Univerzita Karlova v Praze
Přírodovědecká fakulta

Genetická variabilita v růstových, reprodukčních a fotosyntetických charakteristikách rostlin a její změny v důsledku aplikace steroidů

Genetic variability in growth, reproductive and photosynthetic parameters of plants and its changes by exogenously applied steroids

Dizertační práce

RNDr. Olga Rothová

Školitel: RNDr. Dana Holá, Ph.D.
Konzultant: RNDr. Marie Kočová, CSc.

Praha 2014

Prohlašuji, že tato práce byla vypracována samostatně s využitím řádně citovaných literárních zdrojů, pod vedením školitelky a nebyla využita pro získání obdobného nebo jiného akademického titulu.

V Praze dne .....

Tato práce vznikla za finanční podpory projektů č. 501/11/1650 Grantové agentury České republiky, KJB601110611 Grantové agentury Akademie věd České republiky, B/BIO/612612, SVV-2013-267205 a SVV-2014-260081 Grantové agentury Univerzity Karlovy a výzkumného záměru MSM0021620858 Ministerstva školství, mládeže a tělovýchovy České republiky.

Poděkování:

Velmi bych chtěla poděkovat své školitelce i konzultantce Daně Holé a Marii Kočové za trpělivost a laskavost, kterou projevují při naší společné práci v laboratoři i mimo ni, bez jejich podpory by tato práce nikdy nevznikla. Nedílnou součástí laboratoře jsou i studenti, kterým děkuji za to, že celou dobu pomáhají tvořit příjemné pracovní a přátelské ovzduší laboratoře.

Stejně tak bych ráda poděkovala své rodině za podporu, kterou u nich vždy nalézám a bez které bych nemohla dělat práci, kterou mám ráda, jež je však často časově velmi náročná.

Obsah

Abstrakt

Živočišné steroidní hormony jsou velmi dobře známy a jejich výzkum probíhá již dlouhou dobu, v rostlinách však dlouho žádné steroidní látky s biologickou funkcí nebyly identifikovány. Teprve v druhé polovině minulého století byly objeveny brassinosteroidy, u nichž byla později potvrzena hormonální funkce v rostlinách. Stále je však při výzkumu jejich funkce mnoho neznámých. Tato práce předkládá ve své první části důkazy, že brassinosteroidy regulují u kukuřice (*Zea mays* L.) pěstované v polních podmínkách nejen morfologii a výnos, ale i některé vývojové a reprodukční charakteristiky, jako například počet samičích květenství či rychlost vývoje samčích květenství. Konkrétní reakce rostliny však závisí na typu použitého brassinosteroidu, na jeho koncentraci, a v neposlední řadě i na konkrétním genotypu kukuřice a na fázi vývoje rostliny v době postřiku. Vliv brassinosteroidů na primární fotosyntetické procesy v rostlině za těchto podmínek pěstování nebyl prokázán, a to ani na aktivitu fotosystému (PS) 1, ani na aktivitu Hillovy reakce. Nebyly nalezeny ani statisticky průkazné rozdíly v obsahu fotosyntetických pigmentů.

Dalším tématem, řešeným v této práci, byl možný ochranný vliv brassinosteroidů na rostliny vystavené chladu. U kontrolních rostlin došlo při exogenní aplikaci velmi nízkých koncentrací 24-epibrassinolidu ke statisticky průkaznému zvětšení některých listů a zvýšení obsahu fotosyntetických pigmentů, u rostlin vystavených chladu se naopak pozitivní vliv projevil pouze při ošetření vyššími koncentracemi 24-epibrassinolidu, a to pouze u obsahu chlorofylů. Ani tentokrát se nepodařilo zjistit statisticky průkazný vliv na primární fotosyntetické procesy fotosyntézy hodnocené jako aktivita Hillovy reakce či aktivita PS1.

Zcela novým výsledkem práce bylo prokázání biologické aktivity u dalších steroidních látek, které se v některých rostlinách také vyskytují – ekdysteroidů. Při experimentech na rostlinách novozélandského špenátu (*Tetragonia tetragonioides* L.) došlo po ošetření 20-hydroxyekdysonem k statisticky průkaznému zvýšení účinnosti čisté fotosyntézy, ovšem pouze krátkodobému (do čtyř hodin po ošetření). Tyto výsledky poprvé naznačily potenciální biologickou funkci ekdysteroidů, které by se mohly podílet na regulaci fotosyntézy. Při práci s mladými rostlinami špenátu (*Spinacia oleracea* L.) a kukuřice, které mají odlišný endogenní obsah ekdysteroidů, bylo zjištěno, že rostliny

obou druhů ošetřené buď ekdysteroidy nebo brassinosteroidy, reagovaly snížením efektivity celého fotosyntetického elektron-transportního řetězce, ale odpověď PS2 na ošetření byla u obou druhů různá, v řadě charakteristik přímo opačná. Zároveň tato odpověď závisela na vývojovém stádiu listů, přičemž fotosyntetické procesy ve starších listech reagovaly více. Působení nízkých koncentrací ekdysteroidů tedy může ovlivnit účinnost různých částí fotosyntetických procesů obdobně jako u brassinosteroidů, záleží však na rostlinném druhu, vývojovém stádiu a podmínkách pěstování, a navíc různé části elektron-transportního řetězce reagují různým způsobem. Při společném ošetření rostlin oběma látkami však žádné rozdíly oproti kontrole nalezeny nebyly. Je možné, že v některých aspektech signalizace či metabolizmu rostlinné buňky může docházet ke kompetitivnímu účinku těchto příbuzných látek.

Klíčová slova:

Brassinosteroidy, ekdysteroidy, fotosyntéza, morfologie rostlin, primární fotosyntetické procesy, fotosyntetické pigmenty, reprodukce rostlin, vnitrodruhová a mezidruhová genetická variabilita, vývoj rostlin, abiotický stres

Abstract

While animal steroid hormones are very well known and have been studied for a long time, in plants no steroid substances were known until relatively recently. Only in the second half of the past century brassinosteroids were discovered; later on, their hormonal function in plants was confirmed. Still a lot of unknown remains as regards their function in plant cells. This paper presents in its first part the evidence that brassinosteroids control in maize (*Zea mays* L.) grown under field conditions not only its morphology and yield but also some developmental/reproduction characteristics like e.g. number of female inflorescences or speed of the development of male inflorescences. Particular response of a plant depends, however, on the type of applied brassinosteroid, its concentration, and last but not least also on a particular maize genotype and developmental stage of the plant during applicatin. Impact of brassinosteroids on primary photosynthetic processes in plants has not been proven under these conditions, neither on the activity of photosystem (PS) I nor on the Hill reaction. No statistically significant differences in the content of photosynthetic pigments have been found either.

Another topic dealt with in this thesis is the possible protective influence of brassinosteroids on plants subjected to cold. Control plants treated by exogenous application of very low concentrations of 24-epibrassinolide showed a significant enlargement of some leaves and an increase of the content of photosynthetic pigments occurred; in plants subjected to cold, on the contrary, positive influence occurred only after the treatment with higher concentrations of 24-epibrassinolide, and only regarding the chlorophyll content. A significant impact on primary photosynthetic processes assessed as the activity of the Hill reaction or activity of the PS1 was not found here either.

Entirely new result of the thesis has been the proof of a biological activity of other steroid substances also occurring in some plants – ecdysteroids. During the experiments on plants of New Zealand spinach (*Tetragonia tetragonioides* L.), a statistically significant increase of the net photosynthetic rate occurred after the treatment with 20-hydroxyecdysone, but just in a short-term (up to four hours after the treatment). These results have for the first time indicated the potential new biological function of ecdysteroids – regulation of photosynthesis. When working with young plants of common spinach (*Spinacia oleracea* L.) and maize, species with different endogenous content of ecdysteroids, it has been found that plants of both species treated either by ecdysteroids or brassinosteroids responded by decreasing their efficiency of the entire photosynthetic electron-transport chain, but the response of PS2 of each species to the treatment was different and often quite opposite. This response also depended on the developmental stage of leaves (the photosynthetic processes in older leaves responded more). Thus, low concentrations of ecdysteroids can affect the efficiency of different parts of photosynthetic processes similarly to brassinosteroids; however, their effect depends on particular plant species, developmental stage as well as growing conditions; in addition, different parts of the

electron-transport chain respond in different ways. After joint treatment of the plants by both substances, no differences compared to control plants were found. It is possible that a competitive impact of these related substances may occur in some aspects of signaling or metabolism of plant cell.

Key words:

Brassinosteroids, ecdysteroids, photosynthesis, plant morphology, primary photosynthetic processes, photosynthetic pigments, plant reproduction, intraspecific and interspecific genetic variability, plant development, abiotic stress

Seznam zkratek

20E - 20-hydroxyekdyson

24E - 24-epibrassinolid

AAC - 2α,3α,17β-trihydroxy-5α-androstan-6-on

ABA - abscisová kyselina

BR - brassinosteroid(y)

ET - etylén

GA - giberelová kyselina

JA - jasmonová kyselina

Me - metylace

OEC - komplex vyvíjející kyslík

P - fosforylace/defosforylace

P_N – rychlost čisté fotosyntézy

PS - fotosystém

ROS - reaktivní formy kyslíku

RuBisCO – ribulóza-1,5-bisfostátkarboxyláza/oxygenáza

SA- salicylová kyselina

1. Úvod

Naše laboratoř se delší dobu zabývá odpovědí různých genotypů hospodářsky významných rostlin, především jejich fotosyntetického aparátu, na stres způsobený abiotickými faktory (hlavně chladem, nedostatkem či nadbytkem vody). V rámci tohoto výzkumu jsme navázali spolupráci se skupinou Dr. L. Kohouta z Ústavu organické chemie a biochemie AV ČR, v.v.i., která se zabývala syntézou a strukturně-funkční analýzou brassinosteroidů (BR), oxysterolů, které se v malých množstvích vyskytují ve všech rostlinných druzích a plní funkci fytohormonů. Z literatury jsme o této skupině látek věděli, že některé práce naznačují protistresový účinek těchto hormonů, což nás zajímalo. Zda a jak tento účinek souvisí s odezvou různých částí fotosyntetických procesů však zatím není jednoznačně vyřešeno. Téměř nic se také neví o tom, zda různé genotypy jednoho druhu nebo i různé druhy rostlin reagují na tyto hormony stejně nebo různě.

Začali jsme tedy testovat vliv těchto látek nejprve na rostliny pěstované v polních podmínkách a poté se náš zájem soustředil na reakci různých genotypů kukuřice a posléze i dalších druhů na BR při vystavení stresu. Při testování biologické funkce BR s naší laboratoří navázala spolupráci skupina Prof. Ing. T. Macka z Vysoké školy chemicko-technologické, jehož skupina pomocí afinitní chromatografie zjistila v podmínkách *in vitro* specifickou vazbu BR, ale i dalších rostlinných sterolů - ekdysteroidů - k některým proteinům souvisejícím s fotosyntézou nebo se stresovou reakcí rostlin. Role ekdysteroidů v rostlinách není zatím téměř vůbec objasněna a porovnání jejich funkce s rolí BR je velmi zajímavým tématem, na něž bychom se i v budoucnosti dále chtěli soustředit. Předkládaná práce shrnuje první výsledky týkající se této problematiky, na něž budou navazovat další, detailnější publikace, na kterých naše laboratoř v současné době intenzivně pracuje.

2. Cíle práce

V předkládané práci řeším tři základní cíle:

- popsat, jaký je vliv brassinosteroidů na vybrané fotosyntetické, morfologické, vývojové a výnosové charakteristiky inbredních a hybridních genotypů kukuřice, pěstovaných v polních podmínkách; srovnat, zda u různých genotypů bude tato reakce stejná nebo odlišná.

- zjistit, zda exogenní aplikace brassinosteroidů zlepší možnost mladých rostlin kukuřice se vyrovnat se stresem a zda ovlivní primární fotosyntetické procesy a obsah fotosyntetických pigmentů

- zjistit, jestli může exogenní aplikace ekdysteroidů na různé druhy rostlin mít vliv na funkci jejich fotosyntetického aparátu a porovnat případný efekt s reakcí na aplikaci brassinosteroidů, opět především s ohledem na různé části primárních fotosyntetických procesů a na možnou mezidruhovou variabilitu

3. Literární přehled

3.1. Brassinosteroidy jako rostlinné hormony

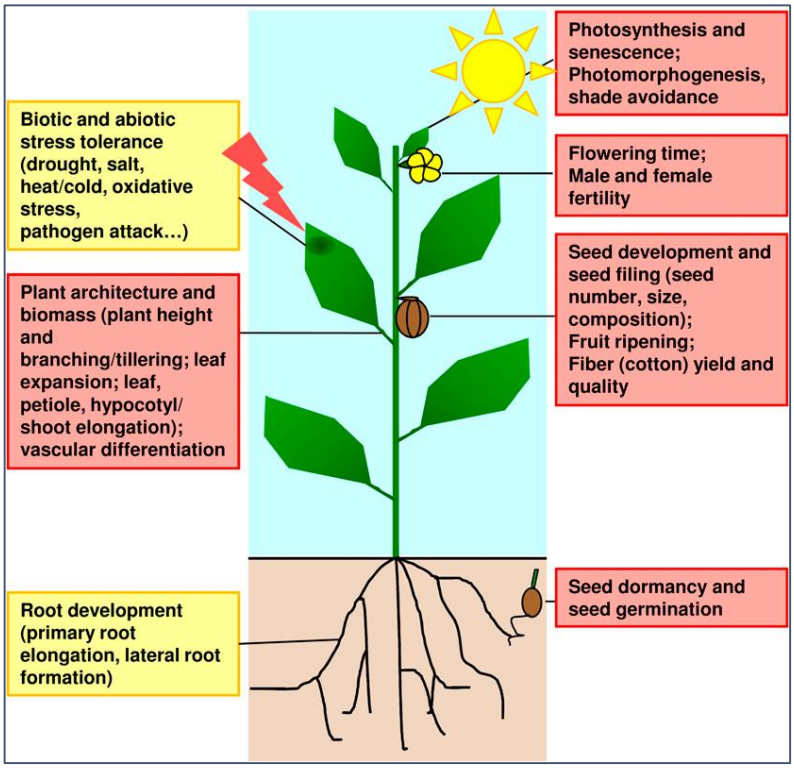
Ačkoliv v živočišné říši jsou steroidní hormony známé již velmi dlouho, v rostlinách žádné podobné látky nebyly dlouhou dobu identifikovány. Až v roce 1979 byla z pylu řepky (*Brassica napus* L.), nasbíraného včelami, izolovaná a charakterizovaná steroidní látka, která měla pozitivní účinek růstového stimulátoru v biologickém testu růstu druhého internodia fazolu (*Phaseolus vulgaris* L.). Byl to první identifikovaný brassinosteroid (BR), nazvaný brassinolid (Grove *et al.*, 1979) a poté byly nalezeny další podobné látky (Abe *et al*, 1982). Protože se tyto látky v rostlinách vyskytují ve velmi malém množství a přímá izolace z rostlin je tedy nevýhodná, po zjištění jejich struktury (Obr. 1) následovala jejich umělá syntéza (Ishiguro *et al.*, 1980; Fung *et* Siddall, 1980) a zároveň byly syntetizovány i látky obdobných struktur (Thompson *et al.*, 1979; Kohout *et al.*, 1991, Kohout, 1994). V současné době je známo více jak 70 BRs (Kohout *et al.*, 1991, Yokota, 1997, Khripach *et al.*, 1999, Fujioka, 1999, Bajguz *et* Tretyn, 2003), které byly nalezeny jak v krytosemenných, tak i v nahosemenných rostlinách i v mechorostech a zelené řase (*Hydrodictyon reticulatum* L.), a to ve všech rostlinných orgánech, ale ve velmi nízkém množství. Nejvíce BRs obsahuje obvykle pyl a často také semena, (1-100 ng na g hmotnosti čerstvé biomasy), zatímco v kořenech a starších listech je těchto látek výrazně méně (cca 0,01-0,1 ng na g) (Griffiths *et al.*, 1995, Clouse *et* Sasse, 1998, Fujioka, 1999).

Obr 1: Chemický vzorec 24-epibrassinolidu, jednoho z velmi běžných BRs (Rothová *et al*., 2014)

V devadesátých letech minulého století byly získány u několika druhů rostlin trpasličí mutanty deficientní v syntéze BR, které se lišily od mutant deficientních na syntézu jiných rostlinných hormonů (Yuan *et al.*, 2010). Jejich získání pomohlo pochopit důležitou roli BR ve vývoji rostlin (Obr. 2) a tyto látky byly záhy přeřazeny z kategorie růstových regulátorů mezi fytohormony. Bylo totiž zjištěno, že BR mají pleiotropní efekt na celé rostliny (Rao *et al*., 2002). Jsou schopny ovlivňovat buněčný růst, dělení buňky, podílí se i na zrání plodů (Clouse *et* Sasse, 1998, Haubrick *et* Assmann 2006, Kartal *et al*. 2009, Rao *et al*. 2002, Mahesh *et al*. 2013), dále na regeneraci buňky (Sasaki, 2002), jejich důležitá role byla popsána při působení na apikální dominanci a senescenci (Sasse, 2003, Rao *et al*. 2002). Podílí se také na indukci diferenciace cévního systému u rostlin (Clouse *et* Sasse 1998, Sasse 2003). Byla popsána indukce růstu pylové láčky po ošetření BR (Clouse *et* Sasse 1998), vliv na klíčení semen (Haubrick *et* Assmann 2006, Őzdemir *et al*. 2004, Rao *et al.* 2002, Sharma *et* Bhardwaj 2007, Vardhini *et* Rao 2003) a také indukce vzniku nových adventivních kořenů (Rao *et al*. 2002). Byl zjištěn i vliv BR na biogenezi buněčné stěny (Sasse 2003), na aktivaci protonových pump v buněčné membráně (Cerrana *et al.* 1984), působení na hladinu nukleových kyselin v buňce (Vardhini *et* Rao 1998, Swamy *et* Rao 2008) i na fosforylaci proteinů (Fedina *et al*. 2008).

