
| $F_0$ | | | | | | | | | | |
| Maximal fluorescence of Photosystem II ($F_m$) | | | | | | | | | | |
| $F_m$ | | | | | | | | | | |
| Maximum quantum efficiency of Photosystem II ($F_v/F_m$) | | | | | | | | | | |
| $F_v/F_m$ | | | | | | | | | | |

Table 5. Overview of the statistical analysis of differences (α = 0.05) in characteristics of maize plants after the 3 days lasting recovery period (38 days after sowing). Kernels of 2023, CE704, 2023xCE704 and CE704x2023 genotype were soaked in 0 M, $10^{-8}$ M, $10^{-10}$ M, $10^{-12}$ M, and $10^{-14}$ M (E0, E8, E10, E12 and E14) 24-epibrassinolide solution previously to sowing. For particular results of statistical analysis see Table S1, Section I. When compared to each other:

values are significantly lower  values are significantly higher

| Parameter | control2/2023 | | | | | recovery/2023 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E0 | E8 | E10 | E12 | E14 | E0 | E8 | E10 | E12 | E14 |
| Lengths of the internodes | | | | | | | | | | |
| Length of the 1st internode | | | | | | | | | | |
| Length of the 2nd internode | | | | | | | | | | |
| Length of the 3rd internode | | | | | | | | | | |
| Lengths of the internodes - difference between the end of the stress period and the end of the recovery period | | | | | | | | | | |
| Δ (length of the 1st internode) | | | | | | | | | | |
| Δ (length of the 2nd internode) | | | | | | | | | | |
| Δ (length of the 3rd internode) | | | | | | | | | | |
| Height of the plants | | | | | | | | | | |
| Height of the plant | | | | | | | | | | |
| Height of the plant - difference between the end of the stress period and the end of the recovery period | | | | | | | | | | |
| Δ (height of the plant) | | | | | | | | | | |
| Lengths of the leaves | | | | | | | | | | |
| Length of the 1st leaf | | | | | | | | | | |
| Length of the 2nd leaf | | | | | | | | | | |
| Length of the 3rd leaf | | | | | | | | | | |
| Length of the 4th leaf | | | | | | | | | | |

| Parameter | control2/2023 | | | | | recovery/2023 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E0 | E8 | E10 | E12 | E14 | E0 | E8 | E10 | E12 | E14 |
| Number of visible leaves | | | | | | | | | | |
| Visible leaves | | | | | | | | | | |
| Number of visible leaves - difference between the end of the stress period and the end of the recovery period | | | | | | | | | | |
| Δ (visible leaves) | | | | | | | | | | |
| Number of fully expanded leaves | | | | | | | | | | |
| Fully expanded leaves | | | | | | | | | | |
| Number of fully expanded leaves - difference between the end of the stress period and the end of the recovery period | | | | | | | | | | |
| Δ (fully expanded leaves) | | | | | | | | | | |
| Development of the 4th leaf - difference between the end of the stress period and the end of the recovery period | | | | | | | | | | |
| Development of the $4^{th}$ leaf | | | | | | | | | | |
| Dry masses (DM) of the leaves | | | | | | | | | | |
| DM of the $1^{st}$ leaf | | | | | | | | | | |
| DM of the $2^{nd}$ leaf | | | | | | | | | | |
| DM of the $3^{rd}$ leaf | | | | | | | | | | |
| DM of the $4^{th}$ leaf | | | | | | | | | | |
| Dry mass (DM) of the shoot residues | | | | | | | | | | |
| DM of the shoot residue | | | | | | | | | | |
| Dry mass (DM) of the shoots (including dry masses of leaves) | | | | | | | | | | |
| DM of the shoot (with leaves) | | | | | | | | | | |
| Dry mass (DM) of the roots | | | | | | | | | | |
| DM of the root | | | | | | | | | | |
| Dry mass (DM) of the whole plants | | | | | | | | | | |
| DM of the whole plant | | | | | | | | | | |
| Shoot to root dry mass (DM) ratios | | | | | | | | | | |
| Shoot-root DM ratio | | | | | | | | | | |
| Relative water content (RWC) | | | | | | | | | | |
| RWC | | | | | | | | | | |
| Specific leaf weight (SLW) | | | | | | | | | | |
| SLW | | | | | | | | | | |
| Pigments content *per* leaf unit area (LA) | | | | | | | | | | |
| Chl *a*/LA | | | | | | | | | | |
| Chl *b*/LA | | | | | | | | | | |
| (Chl *a* + chl *b*)/LA | | | | | | | | | | |
| Carotenoids/LA | | | | | | | | | | |
| Pigments content *per* leaf dry mass (DM) | | | | | | | | | | |
| Chl *a*/DM | | | | | | | | | | |
| Chl *b*/DM | | | | | | | | | | |
| (Chl *a* + chl *b*)/DM | | | | | | | | | | |
| Carotenoids/DM | | | | | | | | | | |

| Parameter | control2/2023 | | | | | recovery/2023 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E0 | E8 | E10 | E12 | E14 | E0 | E8 | E10 | E12 | E14 |
| Chlorophyll *a* to chlorophyll *b* ratio | | | | | | | | | | |
| Chl *a*/chl *b* | | | | | | | | | | |
| Total carotenoids to chlorophyll *a* and chlorophyll *b* ratio | | | | | | | | | | |
| Carotenoids/(chl *a* + chl *b*) | | | | | | | | | | |
| Minimal fluorescence of Photosystem II ($F_0$) | | | | | | | | | | |
| $F_0$ | | | | | | | | | | |
| Maximal fluorescence of Photosystem II ($F_m$) | | | | | | | | | | |
| $F_m$ | | | | | | | | | | |
| Maximal quantum efficiency of Photosystem II ($F_v/F_m$) | | | | | | | | | | |
| $F_v/F_m$ | | | | | | | | | | |

| Parameter | control2/CE704 | | | | | recovery/CE704 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E0 | E8 | E10 | E12 | E14 | E0 | E8 | E10 | E12 | E14 |
| Lengths of the internodes | | | | | | | | | | |
| Length of the 1st internode | | | | | | | | | | |
| Length of the 2nd internode | | | | | | | | | | |
| Length of the 3rd internode | | | | | | | | | | |
| Lengths of the internodes - difference between the end of the stress period and the end of the recovery period | | | | | | | | | | |
| Δ (length of the 1st internode) | | | | | | | | | | |
| Δ (length of the 2nd internode) | | | | | | | | | | |
| Δ (length of the 3rd internode) | | | | | | | | | | |
| Height of the plants | | | | | | | | | | |
| Height of the plant | | | | | | | | | | |
| Height of the plant - difference between the end of the stress period and the end of the recovery period | | | | | | | | | | |
| Δ (height of the plant) | | | | | | | | | | |
| Lengths of the leaves | | | | | | | | | | |
| Length of the 1st leaf | | | | | | | | | | |
| Length of the 2nd leaf | | | | | | | | | | |
| Length of the 3rd leaf | | | | | | | | | | |
| Length of the 4th leaf | | | | | | | | | | |
| Number of visible leaves | | | | | | | | | | |
| Visible leaves | | | | | | | | | | |
| Number of visible leaves - difference between the end of the stress period and the end of the recovery period | | | | | | | | | | |
| Δ (visible leaves) | | | | | | | | | | |
| Number of fully expanded leaves | | | | | | | | | | |
| Fully expanded leaves | | | | | | | | | | |

| Parameter | control2/CE704 | | | | | recovery/CE704 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E0 | E8 | E10 | E12 | E14 | E0 | E8 | E10 | E12 | E14 |
| Number of fully expanded leaves - difference between the end of the stress period and the end of the recovery period | | | | | | | | | | |
| Δ (fully expanded leaves) | | | | | | | | | | |
| Development of the 4th leaf - difference between the end of the stress period and the end of the recovery period | | | | | | | | | | |
| Development of the 4th leaf | | | | | | | | | | |
| Dry masses (DM) of the leaves | | | | | | | | | | |
| DM of the 1st leaf | | | | | | | | | | |
| DM of the 2nd leaf | | | | | | | | | | |
| DM of the 3rd leaf | | | | | | | | | | |
| DM of the 4th leaf | | | | | | | | | | |
| Dry mass (DM) of the shoot residues | | | | | | | | | | |
| DM of the shoot residue | | | | | | | | | | |
| Dry mass (DM) of the shoots (including dry masses of leaves) | | | | | | | | | | |
| DM of the shoot (with leaves) | | | | | | | | | | |
| Dry mass (DM) of the roots | | | | | | | | | | |
| DM of the root | | | | | | | | | | |
| Dry mass (DM) of the whole plants | | | | | | | | | | |
| DM of the whole plant | | | | | | | | | | |
| Shoot to root dry mass (DM) ratios | | | | | | | | | | |
| Shoot-root DM ratio | | | | | | | | | | |
| Relative water content (RWC) | | | | | | | | | | |
| RWC | | | | | | | | | | |
| Specific leaf weight (SLW) | | | | | | | | | | |
| SLW | | | | | | | | | | |
| Pigments content *per* leaf unit area (LA) | | | | | | | | | | |
| Chl *a*/LA | | | | | | | | | | |
| Chl *b*/LA | | | | | | | | | | |
| (Chl *a* + chl *b*)/LA | | | | | | | | | | |
| Carotenoids/LA | | | | | | | | | | |
| Pigments content *per* leaf dry mass (DM) | | | | | | | | | | |
| Chl *a*/DM | | | | | | | | | | |
| Chl *b*/DM | | | | | | | | | | |
| (Chl *a* + chl *b*)/DM | | | | | | | | | | |
| Carotenoids/DM | | | | | | | | | | |
| Chlorophyll *a* to chlorophyll *b* ratio | | | | | | | | | | |
| Chl *a*/chl *b* | | | | | | | | | | |
| Total carotenoids to chlorophyll *a* and chlorophyll *b* ratio | | | | | | | | | | |
| Carotenoids/(chl *a* + chl *b*) | | | | | | | | | | |
| Minimal fluorescence of Photosystem II ($F_0$) | | | | | | | | | | |
| $F_0$ | | | | | | | | | | |

| Parameter | control2/CE704 | | | | | recovery/CE704 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E0 | E8 | E10 | E12 | E14 | E0 | E8 | E10 | E12 | E14 |
| Minimal fluorescence of Photosystem II ($F_0$) | | | | | | | | | | |
| $F_0$ | | | | | | | | | | |
| Maximal fluorescence of Photosystem II ($F_m$) | | | | | | | | | | |
| $F_m$ | | | | | | | | | | |
| Maximal quantum efficiency of Photosystem II ($F_v/F_m$) | | | | | | | | | | |
| $F_v/F_m$ | | | | | | | | | | |

| Parameter | control2/2023xCE704 | | | | | recovery/2023xCE704 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E0 | E8 | E10 | E12 | E14 | E0 | E8 | E10 | E12 | E14 |
| Lengths of the internodes | | | | | | | | | | |
| Length of the 1st internode | | | | | | | | | | |
| Length of the 2nd internode | | | | | | | | | | |
| Length of the 3rd internode | | | | | | | | | | |
| Lengths of the internodes - difference between the end of the stress period and the end of the recovery period | | | | | | | | | | |
| Δ (length of the 1st internode) | | | | | | | | | | |
| Δ (length of the 2nd internode) | | | | | | | | | | |
| Δ (length of the 3rd internode) | | | | | | | | | | |
| Height of the plants | | | | | | | | | | |
| Height of the plant | | | | | | | | | | |
| Height of the plant - difference between the end of the stress period and the end of the recovery period | | | | | | | | | | |
| Δ (height of the plant) | | | | | | | | | | |
| Lengths of the leaves | | | | | | | | | | |
| Length of the 1st leaf | | | | | | | | | | |
| Length of the 2nd leaf | | | | | | | | | | |
| Length of the 3rd leaf | | | | | | | | | | |
| Length of the 4th leaf | | | | | | | | | | |
| Number of visible leaves | | | | | | | | | | |
| Visible leaves | | | | | | | | | | |
| Number of visible leaves - difference between the end of the stress period and the end of the recovery period | | | | | | | | | | |
| Δ (visible leaves) | | | | | | | | | | |
| Number of fully expanded leaves | | | | | | | | | | |
| Fully expanded leaves | | | | | | | | | | |
| Number of fully expanded leaves - difference between the end of the stress period and the end of the recovery period | | | | | | | | | | |
| Δ (fully expanded leaves) | | | | | | | | | | |
| Development of the 4th leaf - difference between the end of the stress period and the end of the recovery period | | | | | | | | | | |
| Development of the 4th leaf | | | | | | | | | | |