U různých druhů rostlin byl také pozorován vliv exogenně aplikovaných brassinosteroidů na regulaci různých fotosyntetických procesů. Autoři popisují ovlivnění rychlosti čisté fotosyntézy (P_N) například u brukve (*Brassica juncea* (L.) Czern.) (Hayat *et al*. 2000, 2001a, 2007, Ali *et al*. 2008b, Fariduddin *et al.* 2009a,b), okurky (*Cucumis sativus* L.) (Yu *et al*. 2004, Xia *et al*. 2006), sóji (*Glycine max* (L.) Merrill) (Zhang *et al.* 2008), rajčete (*Solanum lycopersicum* L.) (Singh *et* Shono 2005), rýže (*Oryza sativa* L.) (Farooq *et al*. 2009), vigny zlaté (*Vigna radiata* (L.)) Wilezek (Fariduddin *et al.* 2003, 2004, Ali *et al*. 2008a) a pšenice (*Triticum aestivum* L.) (Sairam 1994a,b, Ali *et al*. 2008c). Experimentální data také ukazují, že optimální hladina BR je nutná pro normální strukturu a funkci tylakoidní membrány (Krumova *et al.* 2014). Není však zřejmé, zda BR primárně ovlivňují aktivitu pigment-proteinových komplexů fotosyntetického elektron-transportního řetězce, chloroplastové ATP syntázy nebo enzymů katalyzujících jednotlivé kroky fixace CO_2 , či se podílí na regulaci otevírání a zavírání průduchů nebo regulaci syntézy/degradace složek fotosyntetického řetězce (Yu *et al*. 2004, Hayat *et al.* 2007, Ali *et al.* 2008c, Ogweno *et al.* 2008, Fariduddin *et al.* 2009a,b). Vliv BR na fotosyntetické procesy shrnuje např. Holá (2011).

Obr. 2: Vliv BRs na regulaci vývoje rostliny se zaměřením na některé agronomicky důležité znaky (růžové obdélníky - pozitivní vliv, žluté obdélníky - smíšený vliv, např. při různých koncentracích různý) (převzato z Vriet *et al.* 2012)



Jelikož BR a některé jejich syntetické deriváty jsou biologicky aktivní ve velmi nízkých koncentracích, staly se kandidátem pro zemědělské využití v praxi. U obilovin zvyšují počet klasů a počet a hmotnost zrn v klasu (Ali *et al*. 2008b, Hnilička *et al*. 2007, Ramraj *et al.* 1997, Rao *et al.* 2002, Sairam 1994a, b, Takematsu *et* Takeuchi 1989, Torres-Ruiz *et al*. 2007), u luštěnin zvyšují počet lusků a semen v nich (Hayat *et* Ahmad 2003, Ramraj *et al.* 1997, Rao *et al*. 2002, Takematsu *et* Takeuchi 1989, Vardhini *et* Rao 1998). Lepší růst a více semen bylo po ošetření BR zjištěno i u hořčice a řepky i bavlny (Hayat *et al*. 2000, 2001b, Ramraj *et al*. 1997, Rao *et al*. 2002, Takematsu *et* Takeuchi 1989).

Ačkoliv mají BRs na růst, vývoj a výnos rostlin obecně kladný vliv, působení těchto látek je ovlivněno mnoha vnějšími faktory, takže se může stát, že zjištění získaná v kontrolovaných podmínkách nemusí platit v polní kultuře. Většina prací zabývajících se touto tématikou byla prováděna v kontrolovaných podmínkách na velmi mladých rostlinách, bez působení přírodních faktorů reálného prostředí, které samozřejmě reakci celé rostliny velmi ovlivňují. Velmi také záleží na vývojovém stádiu rostliny, během něhož dochází k aplikaci BR (Amzallag 2002, Khripach *et al.* 2000, Núñez *et al*. 2003,

Ramraj *et al.* 1997, Sasse 2003), způsobu, délce a počtu jednotlivých aplikací BR (Fariduddin *et al*. 2003, 2008, Khripach *et al.* 2003, Núñez *et al*. 2003, Ramraj *et al.* 1997, Sasse 2003, Vlašánková *et al*. 2009). Zároveň také velmi záleží na rostlinném druhu a zřejmě i genotypu (Ali *et al*. 2008b, Hnilička *et al.* 2007, Kang *et al.* 2007, Khripach *et al.* 2003, Núñez *et al.* 2003, Ramraj *et al.* 1997, Sairam 1994b, Shahbaz *et al.* 2008, Torres-Ruiz *et al.* 2007, Vardhini *et* Rao 2003, Vlašánková *et al.* 2009, Zhao *et* Chen 2003). Je tedy zřejmé, že použití BRs v zemědělské praxi ještě stále vyžaduje mnoho studia pro zavedení obecně přijímané metody pro polní aplikace.

3.2. Mechanizmy působení brassinosteroidů na molekulární úrovni

Oproti giberelinovým i auxinovým mutantám nemají mutanty v BR syntéze problémy s klíčivostí a ke standardnímu fenotypu je lze navrátit exogenním přidáním BR. Byly získány především u huseníčku (*Arabidopsis thaliana* L.) – mutanty *dwf1*, *cpd/dwf3*, *dwf4*, *dwf5*, *det2/dwf6*, *ste1/dwf7*, u hrachu (*Pisum sativum* L.) – mutanty *lka* a *lkb*, a u rajčete – mutanta *dwarf* (Yokota 1997, Kwon *et* Choe 2005, Zhao *et* Li, 2012). Díky těmto mutantám se později podařilo identifikovat první proteiny signální dráhy BR (Divi *et al.* 2010).

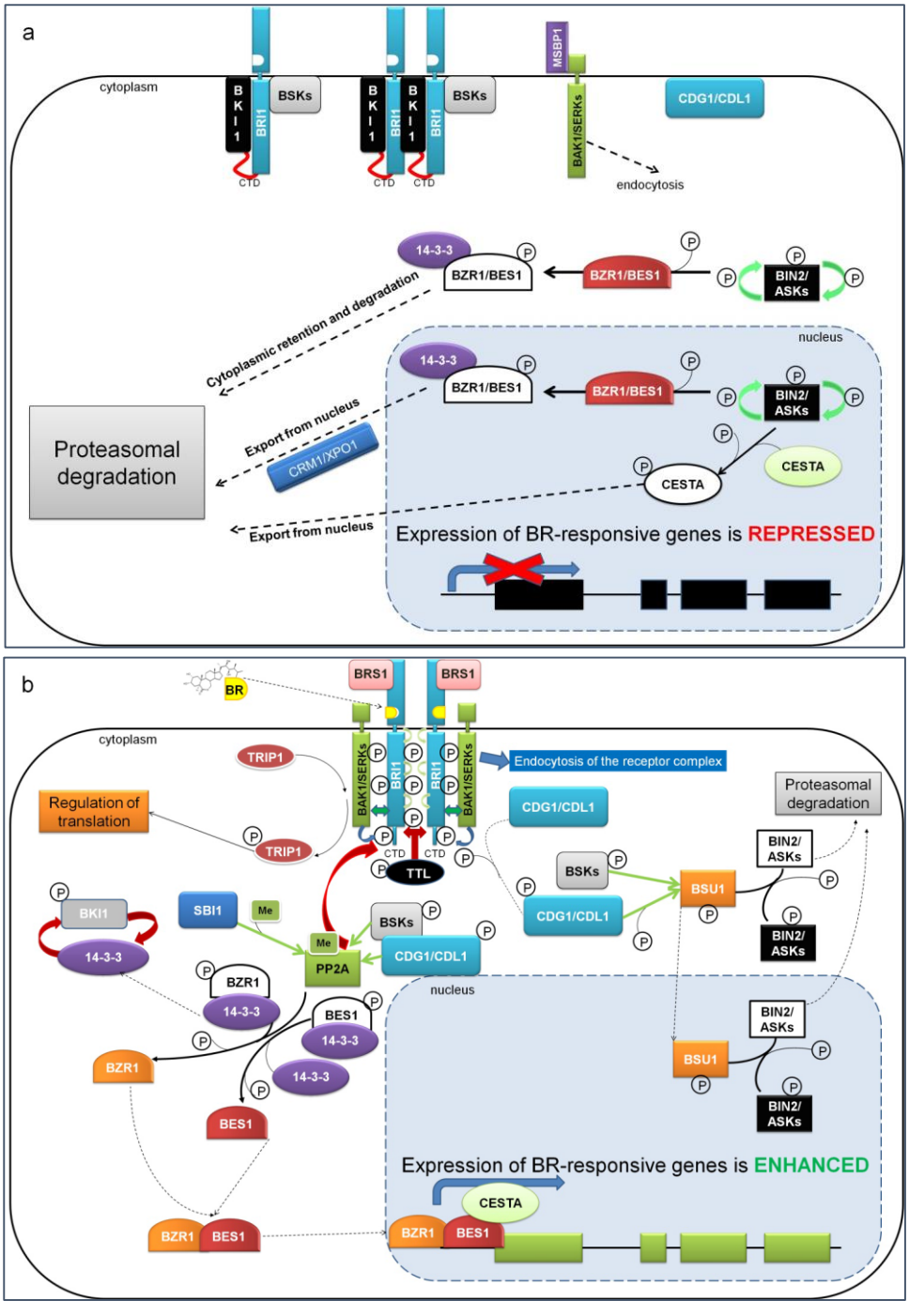
Na rozdíl od živočichů, u nichž jsou receptory pro steroidní hormony umístěny intracelulárně a komplex receptor-steroid reguluje expresi dalších genů přímo v jádře, rostlinné receptory pro BR jsou transmembránové proteiny. Receptorem je BRI1 protein (u *Arabidopsis thaliana* a jeho analogy u dalších druhů rostlin), což je serin/threonin-kináza, jejíž receptorová doména je umístěna na vnější straně buněčné membrány a její intracelulární kinázová doména je aktivována navázáním BR. Signál je dále předáván prostřednictvím fosforylační/defosforylační kaskády a hlavní složky této signální dráhy a jejich funkce byly již vcelku objasněny a jsou schematicky představeny na Obr. 3, který je převzat z jednoho z posledních přehledových článků na toto téma (Gruszka, 2013).

Nejvíce studií popisujících BR-signalizaci je zatím zpracováno na modelovém organizmu *Arabidopsis thaliana*, u kterého bylo také získáno nejvíce BR mutant. Existují však údaje, že některé mechanizmy se mohou lišit u jiných rostlinných druhů, například u rýže (Vriet *et al.* 2013). Geny, které jsou BR regulovány, zatím však nejsou přesně známy. Kódují pravděpodobně především různé transkripční faktory, které pak dále regulují expresi dalších genů.

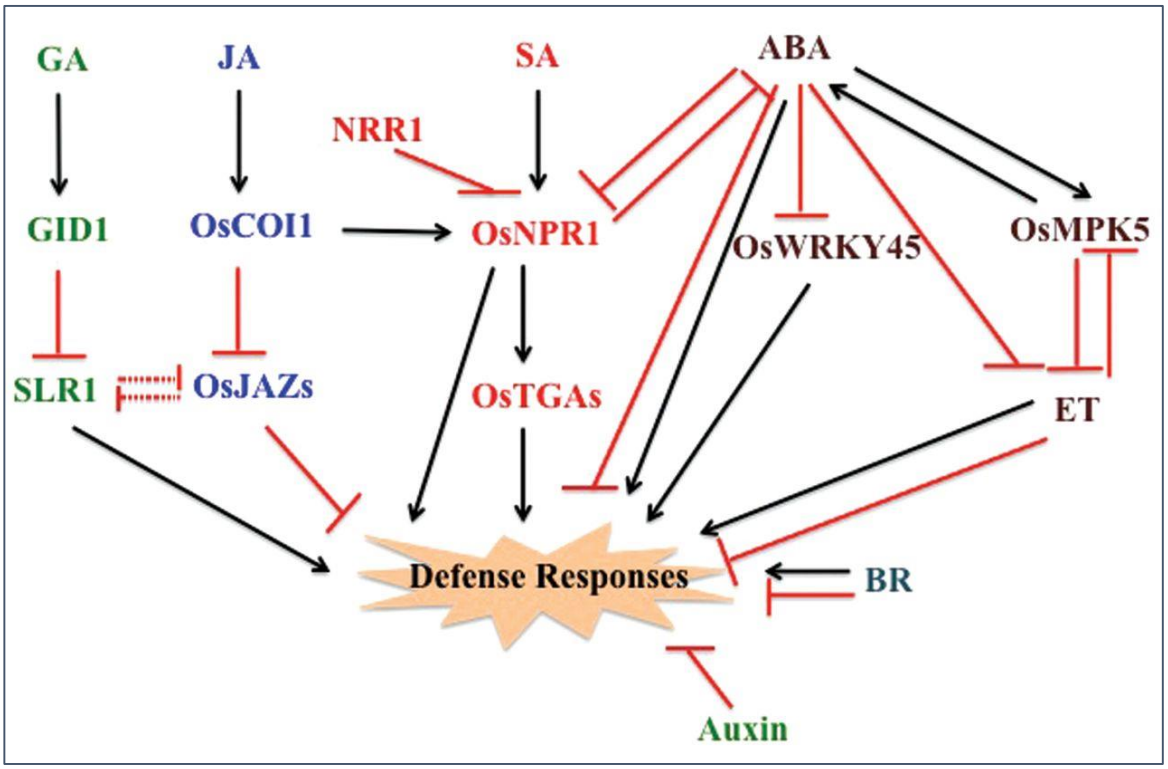
Většina procesů v rostlině, které jsou BR regulovány, je ovlivňována i dalšími skupinami fytohormonů a v mechanizmech součinnosti těchto látek je stále mnoho neznámých. Některé hormony mohou mít jak pozitivní, tak negativní roli a BR mezi ně patří. Ovšem BRs nepůsobí v organizmu osamoceně, ale společně s dalšími rostlinnými hormony. Výsledná odpověď organizmu je vždy regulována velmi komplexně, jak je zřejmé např. ze současných znalostí o roli různých rostlinných hormonů při odpovědi na biotické stresory (Obr. 4) (Yang *et al*. 2013).

Všechny modely, popisující signální dráhu BRs, počítají pouze s externím působením BR na buňku a nepředpokládají žádnou vnitrobuněčnou vazbu BR. Přesto existují *in vitro* studie, popisující specifickou afinitu BR k některým vnitrobuněčným proteinům, například proteinu ribulóza-1,5-bisfosfátkarboxyláza/oxygenáza (RuBisCO), který je jedním ze stěžejních fotosyntetických enzymů (Kamlar *et al*. 2010a). Je ovšem otázkou, zda tato vazba může existovat i v živé buňce.

Obr. 3: Model rozpoznání BRs a signální dráhy (převzato z Gruszka, 2013). *a)* V nepřítomnosti BR je inhibován komplex na C-terminální doméně (CTD) BRI1 kinázy a BKI1 proteinu. Pozitivní regulátory BR signalizace jsou neaktivní, zatímco cytoplazmatická BIN2 kináza je fosforylována a inaktivuje transkripční faktory, což má za následek jejich uchování v cytoplazmě, export z jádra a degradaci. Exprese genů závislých na přítomnosti BR je potlačena. *b)* Po zachycení BR BRI1kinázou se tvoří transmembránový receptorový komplex a je iniciována fosforylační/defosforylační kaskáda. BIN2 kináza je inhibována, což způsobí akumulaci aktivních defosforylovaných forem transkripčních faktorů v jádře a tím i stimulaci genů závislých na přítomnosti BR. Zelené šipky znamenají aktivaci, zatímco červené inhibici. P - fosforylace/defosforylace, Me - metylace.



Obr. 4: Role rostlinných hormonů a jejich spolupráce při odpovědi rýže na biotické stresory. Šipky znamenají pozitivní účinek, červené úsečky ukončené T představují negativní účinek. GA - giberelová kyselina, SA- salicylová kyselina, JA - jasmonová kyselina, ABA - abscisová kyselina, ET - etylén, BR - brassinosteroid (převzato z Yang *et al.*, 2013).

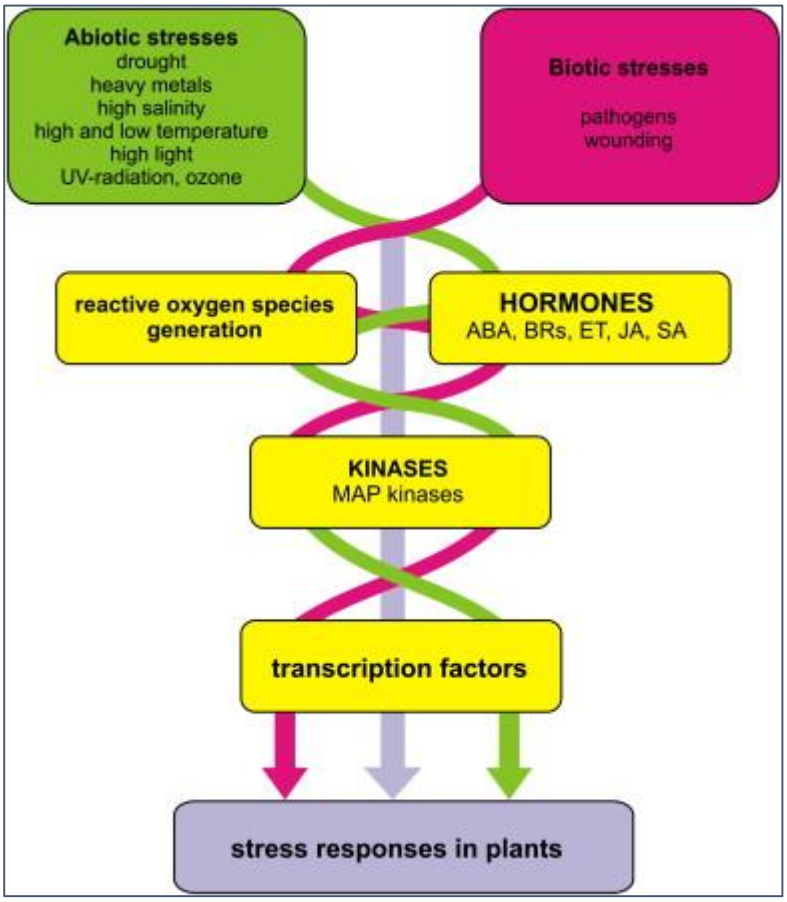


3.3. Role brassinosteroidů v odpovědi rostliny na abiotické stresory

Zatímco BR-spuštěná signální dráha, která vede k regulaci běžného růstu a vývoje rostliny, je v současné době již značně prostudována, mechanizmy, kterými BRs ovlivňují odpověď rostliny na stres, jsou zatím stále velmi nejasné (Hao *et al.* 2013, Fariduddin *et al.* 2014). Rostliny jsou organizmy, které nemohou před nebezpečím utéci, a proto si vyvinuly mnoho mechanizmů, jak mohou nepříznivým podmínkám vzdorovat. Působí na ně jak stresové faktory jak biotické, tak abiotické povahy. Z abiotických faktorů vnějšího prostředí, se rostliny musí vyrovnávat např. s vlivem těžkých kovů, změny ozářenosti, vysokou zasoleností půdy, nedostatkem nebo nadbytkem vody, s příliš vysokou nebo naopak nízkou teplotou. Sucho, nadměrná salinita, extrémní teploty a oxidativní stres mohou způsobovat obdobné typy poškození buněk a vyvolávat podobné fyziologické odpovědi rostlin (Bajguz *et* Hayat, 2009). Odpověď organizmu na stresy je opět velmi komplexní a existují experimentální důkazy, že se jí účastní i BR (Obr. 5). V této oblasti

probíhá již dlouho intenzivní výzkum, přesto je zde stále ještě mnoho neznámého (Khripach *et al*., 1999, Sakurai *et al.*, 1999, Krishna, 2003, Ali *et al*., 2008a,b,c, Bajguz *et* Hayat, 2009; Divi *et* Krishna, 2009; Hayat *et al*., 2010, Yuan *et al*., 2010, Hao *et al*. 2013, Fariduddin *et al*. 2014).

Obr. 5: generalizovaný model signální sítě rostlin, vystavených stresovým podmínkám. ABA - abscisová kyselina, BRs - brassinosteroidy, ET - etylén, JA - jasmonová kyselina, SA - salicylová kyselina (převzato z Bajguz *et* Hayat, 2009)



Pozitivní vliv BR na rostliny v nepříznivých podmínkách prostředí se může projevovat různými způsoby. Při různých typech abiotických stresů jsou ve zvýšené míře produkované reaktivní formy kyslíku (ROS). Tak jako všechny aerobní organizmy, i rostliny mají vyvinuty metabolické dráhy, kterými umí využít svůj energetický potenciál v přítomnosti kyslíku tak, aby zabránily nebezpečným účinkům ROS na makromolekuly. Mají přirozeně vyvinuty i dráhy, kterými tyto látky zneškodňují (Navrot *et al*. 2007). Jako

ochranné prostředky rostliny využívají jednak antioxidativní enzymy, jako například superoxiddismutázu, katalázu, askorbátperoxidázu, monodehydroaskorbátreduktázu, glutathionreduktázu, glutathionperoxidázu aj. (Ruley *et al.* 2004, Šimonovičová *et al.* 2004, Sharma *et* Dubey 2005), a dále neenzymatické antioxidanty, jako jsou prolin, askorbát, glutathion, α-tokoferol, karotenoidy aj. (Vardhini *et* Rao 2003, Őzdemir *et al.* 2004, Sharma *et* Dubey 2005). O tom, jakou roli hrají BRs při regulaci oxidativního stresu v rostlině, se však zatím mnoho neví (Fariduddin *et al.* 2014). Některé experimenty ukazují, že externě aplikované BRs mohou ovlivňovat aktivity antioxidačních enzymů i obsah některých neenzymatických antioxidantů včetně sekundárních metabolitů (Li *et* van Staden 1998, Li *et al.* 1998, 2008, Vardhini *et* Rao 2003, Ahammed *et al.* 2013, Fariduddin *et al.* 2013), stejně tak jako množství antioxidačních enzymů (Núñez *et al.* 2003, Őzdemir *et al.* 2004, Bajguz *et* Hayat 2009, Fariduddin *et al.* 2013). Jiang *et al.* (2012) popisují indukci tolerance vůči stresům právě pozitivní regulací antioxidačního systému prostřednictvím BR. Při pokusech s BR-mutantami se zdá, že exogenně dodávané BR u nemutovaných rostlin potlačují transkripci některých genů souvisejících se stresovou odpovědí nebo potranskripční úpravy jejich produktů, aby tím zajistily normální růst a vývoj rostliny. Avšak stále není zřejmé, zda BR upravují odpověď rostliny na oxidativní stres přímo či nepřímo (Cao *et al*. 2005).

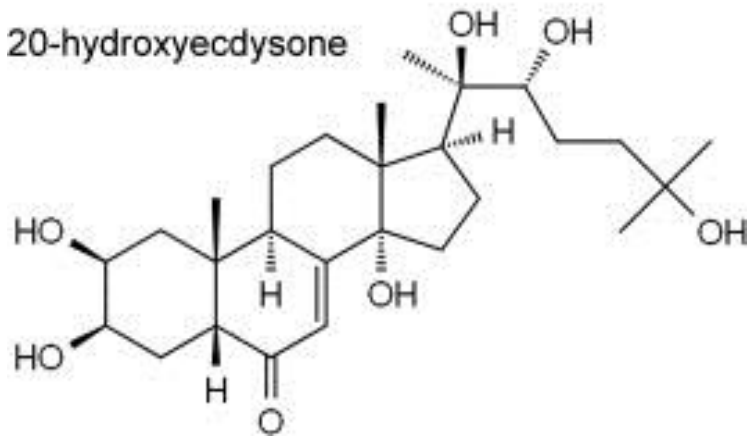
Jednou z možností uplatnění BR v odpovědi rostlin na stresové faktory může být například jejich vliv na fotosyntetické procesy. Exogenní ošetření rostlin BR často zmenšuje stresem indukovaný pokles P_N, což bylo prokázáno u různých druhů rostlin (Sairam 1994a, b, Hayat *et al.* 2007, Ali *et al.* 2008a,b,c, Ogweno *et al.* 2008, Shahbaz *et al.* 2008, Zhang *et al.* 2008 Fariduddin *et al.* 2009a,b, Ma *et* Guo 2014). Avšak přesné mechanizmy tohoto jevu nejsou zatím zdaleka kompletně známy. BRs mohou indukovat syntézu fotosyntetických pigmentů či bránit jejich rozkladu (Hayat *et al.* 2007, Ali *et al.* 2008a,b, Fariduddin *et al.* 2009a,b) nebo zvyšovat efektivitu fotosyntézy například překonáním limitace otevření průduchů a tím zvýšení koncentrace CO_2 v listu (Ali *et al.* 2008b, Shahbaz *et al.* 2008). V současné době se zdá, že blíže pravdě je spíš tato druhá varianta. Aby bylo možné se o mechanizmech vlivu BRs při ochraně fotosyntetického aparátu při stresu dovědět více, bude třeba věnovat pozornost především vlivu BR na vývoj fotosynteticky aktivních chloroplastů, analýze působení BR na různé složky elektronového transportu v chloroplastech, dále analýze vlivu BRs na všechny enzymy, podílející se na fixaci CO_2 (nejen na RuBisCO) a to u rostlin se C_3, C_4 i CAM typem fixace CO_2. Také je důležité získat informace ze strukturně-funkčních analýz, které

fotosyntetické charakteristiky jsou ovlivněny specifickými typy BR. Bude třeba odpovědět i na otázku, proč je obvykle vliv BR na fotosyntézu výraznější při stresových než při optimálních podmínkách (Holá 2011, Fariduddin *et al.* 2014).

3.4. Ekdysteroidy – další steroidní látky obsažené v rostlinách

V rostlinách se kromě BR vyskytují i další steroidní látky, ekdysteroidy, které se od BR odlišují nejen v některých aspektech biosyntézy, ale i výskytem v rostlinách, množstvím, distribucí mezi orgány a podobně. Nejčastěji se vyskytují v kořenech, listech a semenech, někdy byly detekovány i ve stoncích, cibulkách či květech (Dinan *et al.* 2001).

Obr. 6: Chemický vzorec 20-hydroxyekdysonu, nejběžnějšího ekdysteroidu obsaženého v rostlinách (Rothová *et al.* 2014)



Koncentrace ekdysteroidů v rostlinných pletivech může být až 100krát vyšší než u členovců, avšak jejich obsah se velmi odlišuje u různých rostlinných druhů. Závisí i na konkrétním orgánu rostliny a na vývojovém stádiu (Dinan, 2009, Bakrim *et al.* 2008). Nejčastěji se vyskytujícím ekdysteroidem v rostlinách je 20-hydroxyekdyson (20E) (Obr. 6), avšak v rostlinách bylo zjištěno již 460 typů těchto sloučenin a jsou stále objevovány nové. Ne ve všech druzích rostlin se však ekdysteroidy vyskytují v detekovatelném množství, odhady uvažují o jejich přítomnosti cca v 5% rostlin, zatím však tyto odhady jsou stanoveny na základě studia velmi nízného počtu rostlinných druhů. Bylo však

zjištěno, že i druhy, ve kterých se tyto látky nevyskytují, obsahují ve svém genomu geny nutné pro jejich biosyntézu, jejichž transkripce je však potlačena (Dinan, 2009, Lafont *et al.* 2013). Variabilita výskytu jednotlivých typů ekdysteroidů v rostlinách je veliká, závisí na rostlinném druhu, odrůdě, někdy i jednotlivé rostlině, dále na rostlinném orgánu a často i na jeho vývojovém stádiu (Dinan 1995, Dinan 2001, Dinan *et al.* 2001, Bakrim *et al.* 2008, Festucci-Buselli *et al*. 2008, Cheng *et al.* 2010). Množství ekdysteroidů v rostlinách obvykle nepřesahuje 0,01 až 1 % hmotnosti sušiny a většinou je v rostlině obsažen jeden až dva majoritní ekdysteroid a pak více minoritních strukturních analogů (Lafont 1998, Dinan *et al.* 2009).

Funkce ekdysteroidů v rostlině zatím není známá. Nejpřijímanější je hypotéza, podložená mnoha důkazy, že rostlině slouží jako ochranné látky proti fytofágům, především proti fytofágním druhům hmyzu a nematod, u kterých způsobují různé vývojové nebo reprodukční abnormality (Dinan 2001, Dinan 2009, Festucci-Buselli *et al*. 2008). Na druhé straně jsou ekdysteroidy velmi strukturně podobné BRs, a proto se nabízí otázka, zda mají v rostlinách také jinou fyziologickou funkci. Při použití obecných biologických testů, využívaných pro zjištění hormonální aktivity látek v rostlinách nebyly pro ekdysteroidy většinou zjištěny pozitivní výsledky (Dreier *et* Towers, 1988, Macháčková *et al.* 1995). Avšak přestože tyto testy vyšly negativně, slabá aktivita, podobná jako u giberelinů, byla zjištěna u rýže (Macháčková *et al.* 1995) a exogenně aplikované ekdysteroidy měly pozitivní vliv na růst koleoptile a semenáčků u několika rostlinných druhů (Golovatskaya 2004, Bakrim *et al.* 2007). Dále byl po ošetření ekdysteroidy pozorován pozitivní vliv na efektivitu klíčení semen rajčete (Bakrim *et al* 2007), zvýšení aktivity α-amylázy v aleuronu zrn ječmene a dokonce i vliv na obsah DNA, RNA, celkových proteinů, organického a anorganického fosforu či sacharidů, a to u řasy *Chlorella vulgaris* Beyerinck (Bajguz *et* Koronka, 2001). Pomocí afinitní chromatografie bylo pozorováno, že se ekdysteroidy specificky vážou k některým fotosyntetickým a jiným vnitrobuněčným proteinům podobně jako BR (Uhlík *et al*. 2008, Kamlar *et al*. 2010b). Všechna tato data naznačují, že by ekdysteroidy, podobně jako jim strukturně velmi příbuzné BR, mohly mít i roli při regulaci růstu a vývoje rostlin, která zatím není objasněna.

4. Materiál a metody

4. 1. Biologický materiál

- Kukuřice setá (*Zea mays* L.), inbrední rodičovské linie 2023 a CE704 a jejich F1 kříženec (2023×CE704); osivo zakoupeno ze šlechtitelské stanice CEZEA a.s.
- Čtyřboč rozložitá (*Tetragonia tetragonioides* L.); osivo zakoupeno od společnosti SEMO, a.s.
- Špenát setý (*Spinacia oleracea* L. var. Matador); osivo zakoupeno od společnosti SEMPRA Praha, a.s.

4. 2. Použité steroidy

- 24-epibrassinolid (24E) neboli (22*R*,23*R*,24*R*)-2α,3α,22,23-tetrahydroxy-24-methyl-7-oxa-7α-homo-5α-cholestan-6-on; dodal Dr. L. Kohout, ÚOCHAB AV ČR, v.v.i.
- Syntetický androstanový analog castasteronu (AAC) neboli 2α,3α,17β-trihydroxy-5α-androstan-6-on; dodal Dr. L. Kohout, ÚOCHAB AV ČR, v.v.i.
- 20-hydroxyekdyson (20E) neboli (2β,3β,14α,20R,22R,25-hexahydroxy-5β-cholest-7-en-6-one); zakoupeno od Sigma Aldrich

4. 3. Výčet použitých metod

- Měření morfologických, růstových, reprodukčních a výnosových charakteristik rostlin (výška, počet internodií a listů, rozměry listů, specifická hmotnost listu, hmotnost sušiny nadzemní a podzemní části rostliny, sledování vývoje celých rostlin, u kukuřice i samčích i samičích květenství, různé parametry výnosu)
- Stanovení relativního obsahu vody v listu
- Izolace fotosynteticky aktivních chloroplastů z listů a polarografické měření aktivity Hillovy reakce a aktivity fotosystému (PS) 1 v izolovaných chloroplastech
- Polarografické měření rychlosti čisté fotosyntézy na listových terčících
- Gazometrické měření (infračervený analyzátor plynů) rychlosti čisté fotosyntézy, transpirace, vodivosti průduchů a intercelulární koncentrace CO_2
- Spektrofotometrické stanovení obsahu fotosyntetických pigmentů – chlorofyl *a*, chlorofyl *b*, celkové karotenoidy
- Analýza primárních fotosyntetických procesů pomocí fluorescence chlorofylu (fluorescenční přechodové jevy – OJIP analýza)
- Statistická analýza (analýza variance, parametrické i neparametrické *post hoc* testy)

5. Výsledky

5.1. Vliv brassinosteroidů na růstové, vývojové, reprodukční a fotosyntetické charakteristiky rostlin kukuřice rostoucích v polních podmínkách

Toto téma bylo zpracováno v následujících publikacích:

Holá D., Rothová O., Kočová M., Kohout L., Kvasnica M. (2010): The effect of brassinosteroids on the morphology, development and yield of field-grown maize. *Plant Growth Regulation*. 61: 29-43. IF 1,670

Kočová M., Rothová O., Holá D., Kvasnica M., Kohout L. (2010): The effects of brassinosteroids on photosynthetic parameters in leaves of two field-grown maize inbred lines and their F1 hybrid. *Biologia Plantarum* 54: 785-788. IF 1,692

První z publikací byla pilotní studií v naší laboratoři. Shrnuje výsledky čtyřletých polních pokusů, při nichž jsme studovali vliv ošetření rostlin roztoky 24E a AAC o čtyřech koncentracích (10^{-8}, 10^{-10}, 10^{-12}, 10^{-14} M). Dva rodičovské inbrední genotypy kukuřice (2023 a CE704) a jejich kříženec (2023×CE704) jsme ošetřili postřikem do vrcholové růžice listů, a to ve dvou různých vývojových stádiích (V3/4 a V6/7). Ošetření rostlin v mladším vývojovém stádiu způsobilo prodloužení listů od sedmého do desátého včetně, při stejném ošetření ve starším vývojovém stádiu rostlin byl efekt opačný. Rychlost nástupu reprodukční fáze byla různá v závislosti na použitém BR (24E, AAC) a na době, kdy byly BR aplikovány. Naše výsledky ukázaly, že ošetření rostlin v časné fázi vývoje spíše zpomalovalo nástup kvetení, při ošetření v pozdějším stádiu vývoje jsme naopak zaznamenali rychlejší nástup tvorby květů oproti kontrole. Pozorovali jsme rovněž zvýšení počtu sekundárních samičích květenství, které bylo velmi výrazné u rostlin F1 generace, u rodičovských genotypů byly rozdíly závislé na použité látce i její koncentraci. Stejně tak při měření výnosových parametrů byl vliv ošetření BRs výrazný. Závisel však na všech sledovaných proměnných, tj. na typu BR i jeho koncentraci, na vývojovém stádiu rostliny při ošetření i na genotypu rostlin.

Vliv ošetření BRs na obsahy fotosyntetických pigmentů (chlorofylů a karotenoidů) ani na aktivitu Hillovy reakce (která je měřítkem aktivity PS2) nebo aktivitu PS1 se statisticky průkazně neprojevil. Toto téma je zpracované v druhé z uvedených publikací. Vliv BRs na PS1 před uveřejněním tohoto článku nebyl studován, ačkoliv je tento fotosystém velmi důležitou složkou primárních fotosyntetických procesů. Tento výsledek tedy považujeme za velmi důležitý.