| Parameter | control2/2023xCE704 | | | | | recovery/2023xCE704 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E0 | E8 | E10 | E12 | E14 | E0 | E8 | E10 | E12 | E14 |
| Dry masses (DM) of the leaves | | | | | | | | | | |
| DM of the 1st leaf | | | | | | | | | | |
| DM of the 2nd leaf | | | | | | | | | | |
| DM of the 3rd leaf | | | | | | | | | | |
| DM of the 4th leaf | | | | | | | | | | |
| Dry mass (DM) of the shoot residues | | | | | | | | | | |
| DM of the shoot residue | | | | | | | | | | |
| Dry mass (DM) of the shoots (including dry masses of leaves) | | | | | | | | | | |
| DM of the shoot (with leaves) | | | | | | | | | | |
| Dry mass (DM) of the roots | | | | | | | | | | |
| DM of the root | | | | | | | | | | |
| Dry mass (DM) of the whole plants | | | | | | | | | | |
| DM of the whole plant | | | | | | | | | | |
| Shoot to root dry mass (DM) ratios | | | | | | | | | | |
| Shoot-root DM ratio | | | | | | | | | | |
| Relative water content (RWC) | | | | | | | | | | |
| RWC | | | | | | | | | | |
| Specific leaf weight (SLW) | | | | | | | | | | |
| SLW | | | | | | | | | | |
| Pigments content *per* leaf unit area (LA) | | | | | | | | | | |
| Chl *a*/LA | | | | | | | | | | |
| Chl *b*/LA | | | | | | | | | | |
| (Chl *a* + chl *b*)/LA | | | | | | | | | | |
| Carotenoids/LA | | | | | | | | | | |
| Pigments content *per* leaf dry mass (DM) | | | | | | | | | | |
| Chl *a*/DM | | | | | | | | | | |
| Chl *b*/DM | | | | | | | | | | |
| (Chl *a* + chl *b*)/DM | | | | | | | | | | |
| Carotenoids/DM | | | | | | | | | | |
| Chlorophyll *a* to chlorophyll *b* ratio | | | | | | | | | | |
| Chl *a*/chl *b* | | | | | | | | | | |
| Total carotenoids to chlorophyll *a* and chlorophyll *b* ratio | | | | | | | | | | |
| Carotenoids/(chl *a* + chl *b*) | | | | | | | | | | |
| Minimal fluorescence of Photosystem II ($F_0$) | | | | | | | | | | |
| $F_0$ | | | | | | | | | | |
| Maximal fluorescence of Photosystem II ($F_m$) | | | | | | | | | | |
| $F_m$ | | | | | | | | | | |
| Maximal quantum efficiency of Photosystem II ($F_v/F_m$) | | | | | | | | | | |
| $F_v/F_m$ | | | | | | | | | | |
| | | | | | | | | | | |

| Parameter | control2/CE704x2023 | | | | | recovery/CE704x2023 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E0 | E8 | E10 | E12 | E14 | E0 | E8 | E10 | E12 | E14 |
| Lengths of the internodes | | | | | | | | | | |
| Length of the 1st internode | | | | | | | | | | |
| Length of the 2nd internode | | | | | | | | | | |
| Length of the 3rd internode | | | | | | | | | | |
| Lengths of the internodes - difference between the end of the stress period and the end of the recovery period | | | | | | | | | | |
| Δ (length of the 1st internode) | | | | | | | | | | |
| Δ (length of the 2nd internode) | | | | | | | | | | |
| Δ (length of the 3rd internode) | | | | | | | | | | |
| Height of the plants | | | | | | | | | | |
| Height of the plant | | | | | | | | | | |
| Height of the plant - difference between the end of the stress period and the end of the recovery period | | | | | | | | | | |
| Δ (height of the plant) | | | | | | | | | | |
| Lengths of the leaves | | | | | | | | | | |
| Length of the 1st leaf | | | | | | | | | | |
| Length of the 2nd leaf | | | | | | | | | | |
| Length of the 3rd leaf | | | | | | | | | | |
| Length of the 4th leaf | | | | | | | | | | |
| Number of visible leaves | | | | | | | | | | |
| Visible leaves | | | | | | | | | | |
| Number of visible leaves - difference between the end of the stress period and the end of the recovery period | | | | | | | | | | |
| Δ (visible leaves) | | | | | | | | | | |
| Number of fully expanded leaves | | | | | | | | | | |
| Fully expanded leaves | | | | | | | | | | |
| Number of fully expanded leaves - difference between the end of the stress period and the end of the recovery period | | | | | | | | | | |
| Δ (fully expanded leaves) | | | | | | | | | | |
| Development of the 4th leaf - difference between the end of the stress period and the end of the recovery period | | | | | | | | | | |
| Development of the 4th leaf | | | | | | | | | | |
| Dry masses (DM) of the leaves | | | | | | | | | | |
| DM of the 1st leaf | | | | | | | | | | |
| DM of the 2nd leaf | | | | | | | | | | |
| DM of the 3rd leaf | | | | | | | | | | |
| DM of the 4th leaf | | | | | | | | | | |
| Dry mass (DM) of the shoot residues | | | | | | | | | | |
| DM of the shoot residue | | | | | | | | | | |
| Dry mass (DM) of the shoots (including dry masses of leaves) | | | | | | | | | | |
| DM of the shoot (with leaves) | | | | | | | | | | |

| Parameter | control2/CE704x2023 | | | | | recovery/CE704x2023 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E0 | E8 | E10 | E12 | E14 | E0 | E8 | E10 | E12 | E14 |
| Dry mass (DM) of the roots | | | | | | | | | | |
| DM of the root | | | | | | | | | | |
| Dry mass (DM) of the whole plants | | | | | | | | | | |
| DM of the whole plant | | | | | | | | | | |
| Shoot to root dry mass (DM) ratios | | | | | | | | | | |
| Shoot-root DM ratio | | | | | | | | | | |
| Relative water content (RWC) | | | | | | | | | | |
| RWC | | | | | | | | | | |
| Specific leaf weight (SLW) | | | | | | | | | | |
| SLW | | | | | | | | | | |
| Pigments content *per* leaf unit area (LA) | | | | | | | | | | |
| Chl *a*/LA | | | | | | | | | | |
| Chl *b*/LA | | | | | | | | | | |
| (Chl *a* + chl *b*)/LA | | | | | | | | | | |
| Carotenoids/LA | | | | | | | | | | |
| Pigments content *per* leaf dry mass (DM) | | | | | | | | | | |
| Chl *a*/DM | | | | | | | | | | |
| Chl *b*/DM | | | | | | | | | | |
| (Chl *a* + chl *b*)/DM | | | | | | | | | | |
| Carotenoids/DM | | | | | | | | | | |
| Chlorophyll *a* to chlorophyll *b* ratio | | | | | | | | | | |
| Chl *a*/chl *b* | | | | | | | | | | |
| Total carotenoids to chlorophyll *a* and chlorophyll *b* ratio | | | | | | | | | | |
| Carotenoids/(chl *a* + chl *b*) | | | | | | | | | | |
| Minimal fluorescence of Photosystem II ($F_0$) | | | | | | | | | | |
| $F_0$ | | | | | | | | | | |
| Maximal fluorescence of Photosystem II ($F_m$) | | | | | | | | | | |
| $F_m$ | | | | | | | | | | |
| Maximal quantum efficiency of Photosystem II ($F_v/F_m$) | | | | | | | | | | |
| $F_v/F_m$ | | | | | | | | | | |

## 5.2 THE SPRING SEASON

During the spring season we tested effects of spraying the plants of two inbred maize lines with contrastive reaction to water deficit, 2023 and CE704, subjected to water deficit at stage of the fourth leaf visible (22 day old). The treatment solutions consisted of tap water and Tween 20, which was used as a surfactant, and $10^{-5}$ M BRZ or $10^{-8}$ M EBR. The control solution contained no other supplement than Tween 20. Plants were divided into 3 groups according to the treatment applied: “no supplement”, “brassinazole” and “24-epibrassinolide”. This day was designated as “day 0” of the analysis. The very same day, the water supply was withdrawn for half of the plants from each of these groups until the end of the experiment. According to the cultivation conditions, plants were dividend into “drought” and “control” group. Analysis of plant characteristics was done each following day (photosynthetic characteristics) or every other day (morphological characteristics, RWC, pigments content). However, only characteristics from day 0, 2, 4, 6, 8 and 10 are presented here. The role of endogenous brassinosteroids, which biosynthesis was inhibited by BRZ application, and the role of exogenously applied brassinosteroids (EBR) in maize plants subjected to water deficit was investigated. Characteristics were analysed using the one-way completely randomized ANOVA and differences in means were tested by Tukey's HSD or by Tukey-Kramer *post hoc* test for multiple comparisons and considered significant at level of $P \leq 0.05$. Results of statistical analysis can be found in the Section II of the S1 Table in the Supplement (CD enclosed).

### 5.2.1 GENOTYPES

The CE704 genotype plants grew faster than plants of the 2023 genotype (more visible leaves at the same day of the analysis). However, 2023 plants surpassed them in the proportional growth, biomass accumulation and RWC value. They also had higher pigments content (*per* unit leaf dry mass and *per* unit leaf area) up to day 6 of the analysis. At day 8, the pigments content was higher in CE704 plants and at day 10 there were no statistically significant differences between plants of both genotypes. For particular results of statistical analysis see Table S1, Section II.

### 5.2.2 CULTIVATION

Although there were differences in pigments content (*per* unit leaf dry mass and *per* unit leaf area) during the experiment, at day 10 of the analysis there were no statistically significant differences. For particular results of statistical analysis see Table S1, Section II.

### 5.2.3 TREATMENT

#### 5.2.3.1 PLANT GROWTH

In CE704 plants from drought group there was no difference among the treatments. In CE704 control plants and 2023 plants under both cultivation conditions the treatment with no supplement was generally better than the other two, although the effect of the treatment on some growth characteristics was better for no supplement- and brassinazole-treated plants in comparison with EBR-treated. See the overview of statistical analysis in Table 6 at the end of the chapter 5.2.3.

To demonstrate the effect of different treatments on the plant growth, heights of plants at day 0, 2, 4, 6, 8 and 10 were compared (Fig. 14). At day 4 of the analysis, plants of 2023 genotype of both the drought group and the control group were higher after no supplement treatment when compared to BRZ- or EBR-treated plants.

#### 5.2.3.2 DRY MASS ACCUMULATION

Dry mass accumulation was higher in CE704 plants treated with no supplement solution in comparison with BRZ and EBR solution treated plants, what correlated with higher dry mass accumulation of the fourth leaves up to day 6 of the analysis in both, the drought group and the control group. This was similar for plants of 2023 genotype, however at day 8 and 10 of the analysis the dry mass accumulation was higher in no supplement solution and EBR solution treated 2023 drought group plants

when compared to BRZ-treated, what again correlated with higher dry mass accumulation of the fourth leaves. For details see the overview of statistical analysis in Table 6 at the end of the chapter 5.2.3. In Figure 15 there are demonstrated effects of different treatments on dry mass accumulation of plants at day 0, 2, 4, 6, 8 and 10 of the analysis.

#### 5.2.3.3 RELATIVE WATER CONTENT

In control plants of CE704 genotype was the RWC value higher at day 4 after the EBR treatment in comparison with no supplement treatment, and at day 6 after the EBR and BRZ treatment in comparison with no supplement treatment. At day 8 of the analysis, RWC value was higher after the EBR and BRZ treatment when compared to no supplement treatment in control plants of the 2023 genotype. In 2023 drought group plants was the RWC higher after BRZ treatment in comparison with EBR treatment at day 8, and in comparison with no supplement treatment at day 10 (Fig. 16).