Plant Growth Regul (2010) 61:29–43
DOI 10.1007/s10725-010-9446-0

ORIGINAL PAPER

The effect of brassinosteroids on the morphology, development and yield of field-grown maize

Dana Holá · Olga Rothová · Marie Kočová · Ladislav Kohout · Miroslav Kvasnica

Received: 27 July 2009 / Accepted: 4 January 2010 / Published online: 14 January 2010

Abstract The response of two field-grown inbred lines of maize (*Zea mays* L.) and their F1 hybrid to the application of 10^{-8}–10^{-14} M solutions of 24-epibrassinolide or synthetic androstane analogue of castasterone in V3/4 and V6/7 developmental stages was followed during the vegetative and early reproductive phases of plant development. Brassinosteroids (BRs) significantly affected (either positively or negatively, depending on the genotype and the developmental stage they were applied) the height of plants during the early weeks after their application, but not the final plant height nor the number of leaves. Spraying of plants with BRs in V3/4 developmental stage usually also increased the length of the 7th to 10th leaf, whereas the application in V6/7 developmental stage had the opposite effect. The beginning of the reproductive phase of plant development and the course of flowering was strongly influenced by the application of BRs. Treatment of plants in V3/4 stage delayed and treatment of plants in V6/7 stage advanced the dates of anthesis and silking, regardless of the type of BR used, its concentration or plant genotype. The influence of BRs on the development of the secondary ear was the least pronounced in the F1 hybrid; in both inbred lines it strongly depended on the concentrations of BRs used. Various yield parameters were also affected by treatment of plants with BRs, but this effect depended on the developmental stage during which the application of BRs occured, the plant genotype, the type of BR and its concentration.

Keywords Flowering · Genotypes · Growth · *Zea mays* L

D. Holá (✉) · O. Rothová · M. Kočová
Department of Genetics and Microbiology, Charles University in Prague, Faculty of Science, Viničná 5, 128 43 Prague, Czech Republic
e-mail: danahola@natur.cuni.cz

L. Kohout · M. Kvasnica
Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i., Flemingovo sq. 2, 166 10 Prague, Czech Republic

Introduction

Since their discovery in 1979, brassinosteroids (BRs) have become the focus of study of many scientists working in the field of plant physiology, molecular biology, agriculture and even medicine. This is in a large part due to the wide range of biological processes these steroidal compounds can regulate. BRs not only affect both cell division and cell elongation, having thus a significant influence on growth and development (Clouse and Sasse 1998; Haubrick and Assmann 2006; Kartal et al. 2009; Rao et al. 2002; Sasse 2003), but participate also in the induction of the differentiation of plant vascular system (Clouse and Sasse 1998; Sasse 2003), play an important role in the regulation of senescence (Clouse and Sasse 1998; Rao et al. 2002), induce the pollen tube growth (Clouse and Sasse 1998), affect seed germination (Haubrick and Assmann 2006; Özdemir et al. 2004; Rao et al. 2002; Sharma and Bhardwaj 2007; Vardhini and Rao 2003) and induce the formation of adventitious roots (Rao et al. 2002). They are also involved in the regulation of cell wall biosynthesis (Sasse 2003), activation of proton pumps in cellular membranes (Cerrana et al. 1984), they enhance DNA and RNA levels (Vardhini and Rao 1998; Swamy and Rao 2008) and influence the phosphorylation of proteins (Fedina et al. 2008). In plants subjected to stress conditions, BRs affect (positively or negatively) the activity of antioxidant enzymes, synthesis of various osmoprotectants,

synthesis of heat shock proteins, the fatty acid composition of membrane lipids, etc. (Ali et al. 2007, 2008a; Arora et al. 2008; Hayat et al. 2007; Janeczko et al. 2009; Kartal et al. 2009; Mazorra et al. 2002; Özdemir et al. 2004; Shahbaz et al. 2008; Singh and Shono 2005). Photosynthesis, transpiration and nitrogen fixation in plants are also influenced by BRs (Ali et al. 2008b; Fariduddin et al. 2003; Farooq et al. 2009; Hayat et al. 2000; Li et al. 2008; Sairam 1994a, b; Swamy and Rao 2008; Yu et al. 2004), as well as the distribution of assimilates to various plant organs (Fujii and Saka 2001).

The availability of a wide range of synthetic methods for the production of both natural BRs and their synthetic analogues with high biological activity, together with the very low concentrations needed for the manifestation of their effect on plants, make BRs logical candidates for the agricultural application. Exogenously applied BRs have long been known to increase growth and yield in many economically useful plant species. In cereals, BRs promote the number of ears, and in some cases also their length, and the number and weight of kernels per ear (Ali et al. 2008b; Hnilička et al. 2007; Ramraj et al. 1997; Rao et al. 2002; Sairam 1994a, b; Takematsu and Takeuchi 1989; Torres-Ruiz et al. 2007). In leguminous crops, the number of pods per plant and total seed yield increases after the application of exogenous BRs (Hayat and Ahmad 2003; Ramraj et al. 1997; Rao et al. 2002; Takematsu and Takeuchi 1989; Vardhini and Rao 1998). Growth and seed yield of mustard or rapeseed plants is also promoted by their treatment with BRs (Hayat et al. 2000, 2001b; Ramraj et al. 1997; Rao et al. 2002; Takematsu and Takeuchi 1989); the same applies for seed yield in cotton (Ramraj et al. 1997).

Though BRs generally have a positive influence on growth, physiological and yield parameters of plants, their effect depends on various internal and external factors. Many BRs and BRs′ analogues that showed high biological activity in bioassays or controlled-environment experiments failed to stimulate plants grown in the field conditions. This can be explained by various reasons. The time of BRs application (in relation to the developmental stage of plant) has been shown to have an important effect on plant response to BRs treatment (Amzallag 2002; Khripach et al. 2000; Núñez et al. 2003; Ramraj et al. 1997; Sasse 2003). The length of plant exposure to BRs, the frequency of application, the application mode and the type and dose of BRs can also substantially affect the growth/yield promoting activity of these compounds (Fariduddin et al. 2003, 2008; Khripach et al. 2003; Núñez et al. 2003; Ramraj et al. 1997; Sasse 2003; Vlašánková et al. 2009). The degree of BRs influence on plants depends also on plant species and genotype (Ali et al. 2008b; Hnilička et al. 2007; Kang et al. 2007; Khripach et al. 2003; Núñez et al. 2003; Ramraj et al. 1997; Sairam 1994b; Shahbaz et al. 2008; Torres-Ruiz et al. 2007; Vardhini and Rao 2003; Vlašánková et al. 2009; Zhao and Chen 2003).

The purpose of this study was to evaluate the effect of the exogenous application of two types of BRs (the naturally occurring 24-epibrassinolide and the synthetic androstane analogue of castasterone) on the growth, development and yield of field-grown maize plants, and to examine the dependence of this effect on plant genotype, the developmental stage during which the application of BRs occured, and the concentration of BRs.

Materials and methods

Plant material and BRs treatments

Kernels of two dent maize (*Zea mays* L.) inbred lines, 2023 and CE704, and their F1 hybrid 2023 × CE704 (2023 being the maternal parent) were obtained from the Maize Breeding Station CEZEA in Čejč (the Czech Republic). The inbred line CE704 is a poorly producing genotype with short ears and small, pale yellow kernels; it is also characterized by a small height, a rather compact stature, light-green leaves prone to a development of small lesions, and male inflorescences without or with a low number of branches. The inbred line 2023 shows a slightly delayed development (approx. 3-days lag compared to the other two genotypes). The F1 hybrid displays a positive heterosis for both yield and morphological parameters.

Field experiments were conducted at the cultivation grounds of the Department of Genetics and Microbiology, Faculty of Science, Charles University in Prague, the Czech Republic (50°04′N, 14°25′E, altitude 238 m) during the 2004–2007 years. The meteorological conditions of each experimental season are shown in Table 1. Kernels were first sown into low dishes filled with garden soil and placed in glass-covered hot-beds at the end of the April. After approx. 4 weeks, the seedlings were transplanted into the field in a regular pattern (the distance between rows 70 cm, the distance between plants in rows 50 cm). The field was always pre-fertilized with horse manure but no additional fertiliser or other agrochemical was applied during the growing seasons. When necessary, plants were artificially irrigated and manually weeded.

BRs treatments were applied either 41 or 55 days after the date of sowing, representing the early (ET) or later (LT) vegetative developmental stages. Due to the differences among genotypes, the inbred line 2023 was in V3 and V6 stages, whereas the other two genotypes were in V4 and V7 stages for ET and LT, respectively. Foliage of plants was sprayed with 10^{-8}, 10^{-10}, 10^{-12} or 10^{-14} M aqueous BRs solutions or with tap water (control); care was taken to avoid possible contamination. BRs (in the amount

Table 1 Selected meteorological data for the 2004–2007 experimental seasons

Month	Average air temperature (°C)				Total precipitation (mm)				Total sunshine duration (h)			
	2004	2005	2006	2007	2004	2005	2006	2007	2004	2005	2006	2007
April	11.1	11.8	10.3	13.2	9.4	22.9	48.4	1.5	201.8	206.3	167.6	295.3
May	13.6	15.4	15.2	16.6	35.7	42.3	83.5	41.4	198.7	257.3	237.0	253.4
June	17.6	18.5	19.5	20.3	83.2	55.6	55.5	53.9	200.8	247.4	276.0	248.9
July	19.8	20.0	24.7	20.2	26.7	121.2	7.3	49.3	238.2	222.9	345.0	236.9
August	20.8	18.1	17.4	19.4	50.9	61.0	67.1	86.5	244.7	203.8	148.4	234.7
September	15.7	16.7	18.6	13.0	32.6	29.2	11.4	63.8	210.4	211.4	245.1	161.6

representing 10^{-5} M) were initially dissolved in 1 cm^3 of ethanol; to obtain 10^{-4} M stock aqueous solution, 1 cm^3 of dimethyl sulfoxide and 98 cm^3 distilled water was then added. This solution was diluted with tap water to the appropriate concentrations. Two different BRs were tested: 24-epibrassinolide (E), i.e. (22*R*,23*R*,24*R*)-2α,3α,22,23-tetrahydroxy-24-methyl-7a-oxa-5α-cholestan-6-one (according to the IUPAC nomenclature), synthesized as described in Kohout (1994), and synthetic androstane analogue of castasterone (A), i.e. 2α,3α,17β-trihydroxy-5α-androstan-6-one, the use of which was patented—see EP 1401278, ES 2250658, DE 60206379.12, CZ 294343, PL 364495, UA 74760, etc. Experiments were arranged in a split split-plot design with two replicates (main plots = time of BRs application, subplots = genotypes, sub-subplots = BRs treatments). Each subplot consisted of 9 rows with 9 plants per row, the layout of each type of plots in the field was random.

Plant morphology and development

The number of fully developed leaves, the height of whole plant (i.e. the distance from the ground to the ligule of the youngest fully developed leaf or, at the end of experimental season, to the flag leaf) and the length of individual leaves were monitored regularly in 1-week intervals beginning with the day of BRs treatment as Day 0. The date of anthesis (functional maturity) for both male and female flowers was also recorded; anthesis for male flowers being defined as the beginning of pollen shed from the tassel (male inflorescence), whereas for female flowers it represented the first appearance of stigmas (silks) from within the surrounding husks on the primary ear (female inflorescence) and is referred to as silking (Bolaños and Edmeades 1996). The anthesis-silking interval (ASI) was calculated as the difference between these dates. The date of silking and the length of ASI was recorded also for the secondary ear.

The development of each inflorescence was further followed by subdividing it into 3 or 4 stages. For the tassel, the stage 1 was defined as its first visible appearance inside the apical whorl of leaves (the lateral branches still nestled against the central stem), the stage 2 as its full emergence and branching (i.e. the VT stage, Ritchie et al. 1993), and the stage 3 as the anthesis. For the ear, the stage 1 was defined as its first visible appearance in the axil of a leaf (silks still sheltered by husks), the stage 2 as the silking (i.e. the R1 stage, Ritchie et al. 1993), at the stage 3, the silks were fully extended and moist (with most pollen being captured), whereas the stage 4 was characterized by darkening and drying of silks (i.e. the R2 stage, Ritchie et al. 1993). These stages were monitored regularly every second day for the tassel and the primary, secondary and tertiary (when present) ears on each plant. At the end of the flowering period, the total number of visible ears per plant was also recorded.

Yield parameters

At the end of September, the ears from all plants were harvested, allowed to dry out, dehusked and the following parameters were determined for the individual plants: the number of fertile ears (i.e. ears with kernels) per plant, the dry weight of whole ear and cob, the shelling percentage (i.e. 100 × dry weight of shelled kernels/dry weight of whole ear), the dry weight of 100 kernels, the length of whole ear, the number of kernel rows per ear and the number of kernels per row.

Statistical analysis

The data from all four seasons were pooled together and subjected to two-way ANOVA with interactions (BRs treatments, genotypes and the genotype × treatment interactions as the possible sources of variation). Each genotype was then analyzed by one-way ANOVA followed by the LSD test with 0.05 level of probability to determine the statistical significancy of the differences among individual BRs treatments. All statistical evaluations were made with the CoStat (Version 6.204) statistical software (CoHort Software, Monterey, CA, the U.S.A.).

Results

Plant morphology

The differences among genotypes in the height of whole plant started to be pronounced from the V3 stage onwards and were statistically significant (Table 2). At the end of the vegetative stage of development, the height of the F1 hybrid exceeded that of the inbred line 2023 by approx. 20 cm and the inbred line CE704 was by yet additional approx. 10 cm shorter. BRs treatment did not significantly affect this final plant height (Table 2), but the height of the BRs-sprayed plants in some cases differed from that of the control plants during the early weeks after BRs treatment (Fig. 1). The inbred line 2023 was the most positively affected one among the three examined genotypes, particularly after its treatment with E (regardless of the concentration used) in LT stage or with 10^{-14} M solution of E in ET stage (Fig. 1d–f). On the other hand, LT plants of the inbred line CE704 sprayed with 10^{-12} M solution of A slowed their growth during the early weeks after BRs treatment (Fig. 1g). Treatment of plants of this inbred line in ET stage with either E or A also resulted in the lower height of plants during the first 3 weeks after treatment, and similar, even more pronounced effect was observed also for the F1 hybrid (Fig. 1a–d).

Similarly to their differences in the plant height, the three examined genotypes significantly (Table 2) differed in the number of leaves (15–16 in the F1 hybrid, 14–15 in the CE704 and 13–14 in the 2023 inbred lines, not counting the flag leaf). The number of leaves was affected by BRs treatment even less than plant height (Fig. 2). On the other hand, treatment of plants with BRs significantly affected the length of individual leaves, particularly those of lower insertion levels (Table 2). Generally, spraying of plants in ET stage with BRs increased whereas spraying in LT stage decreased the length of leaves 7 to 10, with A usually being the most effective BR (Table 3). The length of other leaves was not significantly affected by BRs treatment and all three genotypes responded to BRs treatment more-or-less similarly (Tables 2, 3).

Inflorescence development and flowering dynamics

Statistically significant differences both among genotypes and among BRs treatments were found for all examined

Table 2 The levels of statistical significance (P) from two-way ANOVA of data for various morphological, developmental and yield parameters of maize

	Treatments (T)	Genotypes (G)	T × G interaction
Plant height	0.234	0	0.984
Number of leaves	0.151	0	0.901
Length of leaf 6	0.009	0	0.975
Length of leaf 7	0	0	0.999
Length of leaf 8	0	0	0.999
Length of leaf 9	0.001	0	1.000
Length of leaf 10	0.001	0	0.999
Length of leaf 11	0.004	0	1.000
Length of leaf 12	0.025	0	1.000
Length of leaf 13	0.218	0	0.997
Length of leaf 14	0.609	0	0.783
Date of the tassel anthesis	0	0	1.000
Date of the primary ear silking	0	0	0.998
Date of the secondary ear silking	0	0	0.589
Anthesis-silking interval for the primary ear	0	0	0.913
Anthesis-silking interval for the secondary ear	0.008	0	0.776
Number of ears per plant at the end of the flowering	0	0	0.211
Number of fertile ears per plant	0.077	0	0.079
Ear length	0	0	0.003
Number of kernel rows per ear	0	0	0.845
Number of kernels per row	0.182	0	0.712
Dry weight of the whole ear	0.198	0	0.780
Dry weight of the cob	0.134	0	0.112
Dry weight of 100 kernels	0.298	0	0.061
Shelling percentage	0.005	0	0.672

Brassinosteroid treatments, genotypes and their interaction were included as possible sources of variation

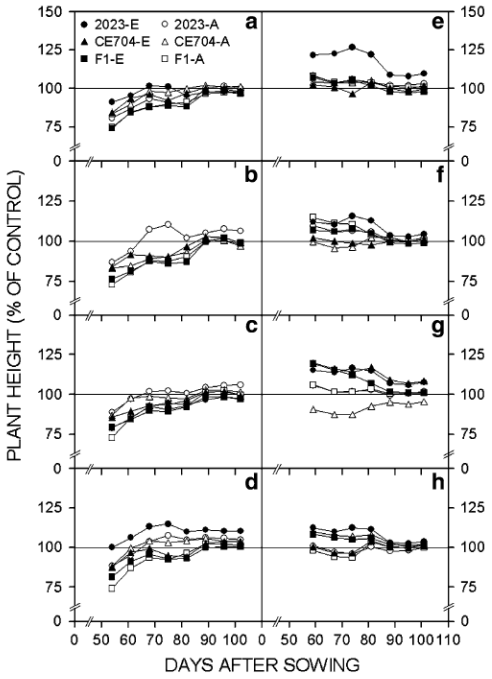


Fig. 1 The changes in the plant height during the development of three maize genotypes (inbred lines 2023 and CE704, F1 hybrid 2023 × CE704) treated by foliar spray with 10^{-8} M (**a**, **e**), 10^{-10} M (**b**, **f**), 10^{-12} M (**c**, **g**) or 10^{-14} M (**d**, **h**) aqueous solutions of 24-epibrassinolide (E) or synthetic androstane analogue of castasterone (A) either 41 (ET stage, **a**–**d**) or 55 (LT stage, **e**–**h**) days after sowing. The mean values calculated from the pooled results of four experimental seasons are shown as the percentage of control (i.e. plants treated with tap water only)

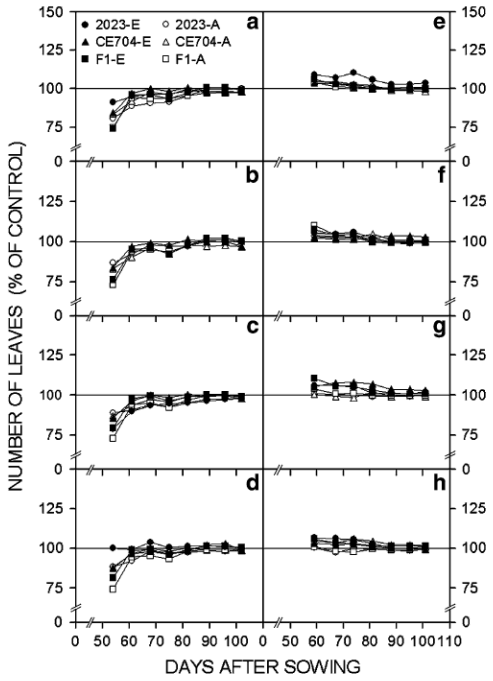


Fig. 2 The changes in the number of fully developed leaves during the development of three maize genotypes (inbred lines 2023 and CE704, F1 hybrid 2023 × CE704) treated by foliar spray with 10^{-8} M (**a**, **e**), 10^{-10} M (**b**, **f**), 10^{-12} M (**c**, **g**) or 10^{-14} M (**d**, **h**) aqueous solutions of 24-epibrassinolide (E) or synthetic androstane analogue of castasterone (A) either 41 (ET stage, **a**–**d**) or 55 (LT stage, **e**–**h**) days after sowing. The mean values calculated from the pooled results of four experimental seasons are shown as the percentage of control (i.e. plants treated with tap water only)

parameters associated with plant flowering (Table 2). The inbred line 2023 showed a delayed silking for both primary (approx. 4 days later compared to the F1 hybrid and the CE704 inbred) and secondary (approx. 6 days later compared to the F1 hybrid and 2 days later compared to the CE704 inbred) ears, and the highest ASI among all three genotypes examined (Table 4). The anthesis of male flowers started approx. 2 days earlier in the F1 hybrid compared to its parental inbreds, which affected the length of its ASI (Table 4). The percentage of plants that developed secondary or tertiary ears was also much lower in both inbreds (approx. 50 or 8%, respectively, compared to approx. 95 or 40% in the F1 hybrid) (Table 5).

BRs treatments significantly and strongly affected the dates of male anthesis and silking (counted as DAS). Generally, treatment of plants in ET stage delayed and treatment of plants in LT stage advanced these dates, regardless of the type of BR used, its concentration or plant genotype. The changes in the length of the period till the start of male anthesis could amount up to 4 days, the changes in the length of the period till the start of primary ear silking up to 6 days (in LT plants of the 2023 inbred line) and the silking of the secondary ear could be advanced or delayed by BRs treatment by 2–9 days, depending on the genotype and the type of BRs treatment (Table 4). Consequently, the length of ASI for the primary ear increased in ET plants by 0.5–1 days and decreased in LT plants by 0.5–1.5 days, with the 2023 inbred line and the F1 hybrid being the most affected genotypes (Table 4). The effect of BRs on the length of ASI for the secondary ear could be examined in more detail only for the F1 hybrid, as about 50% of plants of both inbred lines did not develop this ear, but the changes were similar to those observed for the primary ear ASI (data not shown).

The examination of the progressive development of inflorescences revealed that BRs treatment had the highest impact in the earliest phases of the tassel (Fig. 3) and the primary ear (Fig. 4) development but mostly in the later phases of the secondary ear development (Fig. 5). The development of the primary ear was most affected in the

Table 3 The effect of 24-epibrassinolide (E) and synthetic androstane analogue of castasterone (A) on the length of leaves 7–10 in three maize genotypes (inbred lines 2023 and CE704, F1 hybrid 2023 × CE704)

		Leaf 7 length (cm)			Leaf 8 length (cm)			Leaf 9 length (cm)			Leaf 10 length (cm)		
		2023	CE704	F1 hybrid	2023	CE704	F1 hybrid	2023	CE704	F1 hybrid	2023	CE704	F1 hybrid
Control		46.33 ± 1.51	39.65 ± 2.15	52.52 ± 2.04	50.69 ± 1.47	44.97 ± 1.57	61.07 ± 1.93	51.54 ± 1.44	49.68 ± 1.42	66.56 ± 1.68	50.40 ± 1.34	52.44 ± 1.38	68.51 ± 1.44
ET	E 10^{-8} M	50.11 ± 1.56	38.16 ± 1.65	52.42 ± 2.06	54.32 ± 1.65	45.68 ± 1.94	60.26 ± 2.06	54.53 ± 1.72	50.84 ± 1.98	65.26 ± 1.84	53.05 ± 1.67	53.16 ± 1.87	67.00 ± 1.58
	E 10^{-10} M	48.05 ± 1.67	37.16 ± 1.86	52.42 ± 2.21	51.79 ± 2.00	43.74 ± 1.93	61.11 ± 2.23	52.72 ± 2.22	49.79 ± 2.09	66.05 ± 2.42	50.21 ± 2.25	51.47 ± 2.14	68.16 ± 2.36
	E 10^{-12} M	48.00 ± 1.45	35.47 ± 1.64	50.32 ± 1.88	51.32 ± 1.74	43.32 ± 1.70	59.16 ± 2.23	51.37 ± 2.00	49.21 ± 1.88	65.26 ± 2.10	49.74 ± 2.05	52.42 ± 1.94	68.21 ± 1.99
	E 10^{-14} M	49.68 ± 1.75	37.42 ± 2.05	49.72 ± 2.08	54.32 ± 1.93	45.68 ± 1.76	58.22 ± 2.18	56.58 ± 2.19	51.89 ± 1.74	65.22 ± 2.25	55.53 ± 2.39	56.11 ± 1.53	68.83 ± 2.09
	A 10^{-8} M	49.44 ± 1.80	41.06 ± 1.78	53.21 ± 2.33	52.28 ± 1.87	45.94 ± 2.09	60.42 ± 2.32	52.11 ± 1.83	48.78 ± 2.10	64.95 ± 2.12	49.89 ± 1.74	49.11 ± 1.62	66.58 ± 2.12
	A 10^{-10} M	49.74 ± 2.11	39.79 ± 2.03	54.21 ± 2.56	54.26 ± 2.10	46.47 ± 2.02	60.42 ± 2.92	55.21 ± 2.21	49.68 ± 2.05	64.58 ± 2.58	53.37 ± 2.31	51.53 ± 2.08	66.21 ± 2.29
	A 10^{-12} M	51.37 ± 2.04	38.89 ± 2.19	54.68 ± 2.63	55.37 ± 2.03	46.26 ± 2.08	61.63 ± 2.53	55.89 ± 2.12	52.42 ± 2.13	66.32 ± 2.24	53.53 ± 1.90	55.00 ± 2.15	67.89 ± 1.93
	A 10^{-14} M	48.58 ± 2.16	39.84 ± 2.03	55.21 ± 2.63	52.42 ± 2.27	46.53 ± 2.26	62.26 ± 2.44	52.63 ± 2.08	51.00 ± 2.36	67.63 ± 2.12	50.58 ± 1.94	52.89 ± 2.12	69.00 ± 1.95
LT	E 10^{-8} M	41.33 ± 1.15	34.00 ± 3.07	43.50 ± 2.10	50.10 ± 2.71	40.50 ± 2.58	56.64 ± 2.96	53.60 ± 2.12	46.14 ± 2.17	63.53 ± 2.23	54.07 ± 2.08	50.75 ± 2.37	67.81 ± 2.13
	E 10^{-10} M	44.25 ± 2.53	30.29 ± 1.43	43.00 ± 2.12	51.08 ± 3.07	41.10 ± 2.95	56.60 ± 3.75	50.94 ± 2.73	47.71 ± 3.56	62.93 ± 2.77	49.19 ± 2.62	50.13 ± 3.54	66.44 ± 2.08
	E 10^{-12} M	43.00 ± 1.88	34.00 ± 2.35	49.20 ± 6.27	48.75 ± 2.49	42.50 ± 2.08	58.63 ± 3.63	50.93 ± 2.28	49.94 ± 2.03	67.38 ± 2.03	50.19 ± 2.31	54.00 ± 2.05	70.38 ± 1.46
	E 10^{-14} M	44.22 ± 1.69	38.14 ± 3.54	44.80 ± 4.07	47.92 ± 1.69	42.91 ± 2.46	56.50 ± 3.28	50.00 ± 1.98	47.13 ± 2.03	64.21 ± 2.52	48.50 ± 2.19	49.06 ± 2.29	65.50 ± 2.04
	A 10^{-8} M	43.44 ± 2.08	32.60 ± 1.91	49.33 ± 4.47	45.92 ± 1.61	42.08 ± 2.36	55.36 ± 2.49	47.25 ± 1.72	48.07 ± 1.92	63.13 ± 2.71	46.31 ± 1.96	51.00 ± 2.06	65.31 ± 2.81
	A 10^{-10} M	39.43 ± 2.15	29.83 ± 2.12	44.83 ± 2.51	46.71 ± 2.49	39.90 ± 2.75	49.63 ± 2.71	48.13 ± 2.33	45.07 ± 2.12	58.50 ± 2.72	47.25 ± 2.21	49.25 ± 2.30	62.81 ± 2.90
	A 10^{-12} M	43.25 ± 1.71	33.63 ± 2.46	47.67 ± 4.41	48.46 ± 1.90	38.33 ± 2.48	57.77 ± 3.42	49.00 ± 1.83	45.13 ± 2.67	61.57 ± 3.25	47.69 ± 2.25	48.00 ± 2.59	63.94 ± 2.70
	A 10^{-14} M	45.10 ± 1.81	32.33 ± 2.29	47.00 ± 8.34	47.75 ± 2.11	40.00 ± 2.88	58.83 ± 3.45	49.94 ± 2.47	47.13 ± 3.14	63.44 ± 2.48	47.94 ± 2.69	49.75 ± 3.05	65.44 ± 2.34
LSD ($P = 0.05$)		8.44	10.09	14.19	7.36	7.51	10.29	6.31	6.81	7.55	6.41	6.85	6.31

Plants were sprayed with aqueous solutions of brassinosteroids (concentrations 10^{-8} to 10^{-14} M) or tap water (control) either early (ET) or later (LT) during vegetative phase of their development. The mean values ± SEM calculated from the pooled results of four experimental seasons are shown together with the LSD values significant at $P = 0.05$

Table 4 The effect of 24-epibrassinolide (E) and synthetic androstane analogue of castasterone (A) on the date of anthesis and silking (counted as days after sowing, DAS), and on the anthesis-silking interval (ASI) for the primary ear in three maize genotypes (inbred lines 2023 and CE704, F1 hybrid 2023 × CE704)

		Date of tassel anthesis (DAS)			Date of primary ear silking (DAS)			Date of secondary ear silking (DAS)			ASI (d)		
		2023	CE704	F1 hybrid	2023	CE704	F1 hybrid	2023	CE704	F1 hybrid	2023	CE704	F1 hybrid
Control		84.73 ± 0.70	84.20 ± 0.72	82.88 ± 0.60	88.07 ± 0.79	84.76 ± 0.65	84.08 ± 0.60	93.56 ± 2.06	91.64 ± 1.98	87.37 ± 0.69	2.37 ± 0.36	0.65 ± 0.32	1.20 ± 0.26
ET	E 10^{-8} M	87.02 ± 0.98	86.25 ± 0.88	85.40 ± 0.93	91.92 ± 1.05	87.94 ± 0.84	87.45 ± 0.89	95.07 ± 2.39	93.83 ± 1.31	90.52 ± 0.87	3.59 ± 0.48	1.47 ± 0.35	2.06 ± 0.37
	E 10^{-10} M	87.50 ± 1.00	85.98 ± 0.85	85.67 ± 0.85	91.43 ± 1.00	87.88 ± 0.79	87.59 ± 0.82	100.07 ± 1.78	93.06 ± 0.88	90.45 ± 0.77	3.07 ± 0.52	1.61 ± 0.36	1.93 ± 0.32
	E 10^{-12} M	86.96 ± 0.91	86.20 ± 0.88	85.02 ± 0.78	91.02 ± 1.05	88.62 ± 0.90	87.31 ± 0.73	97.95 ± 1.75	93.94 ± 1.53	91.39 ± 0.69	3.04 ± 0.47	1.88 ± 0.32	2.30 ± 0.35
	E 10^{-14} M	86.19 ± 0.77	86.32 ± 0.93	85.20 ± 0.75	89.85 ± 0.73	87.22 ± 0.81	87.16 ± 0.63	95.11 ± 1.51	92.33 ± 1.04	90.34 ± 0.68	3.45 ± 0.37	1.00 ± 0.37	1.97 ± 0.37
	A 10^{-8} M	88.07 ± 1.05	87.08 ± 0.93	85.71 ± 0.81	92.47 ± 1.00	88.40 ± 0.85	87.60 ± 0.75	98.69 ± 1.45	91.69 ± 2.22	91.22 ± 0.80	1.92 ± 0.48	1.80 ± 0.39	1.89 ± 0.31
	A 10^{-10} M	87.02 ± 1.00	86.55 ± 0.86	85.73 ± 0.83	91.34 ± 0.95	88.27 ± 0.77	87.47 ± 0.69	96.29 ± 1.44	92.42 ± 1.94	89.98 ± 0.75	2.93 ± 0.43	1.83 ± 0.40	1.75 ± 0.43
	A 10^{-12} M	86.81 ± 0.87	86.08 ± 0.86	86.17 ± 0.87	91.17 ± 0.96	87.44 ± 0.82	87.61 ± 0.77	98.28 ± 1.40	91.81 ± 1.19	91.35 ± 0.84	2.56 ± 0.46	1.00 ± 0.41	1.44 ± 0.31
	A 10^{-14} M	87.16 ± 0.78	85.75 ± 0.72	84.91 ± 0.79	90.24 ± 0.86	86.96 ± 0.61	86.56 ± 0.65	95.15 ± 1.57	91.79 ± 1.18	89.40 ± 0.71	3.20 ± 0.44	1.22 ± 0.35	1.65 ± 0.33
LT	E 10^{-8} M	81.33 ± 0.61	80.74 ± 0.73	79.00 ± 0.49	82.06 ± 0.78	80.67 ± 0.65	79.39 ± 0.54	84.83 ± 1.38	84.60 ± 1.80	83.13 ± 0.89	0.97 ± 0.51	0.21 ± 0.43	0.39 ± 0.34
	E 10^{-10} M	81.06 ± 0.47	80.83 ± 0.73	78.84 ± 0.42	82.81 ± 0.76	80.07 ± 0.80	79.46 ± 0.52	86.63 ± 1.61	84.40 ± 2.66	83.76 ± 0.87	1.69 ± 0.56	−0.77 ± 0.40	0.62 ± 0.28
	E 10^{-12} M	81.08 ± 0.56	80.79 ± 0.60	79.08 ± 0.45	82.44 ± 0.77	81.39 ± 0.72	79.75 ± 0.61	86.50 ± 1.95	84.38 ± 1.55	83.30 ± 0.74	1.60 ± 0.49	0.47 ± 0.40	0.67 ± 0.38
	E 10^{-14} M	81.44 ± 0.64	80.88 ± 0.53	79.00 ± 0.44	82.33 ± 0.71	80.94 ± 0.59	79.19 ± 0.64	89.20 ± 2.15	82.38 ± 1.90	83.14 ± 0.73	1.28 ± 0.49	0.16 ± 0.40	0.19 ± 0.40
	A 10^{-8} M	81.58 ± 0.63	80.67 ± 0.66	79.97 ± 0.57	82.54 ± 0.90	81.35 ± 0.82	79.78 ± 0.53	86.29 ± 1.74	84.45 ± 1.11	82.87 ± 0.67	1.26 ± 0.50	0.64 ± 0.46	−0.19 ± 0.34
	A 10^{-10} M	81.44 ± 0.59	81.18 ± 0.68	79.16 ± 0.52	83.32 ± 0.91	81.55 ± 0.83	79.62 ± 0.66	87.00 ± 1.37	85.43 ± 2.34	82.94 ± 0.62	1.69 ± 0.58	0.50 ± 0.47	0.46 ± 0.38
	A 10^{-12} M	82.71 ± 0.59	82.00 ± 0.70	79.42 ± 0.46	84.26 ± 0.82	82.00 ± 0.79	80.11 ± 0.66	85.00 ± 2.43	87.08 ± 1.48	82.88 ± 0.70	1.76 ± 0.57	0.11 ± 0.54	0.69 ± 0.39
	A 10^{-14} M	81.50 ± 0.51	81.00 ± 0.70	79.51 ± 0.48	83.00 ± 0.75	80.74 ± 0.67	80.08 ± 0.54	87.25 ± 1.67	85.50 ± 3.06	82.80 ± 0.77	1.71 ± 0.51	0.26 ± 0.43	0.57 ± 0.39
LSD ($P = 0.05$)		2.63	2.93	2.40	3.05	2.69	2.29	7.54	7.52	2.64	1.55	1.37	1.14

Plants were sprayed with aqueous solutions of brassinosteroids (concentrations 10^{-8} to 10^{-14} M) or tap water (control) either early (ET) or later (LT) during vegetative phase of their development. The mean values ± SEM calculated from the pooled results of four experimental seasons are shown together with the LSD values significant at $P = 0.05$