#### 5.2.3.4 PIGMENTS CONTENT

When compared to both or one of the other treatments, no supplement treatment resulted in higher pigments contents *per* unit leaf dry mass and *per* unit leaf area for CE704 plants at day 4 and 6 of the analysis, and for 2023 plants at day 6 (pigments content *per* unit leaf dry mass) and 8 of the analysis (pigments content *per* unit leaf dry mass and *per* unit leaf area). However, these differences were even up at day 10 of the analysis. See the overview of statistical analysis in Table 6 at the end of the chapter 5.2.3.

The effects of different treatments on pigments content are demonstrated on the sum of chlorophyll *a* and chlorophyll *b per* unit leaf dry mass (Fig. 17) and *per* unit leaf area (Fig. 8) at day 0, 2, 4, 6, 8 and 10 of the analysis.

#### 5.1.3.5 PHOTOSYNTHETIC CHARACTERISTICS

The quantum yield of Photosystem II (QY) value was higher for no supplement-treated CE704 control plants when compared to EBR-treated plants at day 6, and BRZ-treated plants subjected to water deficit at day 4 of the analysis.

Photochemical reflectance index (PRI) value of CE704 plants under both cultivation conditions was higher in no supplement-treated plants than in EBR-treated plants at day 0, and in control no supplement-treated plants higher than in EBR- and BRZ-treated plants at day 6 of the analysis. On the contrary, in 2023 plants from drought group was the PRI value lower in plants treated with no supplement in comparison to BRZ-treated plants at day 8 of the analysis, and in 2023 control plants it was lower in no supplement treated plants than in BRZ treated plants at day 2 and 10, and than BRZ- and EBR-treated plants at day 8 of the analysis.

At day 8 of the analysis, the normalized difference vegetative index (NDVI) value was higher for control plants of the 2023 genotype treated with EBR in comparison with no supplement-treated. At day 4, the NDVI value of BRZ-treated 2023 plants subjected to water deficit was lower than the in plants under both other treatments.

In 2023 control plants, the $F_0$ value was lower in no supplement-treated plants when compared to BRZ-treated at day 4, and when compared to EBR-treated at day 6 of the analysis (Fig. 19). Both, $F_0$ and $F_m$ value were higher in BRZ-treated plants when compared to the other two treatments at day 8 of the analysis. In contrast, $F_V/F_m$ (Fig. 20) value of these plants at day 8 was in BRZ-treated plants lower than in EBR or no supplement-treated plants, and at day 2 of the analysis it was lower than in EBR-treated 2023 plants from drought group. In 2023 plants from control group, the $F_V/F_m$ value was higher in no supplement-treated plants in comparison to plants of the other two treatments at day 6 of the analysis. The $F_V/F_m$ value was lower in CE704 plants treated with BRZ than in plants treated with other two solutions at day 0 of the analysis.

For details of all photosynthetic characteristics mentioned above in this chapter see the overview of statistical analysis in Table 6 at the end of the chapter 5.2.3.

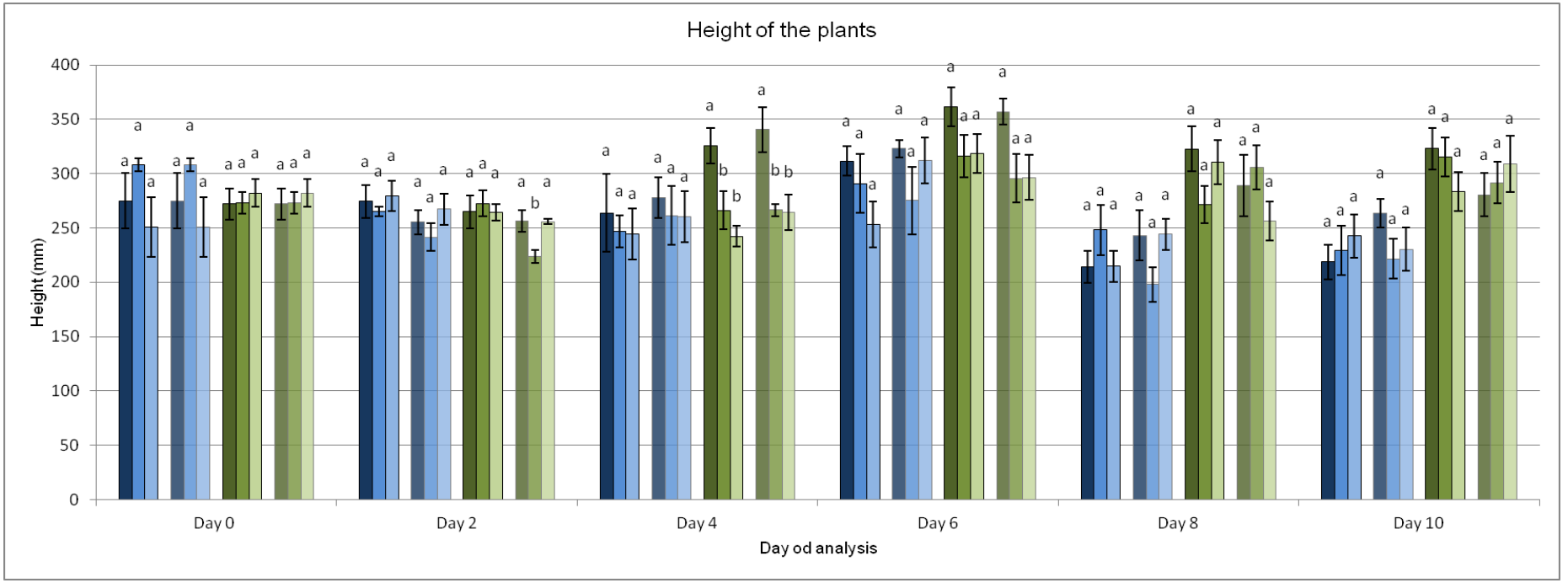


Fig. 14. Height of plants at day 0, 2, 4, 6, 8 and 10 of the analysis. The water supply was withdrawn for plants after they had been sprayed with different treatment solutions at day 0 of the analysis. Each combination of genotype (CE704, 2023), treatment (no supplement, $10^{-5}$ M brassinazole, $10^{-8}$ M 24-epibrassinolide) and cultivation (drought, control) is represented by its mean and the standard error of mean (n = 8). Different letters indicate significant differences between the treatments (one-way ANOVA, Tukey's HSD or Tukey-Kramer test, $P \leq 0.05$).

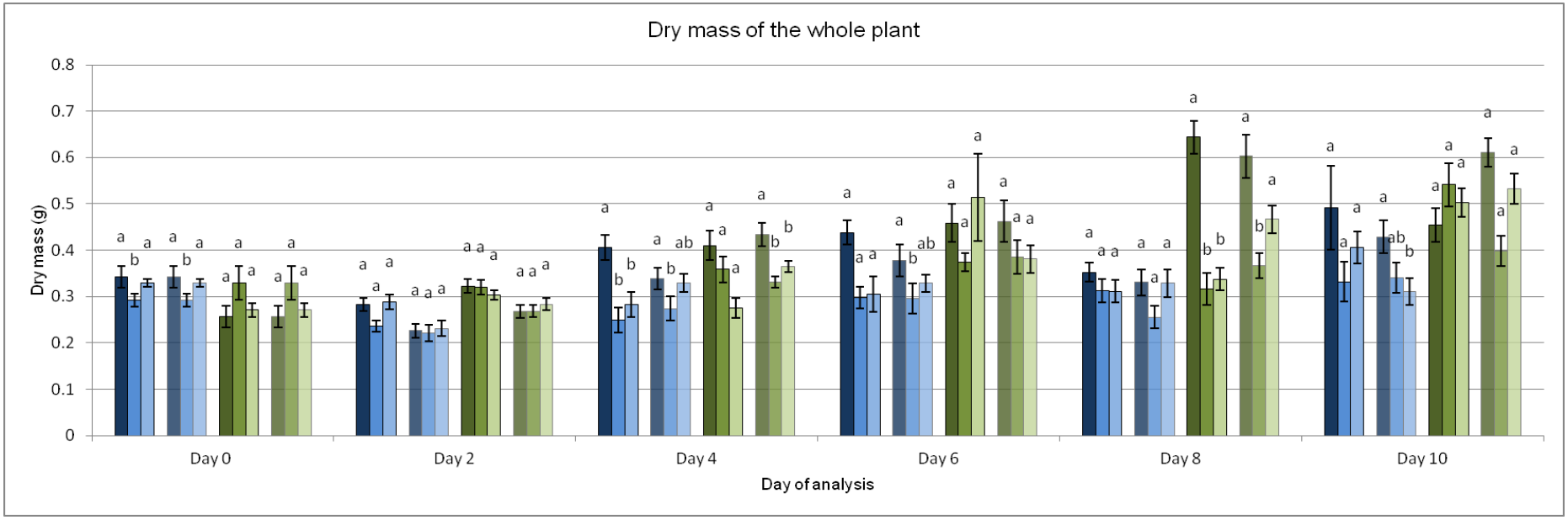


Fig. 15. Dry mass of the whole plants at day 0, 2, 4, 6, 8 and 10 of the analysis. The water supply was withdrawn for plants after they had been sprayed with different treatment solutions at day 0 of the analysis. Each combination of genotype (CE704, 2023), treatment (no supplement, $10^{-5}$ M brassinazole, $10^{-8}$ M 24-epibrassinolide) and cultivation (drought, control) is represented by its mean and the standard error of mean (n = 8). Different letters indicate significant differences between the treatments (one-way ANOVA, Tukey's HSD or Tukey-Kramer test, $P \leq 0.05$).

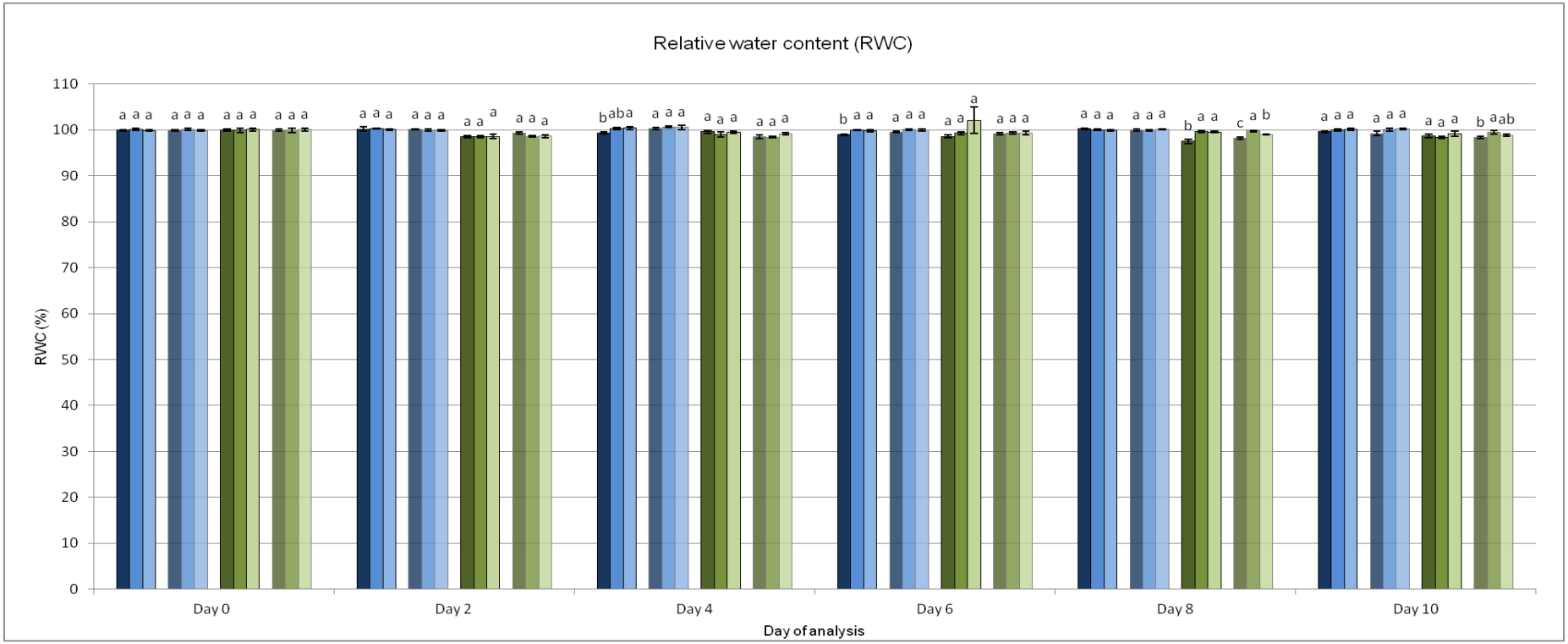


Fig. 16. Relative water content at day 0, 2, 4, 6, 8 and 10 of the analysis. The water supply was withdrawn for plants after they had been sprayed with different treatment solutions at day 0 of the analysis. Each combination of genotype (CE704, 2023), treatment (no supplement, $10^{-5}$ M brassinazole, $10^{-8}$ M 24-epibrassinolide) and cultivation (drought, control) is represented by its mean and the standard error of mean (n = 8). Different letters indicate significant differences between the treatments (one-way ANOVA, Tukey's HSD or Tukey-Kramer test, $P \leq 0.05$).