Table 5 The effect of 24-epibrassinolide (E) and synthetic androstane analogue of castasterone (A) on the number of female inflorescences (ears) in three maize genotypes (inbred lines 2023 and CE704, F1 hybrid 2023 × CE704)

		% of plants with primary ear			% of plants with secondary ear			% of plants with tertiary ear			Number of ears per plant		
		2023	CE704	F1 hybrid	2023	CE704	F1 hybrid	2023	CE704	F1 hybrid	2023	CE704	F1 hybrid
Control		95.65	96.55	98.92	48.91	52.87	94.62	7.61	8.05	40.86	1.57 ± 0.07	1.62 ± 0.07	2.37 ± 0.06
ET	E 10^{-8} M	98.11	98.11	100.00	52.83	62.26	86.79	16.98	7.55	45.28	1.66 ± 0.10	1.71 ± 0.08	2.32 ± 0.10
	E 10^{-10} M	94.34	96.36	98.18	67.92	63.64	94.55	13.21	3.64	47.27	1.75 ± 0.10	1.64 ± 0.08	2.44 ± 0.08
	E 10^{-12} M	92.45	94.55	98.18	67.92	52.73	98.18	20.75	1.82	41.82	1.85 ± 0.11	1.54 ± 0.08	2.41 ± 0.07
	E 10^{-14} M	98.11	98.00	98.39	75.47	74.00	95.16	26.42	6.00	45.16	2.02 ± 0.10	1.76 ± 0.08	2.42 ± 0.07
	A 10^{-8} M	94.23	94.34	100.00	59.62	45.28	96.36	15.38	1.89	41.82	1.75 ± 0.10	1.44 ± 0.08	2.38 ± 0.08
	A 10^{-10} M	96.15	96.30	100.00	63.46	44.44	96.36	13.46	5.56	45.45	1.81 ± 0.09	1.52 ± 0.08	2.42 ± 0.08
	A 10^{-12} M	98.15	98.18	98.18	68.52	58.18	94.55	11.11	5.46	34.55	1.82 ± 0.10	1.67 ± 0.09	2.35 ± 0.08
	A 10^{-14} M	100.00	94.44	100.00	72.73	55.56	98.18	23.64	3.70	50.91	1.96 ± 0.10	1.65 ± 0.08	2.47 ± 0.07
LT	E 10^{-8} M	86.84	88.24	97.30	50.00	58.82	94.59	5.26	2.94	51.35	1.62 ± 0.11	1.58 ± 0.12	2.50 ± 0.09
	E 10^{-10} M	94.74	91.43	100.00	44.74	51.43	97.30	5.26	8.57	37.84	1.53 ± 0.10	1.64 ± 0.11	2.38 ± 0.09
	E 10^{-12} M	92.31	94.29	97.30	56.41	57.14	97.30	7.69	0	37.84	1.65 ± 0.11	1.58 ± 0.09	2.36 ± 0.08
	E 10^{-14} M	92.11	96.97	97.37	50.00	39.39	92.11	2.63	0	31.58	1.50 ± 0.09	1.38 ± 0.08	2.27 ± 0.09
	A 10^{-8} M	94.74	100.00	100.00	52.63	58.82	94.59	13.16	8.82	37.84	1.58 ± 0.12	1.68 ± 0.10	2.32 ± 0.10
	A 10^{-10} M	97.37	100.00	100.00	44.74	44.12	97.30	7.89	5.88	35.14	1.50 ± 0.11	1.47 ± 0.11	2.27 ± 0.09
	A 10^{-12} M	97.37	94.12	97.30	39.47	41.18	97.30	0	2.94	37.84	1.39 ± 0.08	1.48 ± 0.10	2.39 ± 0.08
	A 10^{-14} M	92.11	100.00	100.00	50.00	58.82	94.59	0	2.94	29.73	1.47 ± 0.08	1.56 ± 0.10	2.16 ± 0.09
LSD ($P = 0.05$)											0.33	0.29	0.26

The percentage of plants that developed primary, secondary and tertiary ears, as well as the average number of ears per plant at the end of the flowering period are presented. Plants were sprayed with aqueous solutions of brassinosteroids (concentrations 10^{-8} to 10^{-14} M) or tap water (control) either early (ET) or later (LT) during vegetative phase of their development. The mean values ± SEM calculated from the pooled results of four experimental seasons are shown together with the LSD values significant at $P = 0.05$

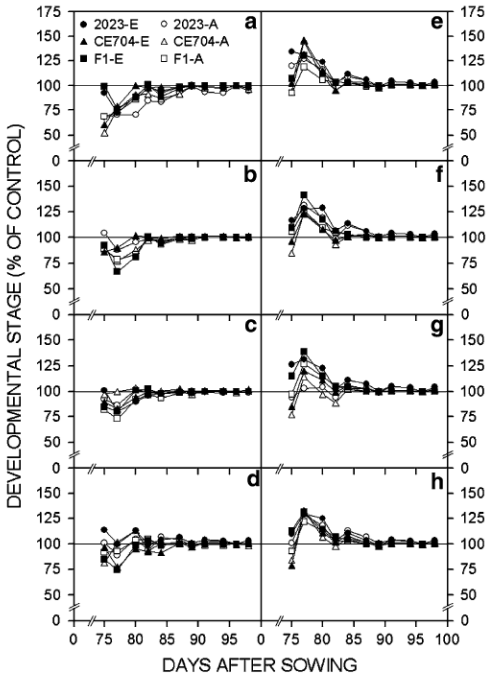


Fig. 3 The changes in the developmental stage of the tassel (male inflorescence) in three maize genotypes (inbred lines 2023 and CE704, F1 hybrid 2023 × CE704) treated by foliar spray with 10^{-8} M (**a**, **e**), 10^{-10} M (**b**, **f**), 10^{-12} M (**c**, **g**) or 10^{-14} M (**d**, **h**) aqueous solutions of 24-epibrassinolide (E) or synthetic androstane analogue of castasterone (A) either 41 (ET stage, **a**–**d**) or 55 (LT stage, **e**–**h**) days after sowing. The mean values calculated from the pooled results of four experimental seasons are shown as the percentage of control (i.e. plants treated with tap water only)

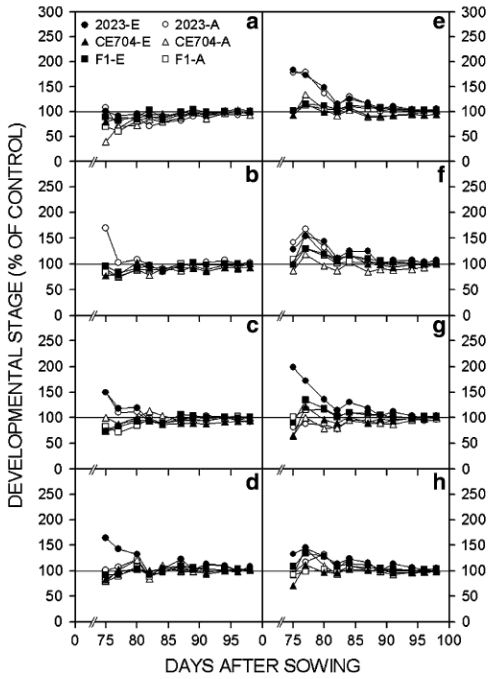


Fig. 4 The changes in the developmental stage of the primary ear (female inflorescence) in three maize genotypes (inbred lines 2023 and CE704, F1 hybrid 2023 × CE704) treated by foliar spray with 10^{-8} M (**a**, **e**), 10^{-10} M (**b**, **f**), 10^{-12} M (**c**, **g**) or 10^{-14} M (**d**, **h**) aqueous solutions of 24-epibrassinolide (E) or synthetic androstane analogue of castasterone (A) either 41 (ET stage, **a**–**d**) or 55 (LT stage, **e**–**h**) days after sowing. The mean values calculated from the pooled results of four experimental seasons are shown as the percentage of control (i.e. plants treated with tap water only)

2023 inbred line (with the exception of treatment of plants in ET stage with 10^{-8} M solutions of BRs) (Fig. 4). The effect of BRs on the development of the secondary ear was the least pronounced in the F1 hybrid; in both inbred lines it strongly depended on the concentrations of BRs used (Fig. 5).

The final number of ears developed by each plant at the end of the flowering was also positively affected by BRs treatment during ET stage, particularly in the 2023 inbred line, with the lowest concentrations of both E and A being the most effective ones (Table 5). On the other hand, treatment of plants in LT stage resulted in the decrease in the ear number per plant; again, spraying of plants with 10^{-14} M solution of BRs usually had the most pronounced effect (Table 5).

Yield parameters

The summary statistical analysis of data confirmed the presence of statistically significant differences among genotypes for all yield parameters (with the F1 hybrid being the „best" one and the inbred line CE704 the „worst" one), but the differences among BRs treatments were statistically significant only for the ear length, the number of kernel rows per ear and the shelling percentage (Table 2). However, this absence of significant BRs effects on other yield parameters resulted from the different—often opposite—response of the 2023 inbred line compared to the other two genotypes, as revealed when the data from individual genotypes were analyzed separately (Tables 6 and 7). The number of fertile ears per plant was positively affected by treatment of plants in ET stage in the inbred line 2023 (particularly using 10^{-8} M or 10^{-14} M concentrations of A) but did not significantly change or even decreased with BRs treatment in the inbred line CE704 or the F1 hybrid; for LT plants, the effect of BRs on the F1 hybrid was more positive but also statistically insignificant (Table 6). The ear length of both CE704 and F1 hybrid decreased after treatment of ET plants with A but was not affected in the 2023 genotype, whereas treatment of plants in LT stage with the same type

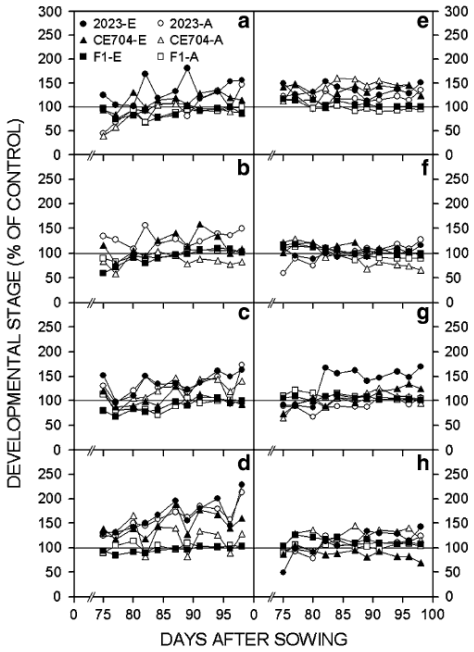


Fig. 5 The changes in the developmental stage of the secondary ear (female inflorescence) in three maize genotypes (inbred lines 2023 and CE704, F1 hybrid 2023 × CE704) treated by foliar spray with 10^{-8} M (**a**, **e**), 10^{-10} M (**b**, **f**), 10^{-12} M (**c**, **g**) or 10^{-14} M (**d**, **h**) aqueous solutions of 24-epibrassinolide (E) or synthetic androstane analogue of castasterone (A) either 41 (ET stage, **a**–**d**) or 55 (LT stage, **e**–**h**) days after sowing. The mean values calculated from the pooled results of four experimental seasons are shown as the percentage of control (i.e. plants treated with tap water only)

of BR (particularly using 10^{-14} M concentration) resulted in the decrease of this parameter in the 2023 but not the other two genotypes (Table 6). BRs treatment of plants in LT stage also negatively affected the number of kernel rows per ear in the CE704 genotype (and usually in the 2023 as well, though these effects were mostly statistically insignificant) (Table 6). The average number of kernels per row was not significantly influenced by BRs treatment of plants (Table 6).

Several positive or negative effects of BRs were observed also for various weight parameters of ears. Generally, the inbred line 2023 responded differently from the CE704 and 2023 × CE704 genotypes and treatment of plants in ET stage showed often inverse effects to the treatment of plants in LT stage. The most pronounced effect was shown by the application of 10^{-14} M solution of A, which significantly increased the dry weight of whole ear and cob in the inbred line 2023 when plants were sprayed in ET stage, but the effect of the same type of treatment on the CE704 inbred line was inverse (and was displayed here also for the weight of 100 kernels) (Table 7).

Discussion

The possibility of influencing plant growth and development by the exogenous application of BRs has been previously often examined in various plant species including maize. However, most of these studies were made with plants grown in the controlled environment conditions and treatment with BRs occured usually either by foliar spray of very young plants or by seed soaking. Application of BRs during later developmental stages has been explored less frequently and only very rarely did someone attempted to compare the effects of BRs application in various stages of plant development. Amzallag (2002) compared the response of sorghum to the early (8-days old plants) or later (18-days old plants) addition of 10^{-8}–10^{-10} M 24-epibrassinolide into root medium and found that 10^{-8} M BR treatment in later stage of plant development affected neither plant biomass nor the length of leaf blade, whereas early treatment significantly decreased both these parameters. Lower concentrations of 24-epibrassinolide had similar (positive) effect on plant biomass but the length of leaves was affected only in the early treated plants. The results of our analysis of field-grown maize plants treated with BRs either in the earlier (V3/4) or later (V6/7) developmental stages also revealed the important role of the time of BRs application on their effect on plant growth and morphology, as well as on the course of flowering or various yield parameters.

The most obvious differences between the results of the exogenous application of BRs in earlier or later stages of maize development were found for the course of flowering and the number of developed female inflorescences. Although the influence of BRs on flowering has been observed in several plant species, more focused studies on BRs′ role in this area have rarely been reported. Recent studies made on *Arabidopsis thaliana* mutants defective in genes involved in BRs signalling revealed the involvement of several components of BR-signalling pathway in the control of floral initiation and showed that the attenuation of BRs′ signalling delays flowering in this plant species (Clouse 2008; Domagalska et al. 2007; Turk et al. 2005; Yu et al. 2008). In our case, the application of 10^{-8}–10^{-14} M 24-epibrassinolide or the synthetic androstane analogue of castasterone in the earlier (V3/4) stages resulted in the delayed flowering and lower number of ears per plants, which agrees with the results of Janeczko et al. (2003), who found that the application of 10^{-8} or 10^{-9} M 24-epibrassinolide (but not 10^{-6} or 10^{-7} M) to 18-days old seedlings of *Arabidopsis* reduced the number of plants in generative

Table 6 The effect of 24-epibrassinolide (E) and synthetic androstane analogue of castasterone (A) on the number of fertile ears per plant, the ear length, the number of kernel rows and the average number of kernels per row in three maize genotypes (inbred lines 2023 and CE704, F1 hybrid 2023 × CE704)

		Number of fertile ears per plant			Ear length (cm)			Number of kernel rows per ear			Number of kernels per row		
		2023	CE704	F1 hybrid	2023	CE704	F1 hybrid	2023	CE704	F1 hybrid	2023	CE704	F1 hybrid
Control		0.98 ± 0.03	0.98 ± 0.03	1.26 ± 0.05	15.14 ± 0.24	12.43 ± 0.22	18.28 ± 0.18	13.10 ± 0.19	12.43 ± 0.17	12.71 ± 0.14	21.66 ± 0.95	17.72 ± 0.81	33.06 ± 0.77
ET	E 10^{-8} M	1.02 ± 0.06	1.02 ± 0.02	1.07 ± 0.04	15.21 ± 0.34	11.97 ± 0.26	17.93 ± 0.30	13.14 ± 0.24	12.18 ± 0.18	12.72 ± 0.18	22.34 ± 1.25	15.92 ± 1.00	31.46 ± 1.35
	E 10^{-10} M	1.04 ± 0.06	0.92 ± 0.05	1.22 ± 0.06	15.81 ± 0.28	12.15 ± 0.35	18.09 ± 0.23	13.18 ± 0.24	12.34 ± 0.18	13.02 ± 0.19	24.82 ± 1.32	18.51 ± 0.73	33.08 ± 1.05
	E 10^{-12} M	1.08 ± 0.07	0.98 ± 0.03	1.17 ± 0.05	15.50 ± 0.30	12.15 ± 0.27	18.31 ± 0.19	13.44 ± 0.25	12.15 ± 0.23	12.87 ± 0.17	24.44 ± 1.16	16.48 ± 0.87	33.17 ± 0.91
	E 10^{-14} M	1.08 ± 0.04	0.94 ± 0.03	1.16 ± 0.05	15.58 ± 0.25	12.58 ± 0.30	18.28 ± 0.17	13.53 ± 0.20	12.28 ± 0.21	13.05 ± 0.16	21.87 ± 1.27	17.85 ± 1.02	33.47 ± 1.04
	A 10^{-8} M	1.15 ± 0.05	0.98 ± 0.04	1.22 ± 0.06	14.85 ± 0.28	11.85 ± 0.34	17.74 ± 0.23	13.41 ± 0.27	12.09 ± 0.28	13.12 ± 0.18	20.41 ± 1.48	17.18 ± 0.88	31.92 ± 0.99
	A 10^{-10} M	1.02 ± 0.04	0.98 ± 0.03	1.18 ± 0.05	15.26 ± 0.23	10.98 ± 0.30	17.90 ± 0.21	13.38 ± 0.25	11.78 ± 0.31	12.83 ± 0.19	22.44 ± 1.10	15.00 ± 0.93	31.75 ± 0.92
	A 10^{-12} M	1.07 ± 0.06	0.98 ± 0.02	1.17 ± 0.05	14.81 ± 0.30	11.81 ± 0.28	17.85 ± 0.17	13.02 ± 0.21	12.10 ± 0.30	12.90 ± 0.16	22.23 ± 1.10	16.77 ± 1.03	33.08 ± 1.02
	A 10^{-14} M	1.13 ± 0.05	1.00 ± 0.00	1.16 ± 0.05	15.66 ± 0.20	11.70 ± 0.23	18.14 ± 0.19	13.34 ± 0.20	12.75 ± 0.25	13.12 ± 0.16	23.71 ± 1.10	18.45 ± 0.76	33.56 ± 0.84
LT	E 10^{-8} M	0.97 ± 0.05	0.94 ± 0.06	1.14 ± 0.06	15.50 ± 0.41	12.70 ± 0.28	18.69 ± 0.19	12.96 ± 0.28	12.00 ± 0.29	12.57 ± 0.21	21.57 ± 1.56	16.15 ± 1.17	34.60 ± 0.93
	E 10^{-10} M	1.03 ± 0.03	0.94 ± 0.04	1.24 ± 0.07	15.13 ± 0.34	12.79 ± 0.28	18.79 ± 0.23	13.27 ± 0.25	12.23 ± 0.19	12.56 ± 0.20	22.81 ± 1.19	18.65 ± 1.51	34.83 ± 0.99
	E 10^{-12} M	1.11 ± 0.06	1.15 ± 0.06	1.36 ± 0.08	15.05 ± 0.35	12.59 ± 0.35	18.57 ± 0.25	12.87 ± 0.25	11.54 ± 0.25	12.53 ± 0.19	21.52 ± 1.58	15.25 ± 1.24	33.76 ± 1.34
	E 10^{-14} M	0.92 ± 0.06	0.97 ± 0.03	1.32 ± 0.08	15.52 ± 0.27	12.77 ± 0.33	19.04 ± 0.28	12.93 ± 0.30	11.82 ± 0.25	12.47 ± 0.21	21.50 ± 1.34	17.61 ± 1.16	35.32 ± 1.33
	A 10^{-8} M	1.03 ± 0.05	1.06 ± 0.04	1.16 ± 0.06	14.71 ± 0.36	12.29 ± 0.38	18.39 ± 0.19	12.69 ± 0.40	11.69 ± 0.23	12.81 ± 0.24	22.22 ± 1.40	15.23 ± 1.20	32.73 ± 1.31
	A 10^{-10} M	1.03 ± 0.06	1.00 ± 0.00	1.19 ± 0.07	14.96 ± 0.42	12.76 ± 0.27	18.40 ± 0.26	13.11 ± 0.27	11.96 ± 0.32	12.49 ± 0.22	22.07 ± 1.56	16.00 ± 1.34	32.63 ± 1.40
	A 10^{-12} M	1.05 ± 0.04	0.97 ± 0.05	1.25 ± 0.08	14.71 ± 0.41	12.22 ± 0.40	18.62 ± 0.26	12.23 ± 0.37	11.83 ± 0.28	12.67 ± 0.24	20.92 ± 1.82	17.96 ± 1.47	34.42 ± 1.41
	A 10^{-14} M	1.00 ± 0.07	0.97 ± 0.03	1.32 ± 0.08	14.02 ± 0.56	12.80 ± 0.28	18.51 ± 0.23	12.76 ± 0.31	12.07 ± 0.26	12.63 ± 0.21	19.80 ± 1.82	18.54 ± 1.23	33.66 ± 1.26
LSD ($P = 0.05$)		0.17	0.12	0.19	1.02	1.01	0.73	0.83	0.84	0.61	4.28	3.53	3.56

Plants were sprayed with aqueous solutions of brassinosteroids (concentrations 10^{-8} to 10^{-14} M) or tap water (control) either early (ET) or later (LT) during vegetative phase of their development. The mean values ± SEM calculated from the pooled results of four experimental seasons are shown together with the LSD values significant at $P = 0.05$

Table 7 The effect of 24-epibrassinolide (E) and synthetic androstane analogue of castasterone (A) on the dry weight of whole ear, the dry weight of cob, the dry weight of 100 kernels and the shelling percentage in three maize genotypes (inbred lines 2023 and CE704, F1 hybrid 2023 × CE704)

		Ear weight (g)			Cob weight (g)			Weight of 100 kernels (g)			Shelling percentage (%)		
		2023	CE704	F1 hybrid	2023	CE704	F1 hybrid	2023	CE704	F1 hybrid	2023	CE704	F1 hybrid
Control		72.16 ± 3.39	48.18 ± 2.14	149.67 ± 4.27	10.97 ± 0.43	12.36 ± 0.52	25.49 ± 0.64	24.35 ± 0.59	20.54 ± 1.01	31.18 ± 0.66	83.75 ± 0.75	74.07 ± 0.48	82.55 ± 0.37
ET	E 10^{-8} M	76.56 ± 5.21	45.45 ± 3.14	146.69 ± 7.74	11.52 ± 0.81	11.49 ± 0.72	24.64 ± 1.06	23.71 ± 0.93	22.42 ± 1.43	32.19 ± 1.07	84.63 ± 0.66	74.12 ± 0.94	81.76 ± 0.97
	E 10^{-10} M	89.70 ± 5.89	48.96 ± 3.81	157.85 ± 6.58	12.94 ± 0.67	11.58 ± 0.91	25.09 ± 1.00	25.95 ± 0.81	19.28 ± 1.28	32.32 ± 0.93	84.15 ± 0.99	76.28 ± 0.77	83.54 ± 0.69
	E 10^{-12} M	86.14 ± 5.45	45.08 ± 2.64	159.52 ± 6.53	12.09 ± 0.61	11.53 ± 0.71	25.49 ± 0.97	24.20 ± 0.83	20.27 ± 1.24	32.71 ± 0.89	85.01 ± 0.81	74.55 ± 0.67	83.29 ± 0.73
	E 10^{-14} M	79.37 ± 4.88	49.89 ± 3.07	160.21 ± 5.61	12.01 ± 0.53	12.26 ± 0.63	26.30 ± 0.74	25.42 ± 0.64	21.27 ± 1.45	32.59 ± 0.90	82.89 ± 1.19	74.71 ± 0.77	82.62 ± 0.74
	A 10^{-8} M	73.66 ± 5.38	41.72 ± 3.10	150.52 ± 5.75	11.13 ± 0.49	9.87 ± 0.57	24.46 ± 0.93	24.95 ± 0.78	17.32 ± 1.17	31.91 ± 0.94	81.96 ± 1.76	75.56 ± 0.72	83.58 ± 0.42
	A 10^{-10} M	83.62 ± 4.73	40.39 ± 3.99	151.26 ± 6.08	12.04 ± 0.59	9.39 ± 0.74	25.02 ± 0.85	25.56 ± 0.83	19.70 ± .157	32.45 ± 0.97	84.65 ± 0.83	75.55 ± 1.09	82.87 ± 0.59
	A 10^{-12} M	77.17 ± 4.29	43.01 ± 2.81	152.86 ± 5.59	11.15 ± 0.58	10.93 ± 0.67	25.02 ± 0.81	25.33 ± 0.60	19.95 ± 1.38	31.91 ± 0.96	84.95 ± 0.60	73.71 ± 1.32	82.97 ± 0.75
	A 10^{-14} M	86.86 ± 4.39	41.97 ± 2.17	162.34 ± 4.14	12.24 ± 0.47	9.99 ± 0.59	27.17 ± 0.67	25.35 ± 0.58	16.78 ± 0.87	32.67 ± 0.93	84.73 ± 0.90	76.15 ± 0.74	83.01 ± 0.42
LT	E 10^{-8} M	74.31 ± 6.22	48.30 ± 3.00	156.31 ± 5.85	11.74 ± 0.81	12.42 ± 0.76	27.99 ± 1.09	24.03 ± 0.83	22.52 ± 1.74	31.21 ± 1.12	82.49 ± 1.30	73.89 ± 0.82	81.95 ± 0.45
	E 10^{-10} M	80.16 ± 4.84	48.54 ± 3.10	156.67 ± 6.07	11.72 ± 0.77	12.14 ± 0.77	27.70 ± 0.95	23.83 ± 0.71	19.75 ± 1.70	31.08 ± 1.00	85.31 ± 0.50	74.76 ± 0.63	82.08 ± 0.40
	E 10^{-12} M	72.64 ± 5.34	46.79 ± 3.99	149.96 ± 7.59	11.27 ± 0.66	11.92 ± 0.92	26.95 ± 1.16	24.65 ± 0.86	22.48 ± 1.68	31.87 ± 1.06	83.16 ± 1.16	73.55 ± 1.13	81.33 ± 0.83
	E 10^{-14} M	71.96 ± 4.98	48.44 ± 3.57	150.67 ± 6.92	11.52 ± 0.78	11.95 ± 0.74	27.20 ± 1.07	23.55 ± 1.03	20.31 ± 1.54	29.56 ± 1.07	83.16 ± 1.14	74.63 ± 0.78	81.52 ± 0.68
	A 10^{-8} M	73.95 ± 6.23	47.28 ± 3.86	148.34 ± 7.39	10.38 ± 0.86	12.22 ± 0.93	26.67 ± 1.25	23.16 ± 0.70	22.55 ± 1.61	31.09 ± 1.16	85.17 ± 0.79	73.70 ± 0.54	81.69 ± 0.48
	A 10^{-10} M	69.33 ± 5.53	51.65 ± 4.13	146.22 ± 8.51	10.98 ± 0.76	13.16 ± 0.89	26.81 ± 1.41	23.32 ± 0.98	22.66 ± 1.60	31.45 ± 1.34	83.08 ± 1.15	73.70 ± 0.68	81.04 ± 0.77
	A 10^{-12} M	64.46 ± 5.80	45.73 ± 4.42	154.71 ± 8.58	9.79 ± 0.68	11.47 ± 0.95	27.35 ± 1.38	23.56 ± 0.90	18.15 ± 1.15	30.62 ± 1.15	82.43 ± 1.58	73.96 ± 0.82	81.53 ± 0.98
	A 10^{-14} M	63.51 ± 5.94	49.96 ± 4.96	148.63 ± 6.98	10.29 ± 0.74	12.97 ± 0.91	25.52 ± 1.11	22.81 ± 1.05	20.22 ± 1.84	29.74 ± 1.13	81.81 ± 1.71	72.35 ± 1.18	82.41 ± 0,52
LSD ($P = 0.05$)		16.48	11.16	20.80	2.07	2.50	3.17	2.54	4.74	3.25	3.45	2.90	2.15

Plants were sprayed with aqueous solutions of brassinosteroids (concentrations 10^{-8} to 10^{-14} M) or tap water (control) either early (ET) or later (LT) during vegetative phase of their development. The mean values ± SEM calculated from the pooled results of four experimental seasons are shown together with the LSD values significant at $P = 0.05$

stage of development, i.e. delayed flowering. Kęsy et al. (2003) described the inhibition of flowering in *Pharbitis nil* seedlings that were treated with 10^{-7} or 10^{-6} M brassinolide or castasterone before or during flowering-inductive dark period. On the other hand, we observed a significant decrease in the length of period to anthesis and silking when maize plants were treated by foliar spray with BRs during V6/7 stages. According to Ritchie et al. (1993), all potential ear shoots in maize are initiated between stages V3 and V5, and the initiation of a tassel occurs during the V5 stage. It is thus possible that BRs negatively influence the flowering *before* the beginning of the inflorescence/flower development but have positive effect on later phases of this development, and that the time boundary between stimulatory and inhibitory effect can be very short. The effective BR concentration apparently also depends on plant species. Moreover, though the general trend in the response of flowering to BRs treatment was similar for all three genotypes examined, some genotypic differences in the lengthening/shortening of the period to the start of the flowering (usually in 1–2 days range) existed as well. Torres-Ruiz et al. (2007) also observed earlier emergence of tassel after BR application in single-cross maize hybrids with male sterility and normal fertility but not in three-way androsterile hybrids.

The interpretation of the results of our measurements of morphological parameters is more difficult. A prevailing trend for the different effects of BRs application in earlier or later developmental stages on plant growth and morphology could be found only for the length of leaf blade, but even then not for all leaves that developed during or after BRs treatments. This rather agrees with the observations of Amzallag (2002), who observed significant influence of 24-epibrassinolide on the 5th to 7th leaf of sorghum but not on leaves of higher or lower (even in plants treated with BR in the stage these leaves were yet developing) insertion levels.

As regards the influence of BRs on plant height or leaf number, previous studies with various plant species usually reported positive results. Arora et al. (2008) found the positive effect of the addition of 10^{-9} M (but not 10^{-7} or 10^{-11} M) solution of 28-homobrassinolide to the growth medium on the shoot length of maize. The shoot length of 7-days old seedlings of Indian mustard (*Brassica juncea*) also increased after presowing treatment of seeds with 10^{-7}–10^{-11} M 24-epibrassinolide, with the 10^{-7} M concentration being the most effective one (Sharma and Bhardwaj 2007). Similarly, thrice repeated foliar spray of *Arachis hypogaea* plants with brassinolide or 24-epibrassinolide solutions in 10^{-6} M concentration range had positive effect on the shoot length (Vardhini and Rao 1998). Wheat raised from kernels treated with 10^{-6} M 28-homobrassinolide also displayed a higher number of leaves per plant (Hayat et al. 2001a). On the other hand, Özdemir et al. (2004) observed a slight decrease in the elongation of rice shoot after soaking of seeds in 3×10^{-6} M 24-epibrassinolide solution, whereas the soaking of rice seeds in low concentrations of 28-homobrassinolide or 24-epibrassinolide promoted the growth of rice plants under low-temperature (Takematsu and Takeuchi 1989) or water stress conditions (Farooq et al. 2009). Vlašánková et al. (2009), who used the same type of BRs as those used in our study, also observed an inhibition of the epicotyl growth in pea and flax seedlings after soaking of seeds in BRs solutions ranging from 10^{-7} to 10^{-9} M concentrations. However, all these studies are somehow disadvantageous in the fact that they were usually made in strictly controlled environment conditions and that the plant height was measured in very young seedlings (7- to 35-days old), i.e. rather soon after BRs treatment. Also, BRs were usually either added into the growth medium or used for the soaking of seeds in their solutions. In our case, the influence of foliar spray of maize plants with BRs on plant height and leaf number was observed solely during the early days after BRs treatment and virtually disappeared at the end of vegetative stage, which does not disagree with the results of the above mentioned studies. The treatment of plants in earlier developmental stage showed more-or-less negative effect, whereas treatment of plants in later developmental stage had more positive effect on maize plant height, but different concentrations or different types of BRs induced different changes. Moreover, strong dependence on the genotype was also observed. Thus, we can only second the statement of Khripach et al. (2000) that „the growth promoting activity of BRs usually takes place only after treatment of plants in the appropriate phase of development and within a certain concentration range, which is different for each plant species and type of BRs".

The dependence of plant response to BRs treatment on the genotype was even more evident for yield parameters. Relatively low number of authors have simultaneously examined the effect of exogenous application of BRs on plants of more than one genotype of the same species. These that have done so, have often found that the response of individual genotypes can be markedly different (Hnilička et al. 2007; Torres-Ruiz et al. 2007). This applied particularly in cases BRs were applied to plants stressed by some unfavourable environmental factor and the susceptible and tolerant genotypes were compared (Kang et al. 2007; Shahbaz et al. 2008; Vardhini and Rao 2003). During our examination of two homozygous inbred lines and one heterozygous F1 hybrid of maize, we observed that the differences between both inbreds were usually more pronounced than those between inbreds and hybrid, and that the response of one inbred line to BRs treatment was often quite opposite to that of the other inbred line (e.g. for the

number of fertile ears per plant, the length of ear, the weight of whole ear and cob or the weight of 100 kernels, as well as the shelling percentage). The response of the F1 hybrid to BRs treatments usually followed that of its *paternal* parent, which was rather unexpected. It could be interesting to make a more detailed examination of the effect of BRs application on various plant parameters in a wider set of parents and hybrids with the aim to ascertain whether (and how) can be the response to these phytohormones inherited.

We can thus conclude that although the exogenous application of BRs to plants can certainly in many ways change plant growth and development and, consequently, influence both total yield and yield parameters, several aspects of the interaction of these compounds with plants rather hinder their wider introduction into agricultural practice. Prior to their large-scale commercial use in some agronomically important species, various detailed studies should be always made, aimed at the determination of the best type of BRs, its most effective concentration, the most convenient and effective mode of application, and the suitable plant age when the respective compound should be applied, always with a particular point of view regarding *which* parameter of plant is expected to be changed by the BRs application. Such studies should be realized in the field conditions (as the controlled-environment studies can often yield quite different results) with several years repetitions (particularly in those areas where a wide between-year climatic variability exists). Moreover, they should be made on plants of the same genotype/s that is expected to be then cultivated commercially. Otherwise, the full exploitation of the potential effectivity of BRs for agricultural use cannot be achieved.

Acknowledgments The authors are grateful to Dr. Jaroslav Poruba from the CEZEA Breeding Station in Čejč, Czech Republic, for the supply of maize kernels, to our students Jana Honnerová, Monika Benešová, Lenka Fridrichová and Zuzana Novosadová for their help with the measurements of yield and growth parameters, and to the technical personnel of the Genetical Garden of the Department of Genetics and Microbiology, Faculty of Science, Charles University in Prague, for taking care of the experimental plants. This study was supported by grants No. KJB601110611 of the Grant Agency of the Academy of Sciences of the Czech Republic, and No. MSM0021620858 from the Ministry of Education, Youth and Sports of the Czech Republic.