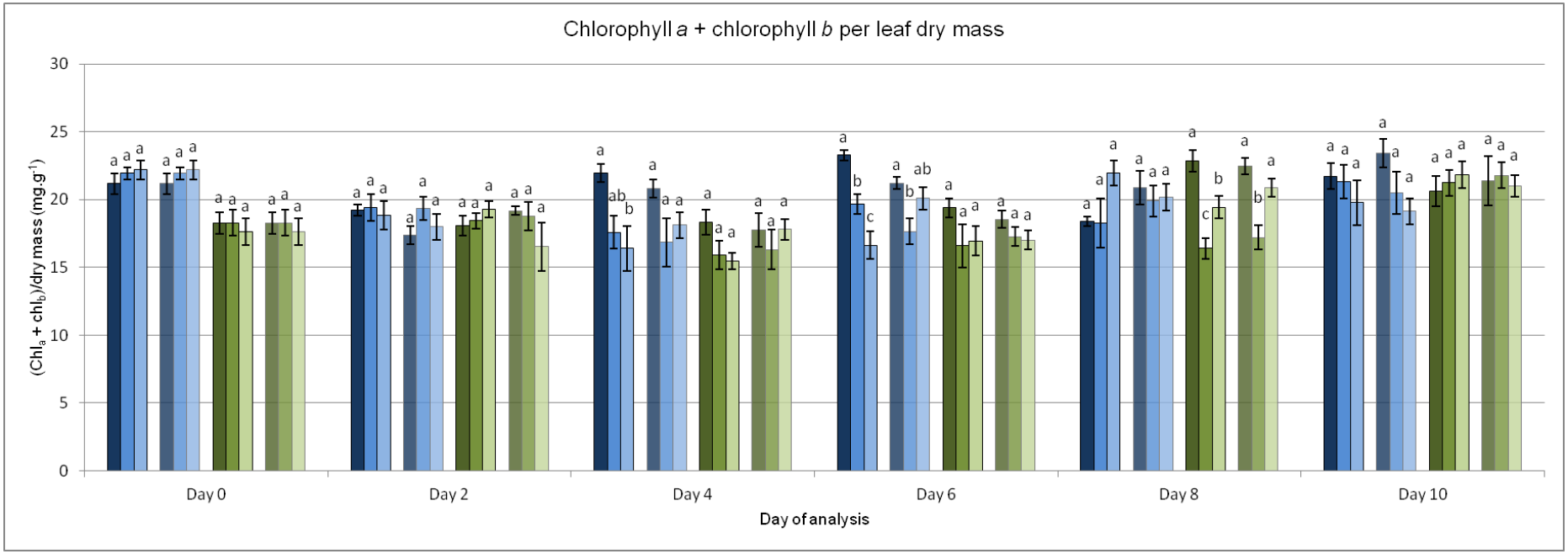


Fig. 17. The sum of chlorophyll *a* and chlorophyll *b* *per* unit leaf dry mass at day 0, 2, 4, 6, 8 and 10 of the analysis. The water supply was withdrawn for plants after they had been sprayed with different treatment solutions at day 0 of the analysis. Each combination of genotype (CE704, 2023), treatment (no supplement, $10^{-5}$ M brassinazole, $10^{-8}$ M 24-epibrassinolide) and cultivation (drought, control) is represented by its mean and the standard error of mean (n = 8). Different letters indicate significant differences between the treatments (one-way ANOVA, Tukey's HSD or Tukey-Kramer test, P ≤ 0.05).

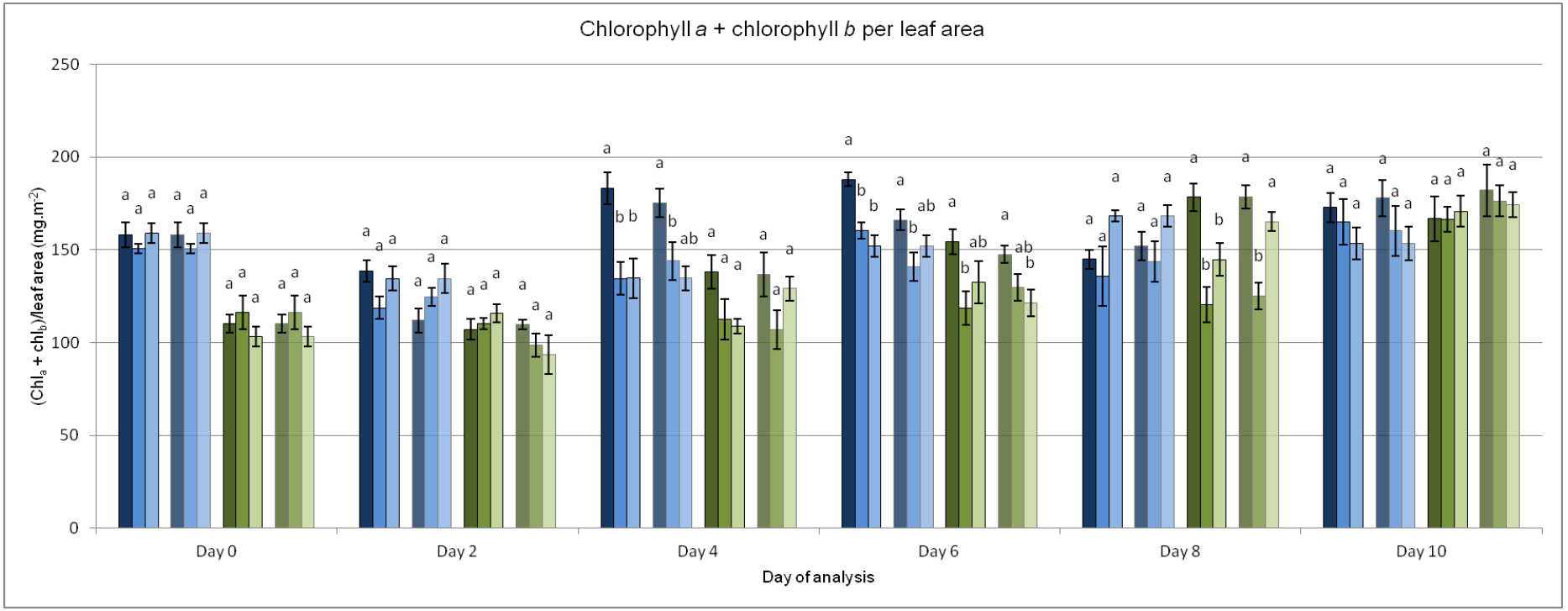


Fig. 18. The sum of chlorophyll *a* and chlorophyll *b* *per* unit leaf area at day 0, 2, 4, 6, 8 and 10 of the analysis. The water supply was withdrawn for plants after they had been sprayed with different treatment solutions at day 0 of the analysis. Each combination of genotype (CE704, 2023), treatment (no supplement, $10^{-5}$ M brassinazole, $10^{-8}$ M 24-epibrassinolide) and cultivation (drought, control) is represented by its mean and the standard error of mean (n = 8). Different letters indicate significant differences between the treatments (one-way ANOVA, Tukey's HSD or Tukey-Kramer test, $P \leq 0.05$).

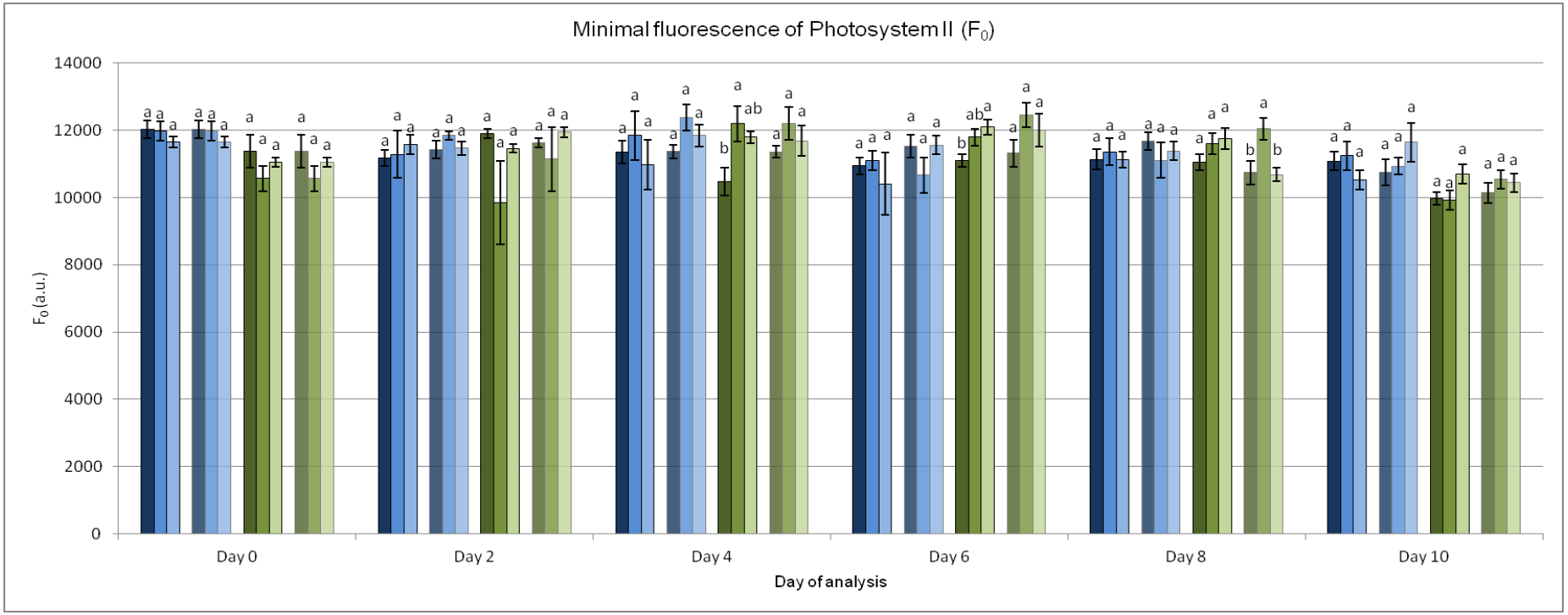


Fig. 19. Minimal fluorescence of Photosystem II ($F_0$) at day 0, 2, 4, 6, 8 and 10 of the analysis. The water supply was withdrawn for plants after they had been sprayed with different treatment solutions at day 0 of the analysis. Each combination of genotype (CE704, 2023), treatment (no supplement, $10^{-5}$ M brassinazole, $10^{-8}$ M 24-epibrassinolide) and cultivation (drought, control) is represented by its mean and the standard error of mean (n = 8). Different letters indicate significant differences between the treatments (one-way ANOVA, Tukey's HSD or Tukey-Kramer test, $P \leq 0.05$).

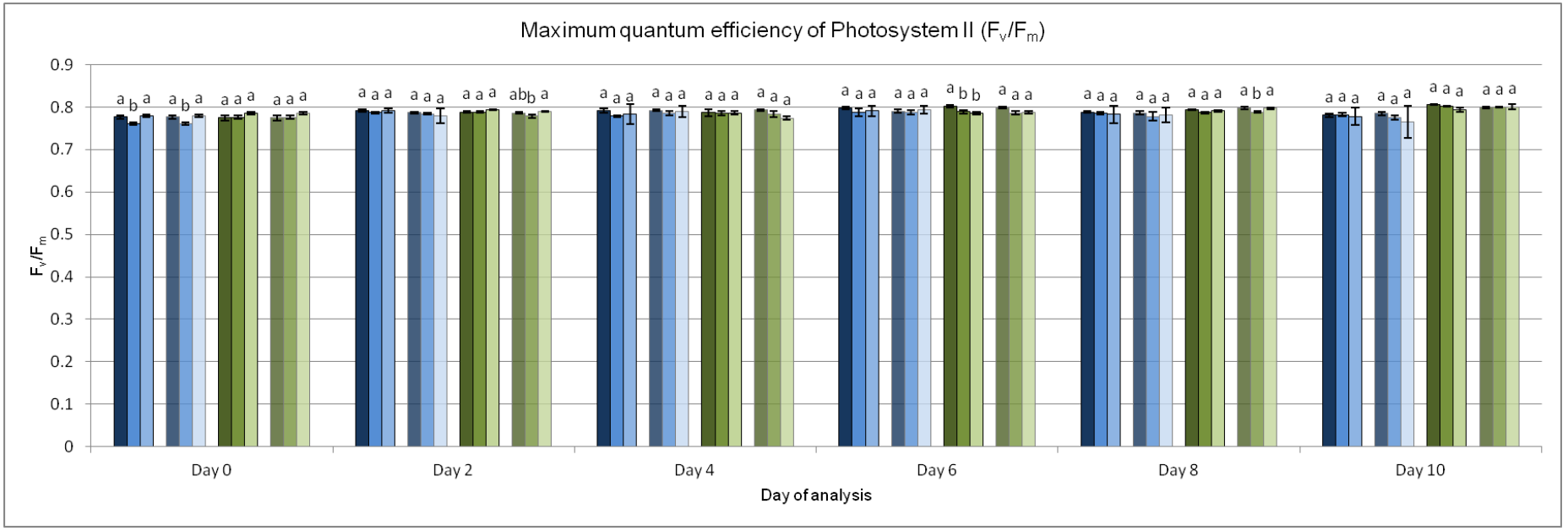


Fig. 20. Maximum quantum efficiency of Photosystem II ($F_v/F_m$) at day 0, 2, 4, 6, 8 and 10 of the analysis. The water supply was withdrawn for plants after they had been sprayed with different treatment solutions at day 0 of the analysis. Each combination of genotype (CE704, 2023), treatment (no supplement, $10^{-5}$ M brassinazole, $10^{-8}$ M 24-epibrassinolide) and cultivation (drought, control) is represented by its mean and the standard error of mean (n = 8). Different letters indicate significant differences between the treatments (one-way ANOVA, Tukey's HSD or Tukey-Kramer test, $P \leq 0.05$).

To demonstrate the effect of different treatments on fluorescence intensity of plants subjected to water deficit and their control group plants, OJIP curve from day 10 of the analysis is shown (Fig. 21). Each of the three peaks represents different process and together they predicate about the photosynthetic efficiency of the plant. The first peak (OJ) phase is suggested to reflex a single turnover photochemical event, the second peak (JI) phase reflects the reduction of the intersystem electron carriers (e.g. plastoquinone, plastocyanin, cytochrome) and the last peak (IP) phase reflects the reduction of Photosystem I electron acceptors (e.g. ferredoxin, NADPH). A typical OJIP shape was found for all the samples. This imply that all analysed plants were photosynthetically active. There is no difference between the treatments, genotypes or cultivation conditions.

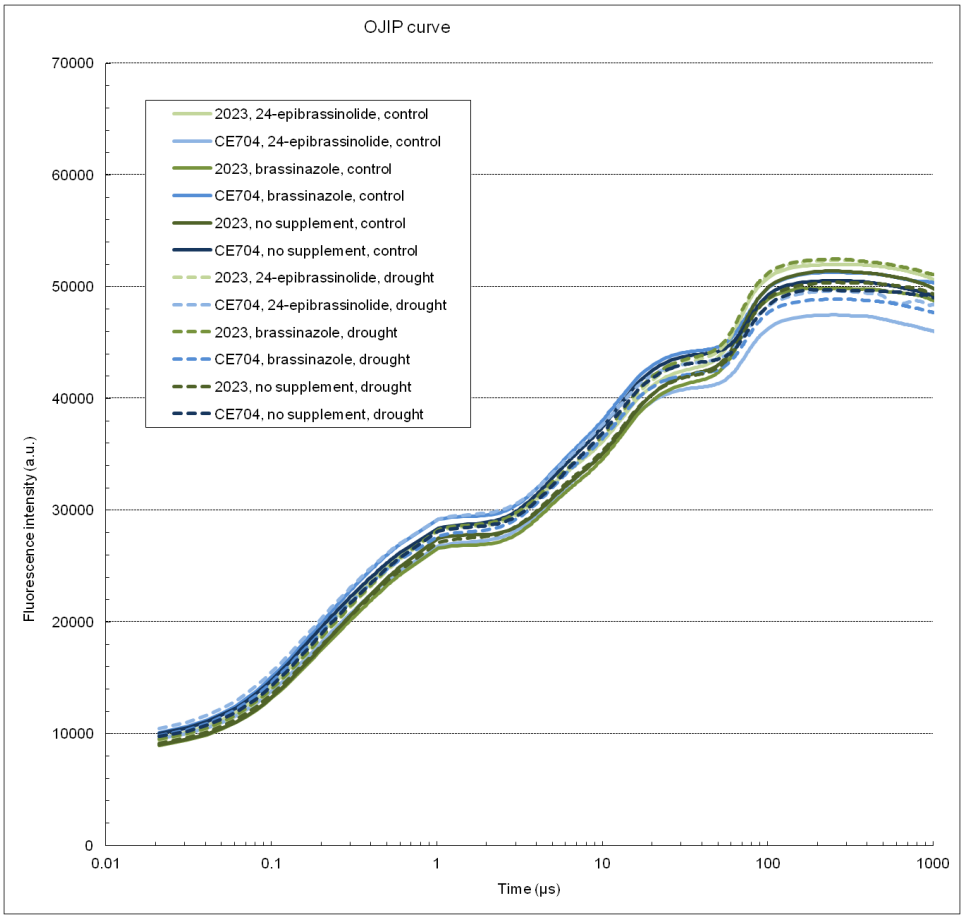


Fig. 21. OJIP curve at day 10 of the analysis. The investigated plants of 2023 and CE704 genotype were subjected to water deficit after they had been sprayed with different treatment solutions ("no supplement", $10^{-5}$ M "brassinazole", $10^{-8}$ M "24-epibrassinolide") at day 10 of the analysis. For each combination of the treatment, cultivation ("drought" group and "control" group) and genotype there were 8 plants analysed.

Table 6. Overview of the statistical analysis of differences (α = 0.05) in characteristics of maize plants treated with "no supplement", $10^{-5}$ M "brassinazole" and $10^{-8}$ M "24-epibrassinolide" solution at day 0 of the analysis (22 days after sowing). The same day, plants of 2023 and CE704 genotypes were subjected to water supply deficit ("drought" group and "control" group). Characteristics were compared among the different treatments at day 2, 4, 6, 8, and 10 of the analysis and where reasonable also at day 0. For particular results of statistical analysis see Table S1, Section II. When compared to each other:

values are significantly lower  values are significantly higher

| Parameter | | control/CE704 | | | drought/CE704 | | | control/2023 | | | drought/2023 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide |
| Lengths of the internodes | | | | | | | | | | | | | |
| Length of the 1st internode | Day 2 | | | | | | | | | | | | |
| Length of the 2nd internode | Day 2 | | | | | | | | | | | | |
| Length of the 3rd internode | Day 2 | | | | | | | | | | | | |
| Length of the 4th internode | Day 2 | | | | | | | | | | | | |
| Length of the 1st internode | Day 4 | | | | | | | | | | | | |
| Length of the 2nd internode | Day 4 | | | | | | | | | | | | |
| Length of the 3rd internode | Day 4 | | | | | | | | | | | | |
| Length of the 4th internode | Day 4 | | | | | | | | | | | | |
| Length of the 1st internode | Day 6 | | | | | | | | | | | | |
| Length of the 2nd internode | Day 6 | | | | | | | | | | | | |
| Length of the 3rd internode | Day 6 | | | | | | | | | | | | |
| Length of the 4th internode | Day 6 | | | | | | | | | | | | |
| Length of the 1st internode | Day 8 | | | | | | | | | | | | |
| Length of the 2nd internode | Day 8 | | | | | | | | | | | | |
| Length of the 3rd internode | Day 8 | | | | | | | | | | | | |
| Length of the 4th internode | Day 8 | | | | | | | | | | | | |
| Length of the 1st internode | Day 10 | | | | | | | | | | | | |
| Length of the 2nd internode | Day 10 | | | | | | | | | | | | |
| Length of the 3rd internode | Day 10 | | | | | | | | | | | | |
| Length of the 4th internode | Day 10 | | | | | | | | | | | | |

| Parameter | | control/CE704 | | | drought/CE704 | | | control/2023 | | | drought/2023 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide |
| Height of the plants | | | | | | | | | | | | | |
| Height of the plant | Day 2 | | | | | | | | | | | | |
| Height of the plant | Day 4 | | | | | | | | | | | | |
| Height of the plant | Day 6 | | | | | | | | | | | | |
| Height of the plant | Day 8 | | | | | | | | | | | | |
| Height of the plant | Day 10 | | | | | | | | | | | | |
| Length of the roots | | | | | | | | | | | | | |
| Length of the root | Day 2 | | | | | | | | | | | | |
| Length of the root | Day 4 | | | | | | | | | | | | |
| Length of the root | Day 6 | | | | | | | | | | | | |
| Length of the root | Day 8 | | | | | | | | | | | | |
| Length of the root | Day 10 | | | | | | | | | | | | |
| Length of the whole plants | | | | | | | | | | | | | |
| Length of the whole plant | Day 2 | | | | | | | | | | | | |
| Length of the whole plant | Day 4 | | | | | | | | | | | | |
| Length of the whole plant | Day 6 | | | | | | | | | | | | |
| Length of the whole plant | Day 8 | | | | | | | | | | | | |
| Length of the whole plant | Day 10 | | | | | | | | | | | | |
| Shoot to root length ratios | | | | | | | | | | | | | |
| Shoot-root length ratio | Day 2 | | | | | | | | | | | | |
| Shoot-root length ratio | Day 4 | | | | | | | | | | | | |
| Shoot-root length ratio | Day 6 | | | | | | | | | | | | |
| Shoot-root length ratio | Day 8 | | | | | | | | | | | | |
| Shoot-root length ratio | Day 10 | | | | | | | | | | | | |
| Lengths of the leaves | | | | | | | | | | | | | |
| Length of the 1st leaf | Day 2 | | | | | | | | | | | | |
| Length of the 2nd leaf | Day 2 | | | | | | | | | | | | |
| Length of the 3rd leaf | Day 2 | | | | | | | | | | | | |
| Length of the 4th leaf | Day 2 | | | | | | | | | | | | |

| Parameter | | control/CE704 | | | drought/CE704 | | | control/2023 | | | drought/2023 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide |
| Lengths of the leaves | | | | | | | | | | | | | |
| Length of the 1st leaf | Day 4 | | | | | | | | | | | | |
| Length of the 2nd leaf | Day 4 | | | | | | | | | | | | |
| Length of the 3rd leaf | Day 4 | | | | | | | | | | | | |
| Length of the 4th leaf | Day 4 | | | | | | | | | | | | |
| Length of the 1st leaf | Day 6 | | | | | | | | | | | | |
| Length of the 2nd leaf | Day 6 | | | | | | | | | | | | |
| Length of the 3rd leaf | Day 6 | | | | | | | | | | | | |
| Length of the 4th leaf | Day 6 | | | | | | | | | | | | |
| Length of the 1st leaf | Day 8 | | | | | | | | | | | | |
| Length of the 2nd leaf | Day 8 | | | | | | | | | | | | |
| Length of the 3rd leaf | Day 8 | | | | | | | | | | | | |
| Length of the 4th leaf | Day 8 | | | | | | | | | | | | |
| Length of the 1st leaf | Day 10 | | | | | | | | | | | | |
| Length of the 2nd leaf | Day 10 | | | | | | | | | | | | |
| Length of the 3rd leaf | Day 10 | | | | | | | | | | | | |
| Length of the 4th leaf | Day 10 | | | | | | | | | | | | |
| Widths of the leaves | | | | | | | | | | | | | |
| Width of the 1st leaf | Day 2 | | | | | | | | | | | | |
| Width of the 2nd leaf | Day 2 | | | | | | | | | | | | |
| Width of the 3rd leaf | Day 2 | | | | | | | | | | | | |
| Width of the 4th leaf | Day 2 | | | | | | | | | | | | |
| Width of the 1st leaf | Day 4 | | | | | | | | | | | | |
| Width of the 2nd leaf | Day 4 | | | | | | | | | | | | |
| Width of the 3rd leaf | Day 4 | | | | | | | | | | | | |
| Width of the 4th leaf | Day 4 | | | | | | | | | | | | |
| Width of the 1st leaf | Day 6 | | | | | | | | | | | | |
| Width of the 2nd leaf | Day 6 | | | | | | | | | | | | |
| Width of the 3rd leaf | Day 6 | | | | | | | | | | | | |
| Width of the 4th leaf | Day 6 | | | | | | | | | | | | |

| Parameter | | control/CE704 | | | drought/CE704 | | | control/2023 | | | drought/2023 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide |
| Widths of the leaves | | | | | | | | | | | | | |
| Width of the 1st leaf | Day 8 | | | | | | | | | | | | |
| Width of the 2nd leaf | Day 8 | | | | | | | | | | | | |
| Width of the 3rd leaf | Day 8 | | | | | | | | | | | | |
| Width of the 4th leaf | Day 8 | | | | | | | | | | | | |
| Width of the 1st leaf | Day 10 | | | | | | | | | | | | |
| Width of the 2nd leaf | Day 10 | | | | | | | | | | | | |
| Width of the 3rd leaf | Day 10 | | | | | | | | | | | | |
| Width of the 4th leaf | Day 10 | | | | | | | | | | | | |
| Number of visible leaves | | | | | | | | | | | | | |
| Visible leaves | Day 2 | | | | | | | | | | | | |
| Visible leaves | Day 4 | | | | | | | | | | | | |
| Visible leaves | Day 6 | | | | | | | | | | | | |
| Visible leaves | Day 8 | | | | | | | | | | | | |
| Visible leaves | Day 10 | | | | | | | | | | | | |
| Dry masses (DM) of the leaves | | | | | | | | | | | | | |
| DM of the 1st leaf | Day 2 | | | | | | | | | | | | |
| DM of the 2nd leaf | Day 2 | | | | | | | | | | | | |
| DM of the 3rd leaf | Day 2 | | | | | | | | | | | | |
| DM of the 4th leaf | Day 2 | | | | | | | | | | | | |
| DM of the 1st leaf | Day 4 | | | | | | | | | | | | |
| DM of the 2nd leaf | Day 4 | | | | | | | | | | | | |
| DM of the 3rd leaf | Day 4 | | | | | | | | | | | | |
| DM of the 4th leaf | Day 4 | | | | | | | | | | | | |
| DM of the 1st leaf | Day 6 | | | | | | | | | | | | |
| DM of the 2nd leaf | Day 6 | | | | | | | | | | | | |
| DM of the 3rd leaf | Day 6 | | | | | | | | | | | | |
| DM of the 4th leaf | Day 6 | | | | | | | | | | | | |

| Parameter | | control/CE704 | | | drought/CE704 | | | control/2023 | | | drought/2023 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide |
| Dry masses (DM) of the leaves | | | | | | | | | | | | | |
| DM of the 1st leaf | Day 8 | | | | | | | | | | | | |
| DM of the 2nd leaf | Day 8 | | | | | | | | | | | | |
| DM of the 3rd leaf | Day 8 | | | | | | | | | | | | |
| DM of the 4th leaf | Day 8 | | | | | | | | | | | | |
| DM of the 1st leaf | Day 10 | | | | | | | | | | | | |
| DM of the 2nd leaf | Day 10 | | | | | | | | | | | | |
| DM of the 3rd leaf | Day 10 | | | | | | | | | | | | |
| DM of the 4th leaf | Day 10 | | | | | | | | | | | | |
| Dry mass (DM) of the shoot residues | | | | | | | | | | | | | |
| DM of the shoot residue | Day 2 | | | | | | | | | | | | |
| DM of the shoot residue | Day 4 | | | | | | | | | | | | |
| DM of the shoot residue | Day 6 | | | | | | | | | | | | |
| DM of the shoot residue | Day 8 | | | | | | | | | | | | |
| DM of the shoot residue | Day 10 | | | | | | | | | | | | |
| Dry mass (DM) of the shoots (including dry masses of leaves) | | | | | | | | | | | | | |
| DM of the shoot (with leaves) | Day 2 | | | | | | | | | | | | |
| DM of the shoot (with leaves) | Day 4 | | | | | | | | | | | | |
| DM of the shoot (with leaves) | Day 6 | | | | | | | | | | | | |
| DM of the shoot (with leaves) | Day 8 | | | | | | | | | | | | |
| DM of the shoot (with leaves) | Day 10 | | | | | | | | | | | | |
| Dry mass (DM) of the roots | | | | | | | | | | | | | |
| DM of the root | Day 2 | | | | | | | | | | | | |
| DM of the root | Day 4 | | | | | | | | | | | | |
| DM of the root | Day 6 | | | | | | | | | | | | |
| DM of the root | Day 8 | | | | | | | | | | | | |
| DM of the root | Day 10 | | | | | | | | | | | | |

| Parameter | | control/CE704 | | | drought/CE704 | | | control/2023 | | | drought/2023 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide |
| Dry mass (DM) of the whole plants | | | | | | | | | | | | | |
| DM of the whole plant | Day 2 | | | | | | | | | | | | |
| DM of the whole plant | Day 4 | | | | | | | | | | | | |
| DM of the whole plant | Day 6 | | | | | | | | | | | | |
| DM of the whole plant | Day 8 | | | | | | | | | | | | |
| DM of the whole plant | Day 10 | | | | | | | | | | | | |
| Shoot to root dry mass (DM) ratios | | | | | | | | | | | | | |
| Shoot-root DM ratio | Day 2 | | | | | | | | | | | | |
| Shoot-root DM ratio | Day 4 | | | | | | | | | | | | |
| Shoot-root DM ratio | Day 6 | | | | | | | | | | | | |
| Shoot-root DM ratio | Day 8 | | | | | | | | | | | | |
| Shoot-root DM ratio | Day 10 | | | | | | | | | | | | |
| Relative water content (RWC) of the 4$^{th}$ leaves | | | | | | | | | | | | | |
| RWC | Day 0 | | | | | | | | | | | | |
| RWC | Day 2 | | | | | | | | | | | | |
| RWC | Day 4 | | | | | | | | | | | | |
| RWC | Day 6 | | | | | | | | | | | | |
| RWC | Day 8 | | | | | | | | | | | | |
| RWC | Day 10 | | | | | | | | | | | | |
| Specific leaf weight (SLW) of the 4$^{th}$ leaves | | | | | | | | | | | | | |
| SLW | Day 0 | | | | | | | | | | | | |
| SLW | Day 2 | | | | | | | | | | | | |
| SLW | Day 4 | | | | | | | | | | | | |
| SLW | Day 6 | | | | | | | | | | | | |
| SLW | Day 8 | | | | | | | | | | | | |
| SLW | Day 10 | | | | | | | | | | | | |
| Pigments content *per* leaf unit area (LA) of the 4$^{th}$ leaves | | | | | | | | | | | | | |
| Chl *a*/LA | Day 0 | | | | | | | | | | | | |
| Chl *b*/LA | Day 0 | | | | | | | | | | | | |
| (Chl *a* + chl *b*)/LA | Day 0 | | | | | | | | | | | | |
| Carotenoids/LA | Day 0 | | | | | | | | | | | | |

| Parameter | | control/CE704 | | | drought/CE704 | | | control/2023 | | | drought/2023 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide |
| Pigments content *per* leaf unit area (LA) of the 4$^{th}$ leaves | | | | | | | | | | | | | |
| Chl *a*/LA | Day 2 | | | | | | | | | | | | |
| Chl *b*/LA | Day 2 | | | | | | | | | | | | |
| (Chl *a* + chl *b*)/LA | Day 2 | | | | | | | | | | | | |
| Carotenoids/LA | Day 2 | | | | | | | | | | | | |
| Chl *a*/LA | Day 4 | | | | | | | | | | | | |
| Chl *b*/LA | Day 4 | | | | | | | | | | | | |
| (Chl *a* + chl *b*)/LA | Day 4 | | | | | | | | | | | | |
| Carotenoids/LA | Day 4 | | | | | | | | | | | | |
| Chl *a*/LA | Day 6 | | | | | | | | | | | | |
| Chl *b*/LA | Day 6 | | | | | | | | | | | | |
| (Chl *a* + chl *b*)/LA | Day 6 | | | | | | | | | | | | |
| Carotenoids/LA | Day 6 | | | | | | | | | | | | |
| Chl *a*/LA | Day 8 | | | | | | | | | | | | |
| Chl *b*/LA | Day 8 | | | | | | | | | | | | |
| (Chl *a* + chl *b*)/LA | Day 8 | | | | | | | | | | | | |
| Carotenoids/LA | Day 8 | | | | | | | | | | | | |
| Chl *a*/LA | Day 10 | | | | | | | | | | | | |
| Chl *b*/LA | Day 10 | | | | | | | | | | | | |
| (Chl *a* + chl *b*)/LA | Day 10 | | | | | | | | | | | | |
| Carotenoids/LA | Day 10 | | | | | | | | | | | | |
| Pigments content *per* leaf dry mass (DM) of the 4$^{th}$ leaves | | | | | | | | | | | | | |
| Chl *a*/DM | Day 0 | | | | | | | | | | | | |
| Chl *b*/DM | Day 0 | | | | | | | | | | | | |
| (Chl *a* + chl *b*)/DM | Day 0 | | | | | | | | | | | | |
| Carotenoids/DM | Day 0 | | | | | | | | | | | | |
| Chl *a*/DM | Day 2 | | | | | | | | | | | | |
| Chl *b*/DM | Day 2 | | | | | | | | | | | | |
| (Chl *a* + chl *b*)/DM | Day 2 | | | | | | | | | | | | |
| Carotenoids/DM | Day 2 | | | | | | | | | | | | |

| Parameter | | control/CE704 | | | drought/CE704 | | | control/2023 | | | drought/2023 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide |
| Pigments content *per* leaf dry mass (DM) of the 4th leaves | | | | | | | | | | | | | |
| Chl *a*/DM | Day 4 | | | | | | | | | | | | |
| Chl *b*/DM | Day 4 | | | | | | | | | | | | |
| (Chl *a* + chl *b*)/DM | Day 4 | | | | | | | | | | | | |
| Carotenoids/DM | Day 4 | | | | | | | | | | | | |
| Chl *a*/DM | Day 6 | | | | | | | | | | | | |
| Chl *b*/DM | Day 6 | | | | | | | | | | | | |
| (Chl *a* + chl *b*)/DM | Day 6 | | | | | | | | | | | | |
| Carotenoids/DM | Day 6 | | | | | | | | | | | | |
| Chl *a*/DM | Day 8 | | | | | | | | | | | | |
| Chl *b*/DM | Day 8 | | | | | | | | | | | | |
| (Chl *a* + chl *b*)/DM | Day 8 | | | | | | | | | | | | |
| Carotenoids/DM | Day 8 | | | | | | | | | | | | |
| Chl *a*/DM | Day 10 | | | | | | | | | | | | |
| Chl *b*/DM | Day 10 | | | | | | | | | | | | |
| (Chl *a* + chl *b*)/DM | Day 10 | | | | | | | | | | | | |
| Carotenoids/DM | Day 10 | | | | | | | | | | | | |
| Chlorophyll *a* to chlorophyll *b* ratio of the 4th leaves | | | | | | | | | | | | | |
| Chl *a*/chl *b* | Day 0 | | | | | | | | | | | | |
| Chl *a*/chl *b* | Day 2 | | | | | | | | | | | | |
| Chl *a*/chl *b* | Day 4 | | | | | | | | | | | | |
| Chl *a*/chl *b* | Day 6 | | | | | | | | | | | | |
| Chl *a*/chl *b* | Day 8 | | | | | | | | | | | | |
| Chl *a*/chl *b* | Day 10 | | | | | | | | | | | | |
| Chlorophyll *a* and chlorophyll *b* to total carotenoids ratio of the 4th leaves | | | | | | | | | | | | | |
| (Chl *a* + chl *b*)/carotenoids | Day 0 | | | | | | | | | | | | |
| (Chl *a* + chl *b*)/carotenoids | Day 2 | | | | | | | | | | | | |
| (Chl *a* + chl *b*)/carotenoids | Day 4 | | | | | | | | | | | | |
| (Chl *a* + chl *b*)/carotenoids | Day 6 | | | | | | | | | | | | |
| (Chl *a* + chl *b*)/carotenoids | Day 8 | | | | | | | | | | | | |
| (Chl *a* + chl *b*)/carotenoids | Day 10 | | | | | | | | | | | | |

| Parameter | | control/CE704 | | | drought/CE704 | | | control/2023 | | | drought/2023 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide |
| Photosystem II quantum yield (QY) of the 4th leaves | | | | | | | | | | | | | |
| QY | Day 0 | | | | | | | | | | | | |
| QY | Day 2 | | | | | | | | | | | | |
| QY | Day 4 | | | | | | | | | | | | |
| QY | Day 6 | | | | | | | | | | | | |
| QY | Day 8 | | | | | | | | | | | | |
| QY | Day 10 | | | | | | | | | | | | |
| Photochemical reflectance index (PRI) of the 4th leaves | | | | | | | | | | | | | |
| PRI | Day 0 | | | | | | | | | | | | |
| PRI | Day 2 | | | | | | | | | | | | |
| PRI | Day 4 | | | | | | | | | | | | |
| PRI | Day 6 | | | | | | | | | | | | |
| PRI | Day 8 | | | | | | | | | | | | |
| PRI | Day 10 | | | | | | | | | | | | |
| Normalized difference vegetative index (NDVI) of the 4th leaves | | | | | | | | | | | | | |
| NDVI | Day 0 | | | | | | | | | | | | |
| NDVI | Day 2 | | | | | | | | | | | | |
| NDVI | Day 4 | | | | | | | | | | | | |
| NDVI | Day 6 | | | | | | | | | | | | |
| NDVI | Day 8 | | | | | | | | | | | | |
| NDVI | Day 10 | | | | | | | | | | | | |
| Minimal fluorescence of Photosystem II ($F_0$) of the 4th leaves | | | | | | | | | | | | | |
| $F_0$ | Day 0 | | | | | | | | | | | | |
| $F_0$ | Day 2 | | | | | | | | | | | | |
| $F_0$ | Day 4 | | | | | | | | | | | | |
| $F_0$ | Day 6 | | | | | | | | | | | | |
| $F_0$ | Day 8 | | | | | | | | | | | | |
| $F_0$ | Day 10 | | | | | | | | | | | | |

| Parameter | | control/CE704 | | | drought/CE704 | | | control/2023 | | | drought/2023 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide | no supplement | brassinazole | 24-epibrassinolide |
| Maximal fluorescence of Photosystem II ($F_m$) of the 4$^{th}$ leaves | | | | | | | | | | | | | |
| $F_m$ | Day 0 | | | | | | | | | | | | |
| $F_m$ | Day 2 | | | | | | | | | | | | |
| $F_m$ | Day 4 | | | | | | | | | | | | |
| $F_m$ | Day 6 | | | | | | | | | | | | |
| $F_m$ | Day 8 | | | | | | | | | | | | |
| $F_m$ | Day 10 | | | | | | | | | | | | |
| Maximum quantum efficiency of Photosystem II ($F_v/F_m$) of the 4$^{th}$ leaves | | | | | | | | | | | | | |
| $F_v/F_m$ | Day 0 | | | | | | | | | | | | |
| $F_v/F_m$ | Day 2 | | | | | | | | | | | | |
| $F_v/F_m$ | Day 4 | | | | | | | | | | | | |
| $F_v/F_m$ | Day 6 | | | | | | | | | | | | |
| $F_v/F_m$ | Day 8 | | | | | | | | | | | | |
| $F_v/F_m$ | Day 10 | | | | | | | | | | | | |

# 6. DISCUSSION

## 6.1 THE AUTUMN SEASON EXPERIMENT EVALUATION

During the autumn experimental season it was investigated which of the EBR (24-epibrassinolide, a stable BR with natural occurence) concentrations was the most effective in helping the maize plants to overcome the stress induced by water deficit and to recover after the return under optimal growth conditions, and also if soaking of kernels is a suitable application for further investigation of BR action in the experimental research or for use in larger scale in agriculture.

Although the difference in treatments was evaluated, the effect of genotypes cannot be omitted in the evaluation of this season experiment. As mentioned previously, hybrids (2023xCE704 and CE704x2023) were superior to their parental lines, especially when talking about developmental and growth characteristics (e.g. number of visible leaves, plant length and dry mass accumulation) as well as pigments content *per* unit leaf dry mass or area (Table S1, Section I). These characteristics are associated with better photosynthesis performance and directly affect the yield of plants. Heterosis or hybrid vigour is a long known phenomenon. It describes the tendency of increased or improved function of different biological qualities in the hybrid offspring when compared to parental lines.

The results of autumn season are in the agreement with those of, for example, CHOHAN *et al.* (2012), who observed heterosis in normal and water stress regime grown maize yield arising from improved plant morphology and photosynthesis, and WANG *et al.* (2009), who observed higher photosynthetic rate and related photosynthetic traits, and subsequently the yield in maize hybrids of normal maize cross-pollinated by high oil maize. LOPES *et al.* (2011) reviewed that heterosis in maize is associated with higher yield potential and it confers adaptation to a wide range of growth conditions, and proposed that traits associated with heterosis have potential to reveal new mechanisms which contribute to drought tolerance.

When considering the drought tolerance of inbred parental lines (2023 and CE704), the large majority of results (Table S1, Section I) corresponds to results

of BENEŠOVÁ *et al.* (2012). Among other characteristics, they also published that in 2023 drought stress sensitive genotype the stomatal closure and decrease in transpiration rate occurred even after mild drought conditions, while the drought stress tolerant genotype CE704 maintained opened stomata and efficient transpiration, what allowed efficient photosynthesis under these conditions. This is all in the agreement with LOPES *et al.* (2011) and TARDIEU (2012), who proposed that genotypes with an early stomatal closure and decreased transpiration rate are better adapted to survive severe and longer lasting water deficit, while genotypes with opened stomata and increased transpiration have increasing yield potential under mild to moderate water deficit.

Different strategy to deal with the water deficit of the sensitive 2023 genotype and the tolerant CE704 genotype could be seen also in their diverse morphology. The 2023 plants have bigger leaves providing shade which helps to preserve water supply for longer time, while leaf area of the CE704 plants is smaller, what reduces evapotranspiration.

When comparing plants subjected to water deficit and control plants, expected differences are evident (e.g. retarded development, decreased RWC and shoot-root dry mass ratio, and changed fluorescence characteristics in plants under water deficit), however, biomass accumulation was not significantly affected during this period (Table S1, Section I). The fact that there were almost no significant differences when comparing plants recovered after water deficit and control plants well watered during the whole period of the experiment implicates, that the mechanisms which help the sensitive and the tolerant genotype to cope with stress are counteracting on many levels.

Soaking of kernels in EBR solution of concentration from 0 M to $10^{-14}$ M before sowing had no effect on plants germination under optimal conditions (Table S1, Section I). This is supported by ASAMI *et al.* (2000) who published that BRZ, BR biosynthesis inhibitor, had no effect on germination of *Arabidopsis* plants. However, when plants grown under suboptimal conditions, HBR and EBR treatment improved germination of susceptible sorghum varieties under osmotic stress (VARDHINI and RAO 2003).

When focusing on the growth velocity (e.g. number of visible leaves) and intensity (e.g. plant height), the positive effect of $10^{-8}$ M EBR treatment on plants under water deficit conditions is significant when compared to control plants treated with tap water before the stress period, and positive effect of $10^{-8}$ M and $10^{-10}$ M EBR treatment when compared to control plants after the stress period in 2023, CE704 and CE704x2023 plants. However, almost no significant differences in these characteristics among plants treated with various EBR concentrations were observed after the recovery period (Table S1, Section I). This is in consistency with findings of VLAŠÁNKOVÁ *et al.* (2009), when the slight stimulatory effect of seed soaking in $2.4x10^{-8}$ EBR on epicotyl growth of pea and flax seedlings was observed. Higher concentrations led to the inhibition of growth of epicotyls and roots of these seedlings.

RWC value of plants subjected to water deficit was lower than in plants grown under optimal moisture conditions. Generally, there was no difference in RWC value of plants treated with different EBR concentration, the only one was significant in well-watered CE704 control plants when treated with $10^{-14}$ M EBR in comparison with 0 M EBR-treated plants at the end of the recovery period (Table S1, Section I). This is in the conflict with published experiments when under drought conditions increase in RWC was observed after HBR treatment in wheat plants (SAIRAM 1994a, 1994b), after BL treatment in drought resistant maize plants (LI and VAN STADEN 1998a) and in *Robinia pseudoacacia* seedlings (LI *et al.* 2008), after EBR treatment in *Chorispora bungeana* plants (LI *et al.* 2012) or in tomato plants (YUAN *et al.* 2010), independently of the way of BR application (seed soaking or leaf spraying). However, similarly to the autumn season there was noted no significant difference between the RWC values when maize plants sprayed with the synthetic BR and non-treated were compared in the experiment of KUKLÍKOVÁ (2011) performed at the same Laboratory of Plant Genetics. This implies that it is not the type of BR or the way of EBR application what is responsible for increased RWC values in plants under water deficit conditions, but the growth conditions in the combination with the species of BR-treated plant at certain stage of development, respectively its endogenous BRs content.

In 2023xCE704 plants treated with EBR the $10^{-14}$ M EBR treatment was more efficient than $10^{-10}$ M EBR treatment in plants subjected to water deficit when chlorophylls content *per* leaf dry mass and maximal fluorescence of Photosystem II ($F_m$) were in question. No other significant differences in EBR treatment regarding fluorescent and photosynthetic characteristics were not observed (Table S1, Section I). This is again in the conflict with the published results when under water deficit conditions HBR spraying increased chlorophyll content and photosynthesis rate in wheat plants (SAIRAM 1994a, 1994b), spraying of BL increased leaf photosynthesis, chlorophyll content and maximum quantum yield of Photosystem II in soybean plants (ZHANG *et al.* 2008) or EBR spraying increased chlorophyll content and maximum quantum yield of Photosystem II ($F_v/F_m$) in *Chorispora bungeana* plants (LI *et al.* 2012). This could be explained by different application of BRs (spraying vs. seed soaking) or longer period between the BR application and photosynthetic characteristics evaluation. However, no differences were observed in the experiment of KUKLÍKOVÁ (2011) performed at the same Laboratory of Plant Genetics with the application of synthetic BR by spraying, what offers an explanation already given for the contrast between the experiments performed in our laboratory and those published concerning RWC values.

When compared to non-EBR treated plants (and in some cases to $10^{-14}$ M EBR-treated plants) under water deficit conditions, soaking of kernels before sowing in $10^{-8}$ M or in some cases in $10^{-10}$ M EBR positively influenced growth velocity, intensity and dry mass accumulation. However, RWC and photosynthetic parameters (chlorophylls content and chlorophyll *a* fluorescence characteristics), which are considered to be closely related to yield characteristics, were not impaired or improved by these treatments. To sum up, the $10^{-8}$ M concentration of EBR solution for maize kernels soaking was the most effective in helping the plants to overcome water deficit. Almost no significant differences in these characteristics among plants treated with various EBR concentrations (0 M, $10^{-8}$ M, $10^{-10}$ M, $10^{-12}$ M and $10^{-14}$ M) were observed after the recovery period.

When compared to the experiment of KUKLÍKOVÁ (2011) with the synthetic BR applied by spraying, the application of EBR by soaking the maize kernel previously to the sowing had no additional significant or evident contribution to plants grown

under water deficit conditions, recovered or grown under optimal soil moisture conditions during the whole period of the experiment. Likewise, in rice plants grown under drought stress conditions foliar spraying of EBR had better effect than seed soaking (FAROOQ *et al.* 2009a). Interestingly, JANECZKO and SWACZYNOVÁ (2010) observed that while the spraying of wheat plants with $10^{-7}$ M EBR had no effect, seed soaking in EBR of this concentration resulted in dry mass accumulation of leaves and roots, although the EBR content in the tissue was not changed in comparison with the control. The $2x10^{-6}$ M EBR application by seed soaking or root drenching further significantly increased its content in plants and resulted in the inhibition of plant growth.

Although in previously mentioned experiment seed soaking showed up to be more effective way of EBR application, based on the results of autumn season experiment presented in this work and correlated to experiment of KUKLÍKOVÁ (2011), application of EBR by soaking of maize kernels in its solution have not been proved to have any advantages when compared to application by spraying. It is not probable that different duration of soaking or different concentrations or any additive would provide different results under growth conditions given. Therefore for further investigation of EBR action in maize plants under water deficit conditions the foliar application is recommended.

## 6.2 THE SPRING SEASON EXPERIMENT EVALUATION

During the spring experimental season, the role of exogenous application and endogenous biosynthesis of BRs were investigated by spraying the maize plants subjected to water deficit by $10^{-8}$ M EBR and $10^{-5}$ M BRZ (brassinazole, a BR biosynthesis inhibitor). This experiment was focused on changes in the RWC and photochemical characteristics, which are considered to be important indicators of yield characteristics which are impaired by the drought the most. Germination, development and growth of plants were not considered to be important as they were mostly the result of genotypes performance during the period of the plant growth before the plants treatment and water supply arresting.

In Figure 4 it can be seen that the relative soil moisture of drought tolerant CE704 plants subjected to water deficit decreased under 10 % at day 4 of the analysis, while the relative soil moisture of drought sensitive CE704 plants subjected to water deficit decreased so at day 6 of the analysis. While at day 10 of the analysis was the soil moisture very similar between the treatments for particular combination of genotype and cultivation conditions, at day 7 there could be seen interesting changes caused the most probably by heavy rain.

During the day 4 and 6 of the analysis, RWC of CE704 plants grown under optimal soil moisture conditions decreased in plants treated with no supplement. The similar effect was observed in 2023 plants grown under optimal soil moisture conditions treated with no supplement at day 8, and in 2023 plants grown under water deficit conditions at day 8 and 10 of the analysis (Table S1, Section II). The mechanism which enables lower decrease of RWC in BRZ- and EBR-treated plants when compared to non-treated during the days following after the drought stress onset in drought sensitive as well as in drought resistant maize plants remains to be suggested. It appears to be interesting that decrease in endogenous BRs levels after BRZ treatment and exogenous BR treatment implicate the same action in maize plant subjected to drought stress. This seems to be in the agreement with conclusions of KAGALE *et al.* (2007) that the effect of EBR on plant stress response is not comparable to a switch being turned on and off, but it is more likely that EBR augments plant responses to drought stress, what results in higher plant tolerance to this stress, and that this involves changes in the expression of genes encoding both structural and regulatory proteins.

During the day 4 and 6 of the analysis, total chlorophyll content *per* unit leaf area and *per* unit dry mass in CE704 plants grown under both the optimal soil moisture and the water deficit conditions was increased in plants treated with no supplement. In 2023 plants was the total chlorophyll content *per* unit leaf area and *per* dry mass increased in plants treated with no supplement and grown under both the optimal soil moisture and the water deficit conditions, and in EBR-treated 2023 plants under water deficit conditions at day 8 of the analysis (Table S1, Section II). When compared to RWC values, the opposite trend of RWC and of total chlorophyll content

at the same time and under the same conditions implies the possibility that this antagonism somehow contributes to the constant photosynthesis rate maintenance.

An early inhibitory effect of Brz2001 on the chlorophylls content was observed in *Chlorella vulgaris* culture after the cultivation under light started, later this effect was stimulatory (BAJGUZ, ASAMI 2004). EBR increased the content of photosynthetic pigments in *Wolffia arrhiza* plants, while Brz220 addition reduced it (BAJGUZ, ASAMI 2005). The current literature does not provide many examples of BRs biosynthesis inhibitors effects on chlorophyll content. Despite that, the effect of endogenous or exogenous BRs on chlorophyll content apparently depends on the plants species among the other factors.

When talking about the fluorescence characteristics of chlorophyll *a* and the primary photosynthetic processes, the only change in more than one of them at the same day was observed only in plants of drought sensitive 2023 genotype subjected to water drought at day 8 of the analysis. Photochemical reflectance index (PRI) value was higher in BRZ-treated plants when compared to those treated with no supplement and Maximum quantum efficiency of Photosystem II ($F_v/F_m$) was higher in BRZ-treated plants when compared to EBR-treated or with no supplement-treated plants. The trend was exactly opposite when talking about the minimal ($F_0$) and maximal ($F_m$) fluorescence of Photosystem II. Anyhow, at day 10 there were almost no differences between the treatments in particular genotype and growth conditions combination (Table S1, Section II).

Repeatedly sprayed EBR increased, while BRZ decreased quantum yield of Photosystem II in cucumber plants (XIA *et al.* 2009). In dark-grown *Arabidopsis* seedling BRZ application increased the expression of light regulated genes coding the small subunit of RuBisCo (*rbcS*), chlorophyll *a*/*b* binding protein (*cab*), *psbA* gene for protein of Photosystem II (ASAMI *et al.* 2000), and induced the initial step of plastid differentiation, which occurs prior to the development of thylakoid membranes (NAGATA *et al.* 2000). It is certain that both exogenously applied and endogenously synthesized BRs play a role in plant photosynthesis. From the spring season experiment it is obvious that in both maize genotypes with different sensitivity to water deficit, CE704 and 2023, BRs play an important role in the constant

photosynthesis rate maintenance. However, based on results of this experiment and the current state of knowledge of this problem, it seems to be very complex and further experiments are needed to propose any explanatory theory.

Both exogenous and endogenous BRs enhanced the stress tolerance to oxidative damage during the water stress in maize leaves (ZHANG *et al.* 2011). Contrariwise, in pea plants subjected to water stress there was no difference between the WT plants and in their BR-deficient and BR-perception mutant in endogenous ABA levels, growth characteristics and water potential before and after 14 days of water deficit. Therefore they concluded that in pea plants changes in endogenous BRs levels are not normally plant's response to water stress.

It is known that endogenous BRs contents are checked and balanced constantly *via* control of BR biosynthesis and inactivation rates in normally growing plant. Increase in endogenous BR contents results in feedback regulation at mRNA levels of these BR metabolic genes to maintain the BR homeostasis. This homeostasis is finely modulated by the feedback expression of multiple BR metabolic genes, each of which is involved not only in BR-specific biosynthesis and inactivation, but also in sterol biosynthesis (TANAKA *et al.* 2005). The secondary effect of BRZ in rice was observed when its application in higher concentration caused GA deficiency due to inhibition of GA biosynthesis (SEKIMATA *et al.* 2001). Already ASAMI and YOSHIDA (1999) pointed out limitations of BRZ use. At higher concentration the morphology of BRZ-treated plants cannot be restored perfectly to that of non-treated plants by BL treatments, possibly because of non-specific effect(s) on other aspects of plant metabolism, and plants that are larger than *Arabidopsis* tend to require higher concentrations of BRZ to bring about a change in morphology, possibly because of its movement through the plant.

During the spring season experiment the BRZ was use for the first time in our laboratory. Therefore it is possible that experiments performed with a scale of BRZ concentrations could bring more light into the role of endogenous BRs in maize plants subjected to drought stress than the experiment with only one BRZ concentration ($10^{-5}$ M) chosen according to the literature. Anyway, results of this experiment brought desired evidence that this question is worth of further

investigation, preferably with other yield and drought-tolerance related characteristics investigation. For example, antioxidant system is often discussed in the literature and currently it is also investigated in EBR-treated maize in our laboratory. Experiments with BRZ-treated maize would be the logical continuation of this effort.

## 6.3 DROUGHT STRESS TOLERANCE AND BRASSINOSTEROIDS

Drought, salinity, extreme temperatures and oxidative stress are often interconnected and may induce similar cellular damage (BAJGUZ, HAYAT 2008). Drought as an abiotic stress leads to morphological, physiological, biochemical and molecular changes. These vary according to the plants species, cultivar, organ, tissue, cell type, stage of growth, water deficit and drought stress duration and type, and other factors. Understanding how plants tolerate water loss has important consequences not only for plant biology in general but it is also a necessary prerequisite for developing strategies for improving drought tolerance and maintaining biomass and yield (OLIVER *et al.* 2010). Fully drought resistant crop plants would be beneficial, but selection breeding has not produced them. The recently released oilseed rape and maize which drought resistance probably due to delayed stress onset and genetically modified plants may not be able to cope with drought better than selection-bred cultivars (LAWLOR 2012).

BRs help plants to deal with stress on morphological, physiological and molecular level. Their action is dependent on various factors, besides those above mentioned, for example, on the way of exogenous application and its possible repetition, the concentration, or the additive(s) used. Exogenous application of endogenous BRs inhibitors helps to clarify the role of BRs in plants complementing the use of BR-deficient mutants and BR-deficient or BR-insensitive transgenic plants. Understanding the role and action of BRs in plant drought tolerance and resistance is important not only for the basic research but could become an important prerequisite for breeding drought tolerant crops under specific environmental conditions.

## 7. CONCLUSIONS

When focused on developmental and growth characteristics, the heterosis was observed during the autumn season in 2023xCE704 and CE704x2023 hybrid plants when compared to their parental lines. Different strategy of drought sensitive (2023) and drought resistant (CE704) plants to deal with the water deficit was observed.

Soaking of maize kernels before the sowing in solution with different EBR concentrations did not affected RWC and photosynthetic parameters (i.e. chlorophylls content and chlorophyll a fluorescence characteristics), while $10^{-8}$ M EBR treatment had a positive effect on growth velocity and intensity of plants before the stress period, and $10^{-8}$ M and $10^{-10}$ M EBR treatment on growth velocity and intensity of plants during the water deficit period when compared to well-watered plants of 2023, CE704 and CE704x2023 genotype. After the recovery under optimal conditions there were no differences among the treatments.

Application of EBR by soaking the maize kernels in solution with its different concentrations (0 M to $10^{-14}$ M) have not been proved to have any advantages in comparison with application by spraying. However, as this way of application did not affected plants germination under optimal soil moisture conditions, its positive effect on plants germination under water deficit conditions could be possible.

When compared to treatment with no supplement, $10^{-5}$ M BRZ and $10^{-8}$ M EBR treatment lowered the decrease of RWC in plants of both genotypes contrastive in drought stress sensitivity (2023 and CE704) during the days following after drought stress onset, while this trend was exactly the opposite in total chlorophyll content *per* unit leaf dry mass and area. This antagonism may contribute to constant photosynthesis rate maintenance in maize plants subjected to water deficit.

Although it is not possible to deduce the role of exogenously applied and endogenous BRs in maize plants subjected to water deficit based on the results of spring season experiment, these confirmed that the problem is worth of further investigation.

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