References

Ali B, Hayat S, Ahmad A (2007) 28-homobrassinolide ameliorates the saline stress in chickpea (*Cicer arietinum* L.). Environ Exp Bot 59:217–223. doi:10.1016/j.envexpbot.2005.12.002

Ali B, Hasan SA, Hayat S, Hayat Q, Yadav S, Fariduddin Q, Ahmad A (2008a) A role for brassinosteroids in the amelioration of aluminium stress through antioxidant system in mung bean (*Vigna radiata* L. Wilczek). Environ Exp Bot 62:153–159. doi:10.1016/j.envexpbot.2007.07.014

Ali Q, Athar HR, Ashraf M (2008b) Modulation of growth, photosynthetic capacity and water relations in salt stressed wheat plants by exogenously applied 24-epibrassinolide. Plant Growth Regul 56:107–116. doi:10.1007/s10725-008-9290-7

Amzallag GN (2002) Brassinosteroids as metahormones: evidence for their specific influence during the critical period in *Sorghum* development. Plant Biol 4:656–663. doi:10.1055/s-2002-37397

Arora N, Bhardwaj R, Sharma P, Arora HK (2008) Effect of 28-homobrassinolide on growth, lipid peroxidation and antioxidative enzyme activities in seedlings of *Zea mays* L. under salinity stress. Acta Physiol Plant 30:833–839. doi:10.1007/s11738-008-0188-9

Bolaños J, Edmeades GO (1996) The importance of the anthesis-silking interval in breeding for drought tolerance in tropical maize. Field Crops Res 48:65–80. doi:10.1016/0378-4290(96)00036-6

Cerrana R, Lado P, Anastasia M, Ciuffreda P, Allevi P (1984) Regulating effects of brassinosteroids and of sterols on growth and H^+ secretion in maize roots. J Plant Physiol 114:221–225

Clouse SD (2008) The molecular intersection of brassinosteroid-regulated growth and flowering in Arabidopsis. Proc Natl Acad Sci USA 105:7345–7346. doi:10.1073/pnas.0803552105

Clouse SD, Sasse JM (1998) Brassinosteroids: essentials regulators of plant growth and development. Annu Rev Plant Physiol Plant Mol Biol 49:427–451. doi:10.1146/annurev.arplant.49.1.427

Domagalska MA, Schomburg FM, Amasino RM, Vierstra RD, Nagy F, Davis SJ (2007) Attenuation of brassinosteroid signaling enhances *FLC* expression and delays flowering. Development 134:1841–1850. doi:10.1242/dev.02866

Fariduddin Q, Ahmad A, Hayad S (2003) Photosynthetic response of *Vigna radiata* to pre-sowing seed treatment with 28-homobrassinolide. Photosynthetica 41:307–310. doi:10.1023/B:PHOT.0000011968.78037.b1

Fariduddin Q, Hasan SAS, Ali B, Hayat S, Ahmad A (2008) Effect of modes of application of 28-homobrassinolide on mung bean. Turk J Biol 32:17–21

Farooq M, Wahid A, Basra SMA, Din I (2009) Improving water relations and gas exchange with brassinosteroids in rice under drought stress. J Agron Crop Sci 195:262–269. doi:10.1111/j.1439-037X.2009.00368.x

Fedina EO, Karimova FG, Tarchevsky IA, Toropygin IY, Khripach VA (2008) Effect of epibrassinolide on tyrosine phosphorylation of the Calvin cycle enzymes. Russ J Plant Physiol 55:193–200. doi:10.1134/S1021443708020052

Fujii S, Saka H (2001) Distribution of assimilates to each organ in rice plants exposed to low temperature at the ripening stage, and the effect of brassinolide on the distribution. Plant Product Sci 4:136–144

Haubrick LL, Assmann SM (2006) Brassinosteroids and plant function: some clues, more puzzles. Plant Cell Environ 29:446–457. doi:10.1111/j.1365-3040.2005.01481.x

Hayat S, Ahmad A (2003) Soaking seeds of *Lens culinaris* with 28-homobrassinolide increased nitrate reductase activity and grain yield in India. Ann Appl Biol 143:121–124. doi:10.1111/j.1744-7348.2003.tb00276.x

Hayat S, Ahmad A, Mobin M, Hussain A, Fariduddin Q (2000) Photosynthetic rate, growth and yield of mustard plants sprayed with 28-homobrassinolide. Photosynthetica 38:469–471. doi:10.1023/A:1010954411302

Hayat S, Ahmad A, Hussain A, Mobin M (2001a) Growth of wheat seedlings raised from the grains treated with 28-homobrassinolide. Acta Physiol Plant 23:27–30. doi:10.1007/s11738-001-0018-9

Hayat S, Ahmad A, Mobin M, Fariduddin Q, Azam ZM (2001b) Carbonic anhydrase, photosynthesis, and seed yield in mustard plants treated with phytohormones. Photosynthetica 39:111–114. doi:10.1023/A:1012456205819

Hayat S, Ali B, Hasan SA, Ahmad A (2007) Brassinosteroid enhanced the level of antioxidants under cadmium stress in *Brassica juncea*. Environ Exp Bot 60:33–41. doi:10.1016/jenvexpbot.2006.06.002

Hnilička F, Hniličková H, Martinková J, Bláha L (2007) The influence of drought and the application of 24-epibrassinolide on the formation of dry matter and yield in wheat. Cereal Res Commun 35:457–460. doi:10.1556/CRC.35.2007.2.73

Janeczko A, Filek W, Biesaga-Kościelniak J, Marcińska I, Janeczko Z (2003) The influence of animal sex hormones on the induction of flowering in *Arabidopsis thaliana*: comparison with the effect of 24-epibrassinolide. Plant Cell Tiss Organ Cult 72:147–151. doi:10.1023/A:1022291718398

Janeczko A, Hura K, Skoczowski A, Idzik I, Biesaga-Kościelniak J, Niemczyk E (2009) Temperature-dependent impact of 24-epibrassinolide on the fatty acid composition and sugar content in winter oilseed rape callus. Acta Physiol Plant 31:71–79. doi:10.1007/s11738-008-0202-2

Kang Y, Guo S, Li J, Duan J (2007) Effects of 24-epibrassinolide on antioxidant system in cucumber seedling roots under hypoxia stress. Agric Sci China 6:281–289. doi:10.1016/S1671-2927(07)60046-7

Kartal G, Temel A, Arican E, Gozukirmizi N (2009) Effects of brassinosteroids on barley root growth, antioxidant system and cell division. Plant Growth Regul 58:261–267. doi:10.1007/s10725-009-9374-z

Kęsy J, Trzaskalska A, Galoch E, Kopcewicz J (2003) Inhibitory effect of brassinosteroids on the flowering of the short-day plant *Pharbitis nil*. Biol Plant 47:597–600. doi:10.1023/B:BIOP.0000041069.27805.89

Khripach V, Zhabinskii V, deGroot A (2000) Twenty years of brassinosteroids: steroidal plant hormones warrant better crops for the XXI century. Ann Bot 86:441–447. doi:10.1006/anbo.2000.1227

Khripach VA, Zhabinskii VN, Khripach NB (2003) New practical aspects of brassinosteroids and results of their ten-year agricultural use in Russia and Belarus. In: Hayat S, Ahmad A (eds) Brassinosteroids: bioactivity and crop productivity. Kluwer, Dordrecht-Boston-London, pp 189–230

Kohout L (1994) New method for preparation of brassinosteroids. Coll Czech Chem Commun 59:457–460. doi:10.1135/cccc19940457

Li KR, Wang HH, Han G, Wang QJ, Fan J (2008) Effect of brassinolide on the survival, growth and drought resistance of *Robinia pseudoacacia* seedlings under water stress. New Forests 35:255–266. doi:10.1007/s11056-007-9075-2

Mazorra LM, Núñez M, Hechavarria M, Coll F, Sánchez-Blanco MJ (2002) Influence of brassinosteroids on antioxidant enzymes activity in tomato under different temperatures. Biol Plant 45:593–596. doi:10.1023/A:1022390917656

Núñez MV, Robaina CR, Coll FM (2003) Synthesis and practical applications of brassinosteroid analogs. In: Hayat S, Ahmad A (eds) Brassinosteroids: bioactivity and crop productivity. Kluwer, Dordrecht-Boston-London, pp 87–118

Özdemir F, Bor M, Demiral T, Türkan I (2004) Effects of 24-epibrassinolide on seed germination, seedling growth, lipid peroxidation proline content and antioxidative system of rice (*Oryza sativa* L.) under salinity stress. Plant Growth Regul 42:203–211. doi:10.1023/B:GROW.0000026509.25995.13

Ramraj VM, Vyas BN, Godrej NB, Mistry KB, Swami BN, Singh N (1997) Effects of 28-homobrassinolide on yields of wheat, rice, groundnut, mustard, potato and cotton. J Agric Sci 128:405–413

Rao AAR, Vardhini BV, Sujatha E, Anuradha S (2002) Brassinosteroids—a new class of phytohormones. Curr Sci 82:1239–1245

Ritchie SW, Hanway JJ, Benson GO (1993) How a corn plant develops. Spec Rep No 48. Iowa State University Extension, Ames

Sairam RK (1994a) Effect of homobrassinolide application on metabolic activity and grain yield of wheat under normal and water-stress condition. J Agron Crop Sci 173:11–16

Sairam RK (1994b) Effect of homobrassinolide application on plant metabolism and grain yield under irregated and moisture-stress conditions of two wheat varieties. Plant Growth Regul 14:173–181. doi:10.1007/BF00025220

Sasse JM (2003) Physiological actions of brassinosteroids: an update. J Plant Growth Regul 22:276–288. doi:10.1007/s00344-033-0062-3

Shahbaz M, Ashraf M, Athar HR (2008) Does exogenous application of 24-epibrassinolide ameliorate salt induced growth inhibition in wheat (*Triticum aestivum* J.)? Plant Growth Regul 55:51–64. doi:10.1007/s10725-008-9262-y

Sharma P, Bhardwaj R (2007) Effect of 24-epibrassinolide on seed germination, seedling growth and heavy metal uptake in *Brassica juncea* L. Gen Appl Plant Physiol 33:59–73

Singh I, Shono M (2005) Physiological and molecular effects of 24-epibrassinolide, a brassinosteroid on thermotolerance of tomato. Plant Growth Regul 47:111–119. doi:10.1007/s10725-005-3252-0

Swamy KN, Rao SSR (2008) Influence of 28-homobrassinolide on growth, photosynthesis metabolite and essential oil content on geranium (*Pelargonium graveolens* (L.) Herit). Amer J Plant Physiol 3:173–179

Takematsu T, Takeuchi Y (1989) Effect of brassinosteroids on growth and yields of crops. Proc Japan Acad 65:149–152. doi:10.2183/pjab.65.149

Torres-Ruiz BL, Espinosa-Calderón A, Mendoza-Rodríguez M, Rodríguez de la OJL, Irizar-Garza MB, Castellanos-Ruiz JS (2007) Effecto de brasinoesteroides en híbridos de maíz androestériles y fértiles. Agronomía Mesoamericana 18:155–162

Turk EM, Fujioka S, Seto H, Shimada Y, Takatsuto S, Yoshida S, Wang H, Torres QI, Ward JM, Murthy G, Zhang J, Walker JC, Neff MM (2005) *BAS1* and *SOB7* act redundantly to modulate Arabidopsis photomorphogenesis via unique brassinosteroid inactivation mechanisms. Plant J 42:23–34. doi:10.1111/j.1365-313X.2005.02358.x

Vardhini BV, Rao SSR (1998) Effect of brassinosteroids on growth, metabolite content and yield of *Arachis hypogaea*. Phytochemistry 48:927–930. doi:10.1016/S0031-9422(97)00710-3

Vardhini BV, Rao SSR (2003) Amelioration of osmotic stress by brassinosteroids on seed germination and seedling growth of three varieties of sorghum. Plant Growth Regul 41:25–31. doi:10.1023/A:1027303518467

Vlašánková E, Kohout L, Klemš M, Eder J, Reinöhl V, Hradilík J (2009) Evaluation of biological activity of new synthetic brassinolide analogs. Acta Physiol Plant 31:987–993. doi:10.007/s11738-009-0314-3

Yu JQ, Huang LF, Hu WH, Zhou YH, Mao WH, Ye SF, Nogués S (2004) A role for brassinosteroids in the regulation of photosynthesis in *Cucumis sativus*. J Exp Bot 399:1135–1143. doi:10.1093/jxb/erh124

Yu X, Li L, Li L, Guo M, Chory J, Yin Y (2008) Modulation of brassinosteroid-regulated gene expression by jumonji domain-containing proteins ELF6 and REF6 in *Arabidopsis*. Proc Natl Acad Sci USA 105:7618–7623. doi:10.1073/pnas.0802254105

Zhao YJ, Chen J (2003) Studies on physiological action and application of 24-epibrassinolide in agriculture. In: Hayat S, Ahmad A (eds) Brassinosteroids: bioactivity and crop productivity. Kluwer, Dordrecht-Boston-London, pp 159–170

BIOLOGIA PLANTARUM 54 (4): 785-788, 2010

BRIEF COMMUNICATION

The effects of brassinosteroids on photosynthetic parameters in leaves of two field-grown maize inbred lines and their F1 hybrid

M. KOČOVÁ[1], O. ROTHOVÁ[1], D. HOLÁ[1]*, M. KVASNICA[2] and L. KOHOUT[2]

Charles University in Prague, Faculty of Science, Viničná 5, CZ-12843 Prague, Czech Republic[1]
Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, CZ-16610 Prague, Czech Republic[2]

Abstract

The effect of foliar spray with 10^{-12} M aqueous solutions of 24-epibrassinolide or a synthetic androstane analogue of castasterone on the activity of photosystem (PS) 1, the Hill reaction activity, the content of photosynthetic pigments and the specific leaf mass was examined for three different leaves developed after brassinosteroid (BR) treatment in two inbred lines of field-grown maize and their F1 hybrid. The brassinosteroids significantly affected neither the efficiency of photosynthetic electron transport, nor the content of chlorophylls or carotenoids.

Additional key words: carotenoids, chlorophylls, 24-epibrassinolide, heterosis, Hill reaction, photosystem 1.

More than 70 structurally and functionally related brassinosteroids (BRs) have been characterized from various natural sources since their discovery in 1979 (Grove *et al.* 1979). These organic compounds with structure based on polyhydroxylated sterols show multiple effects on plant physiology, development and growth and are now included among main groups of phytohormones. Several excellent reviews concerning BRs action in plants at the molecular, cellular or whole plant level have appeared in the last years (*e.g.* Wang and He 2004, Haubrick and Assman 2006, Bajguz and Hayat 2009). One of the diverse functions of BRs in higher plants is their possible involvement in the regulation of photosynthesis. The application of BRs enhanced the net photosynthetic rate in several plant species, *e.g., Brassica juncea* (Hayat *et al.* 2000, 2001, 2007, Ali *et al.* 2008b, Fariduddin *et al.* 2009a,b), *Cucumis sativus* (Yu *et al.* 2004, Xia *et al.* 2006), *Glycine max* (Zhang *et al.* 2008), *Lycopersicon esculentum* (Singh and Shono 2005), *Oryza sativa* (Farooq *et al.* 2009), *Vigna radiata* (Fariduddin *et al.* 2003, 2004, Ali *et al.* 2008a) or *Triticum aestivum* (Sairam 1994a,b, Ali *et al.* 2008c). The majority of these studies was made on very young plants cultivated in controlled conditions (often in combination with some unfavourable environmental factor), so a care should be taken when extrapolating these results to plants grown at natural conditions. Moreover, it is still not entirely clear, whether BRs primarily affect the activities or contents of the pigment-protein complexes of photosynthetic electron-transport chain, the chloroplast ATP synthase or the enzymes catalyzing the individual steps of CO_2 fixation, or whether they perhaps participate in the regulation of stomatal function, the synthesis or degradation of photosynthetic pigments or some other parts of photosynthetic processes (Yu *et al.* 2004, Hayat *et al.* 2007, Ali *et al.* 2008c, Ogweno *et al.* 2008, Fariduddin *et al.* 2009a,b). Thus, we have decided to examine the possible effects of applied BRs on the primary

Received 7 August 2009, *accepted* 15 February 2010.

Abbreviations: AAC - 2α,3α,17β-trihydroxy-5α-androstan-6-one; BR - brassinosteroid; Car - carotenoids; Chl - chlorophyll; DAS - days after sowing; DCPIP - 2,6-dichlorophenol indophenol; DMF - *N,N*-dimethyl formamide; DMSO - dimethyl sulfoxide; 24-EPI - 24-epibrassinolide; HRA - Hill reaction activity; PAR - photosynthetically active radiation; PS - photosystem; SLM - specific leaf mass.

Acknowledgements: The authors are grateful to Dr. Jaroslav Poruba from the CEZEA Breeding Station in Čejč, Czech Republic, for the supply of maize kernels, and to our students Lenka Fridrichová and Zuzana Novosadová for their help with the measurements of photosynthetic parameters. This study was supported by grants No. KJB601110611 of the Grant Agency of the Academy of Sciences of the Czech Republic, No. MSM0021620858 from the Ministry of Education, Youth and Sports of the Czech Republic, and IOCB research project Z4 055 0506.

* Corresponding author; e-mail: danahola@natur.cuni.cz

photochemistry (particularly the activity of PS 1, as this has not yet been previously studied) and the contents of photosynthetic pigments in leaves of field-grown maize.

Zea mays L. plants were cultivated at the experimental field of the Department of Genetics and Microbiology, Faculty of Science, Charles University in Prague, Czech Republic (54°04´ N, 14°25´ E, altitude 238 m) during four years (2004 - 2007). Kernels of two maize inbred lines (2023 and CE704) and their F1 hybrid (2023×CE704) were first sown into containers filled with garden soil and placed in glass-covered hot-beds at the end of April. After 4 weeks the seedlings were transplanted into the field in a regular pattern (70 × 50 cm). The leaves of plants were sprayed with tap water (control) or with 10^{-12} M aqueous solutions of either 24-epibrassinolide (24-EPI) or synthetic androstane analogue of castasterone (AAC) 41 d after sowing (DAS). BRs were synthesized as described by Kohout (1994). Experiments were arranged in a split-plot design with two replicates during each experimental season; each subplot consisted of 9 plants.

Photosynthetic parameters were measured in leaves 2, 4 and 6, counting the youngest fully developed leaf at the time of BRs treatment as leaf 0. The measurements were made at 72 DAS for leaf 2, 82 DAS for leaf 4 and 94 DAS for leaf 6; at this time points the respective leaves were fully developed and showed maximum photosynthetic efficiency. At each sampling date, the leaves from 9 plants per each treatment were cut off and the middle parts of leaf blades were used for all subsequent analyses. The isolation of chloroplasts and the polarographical measurements (Clark-type oxygen electrode, *Theta ´90*, Prague, Czech Republic) of the activities of photosystem (PS) 1 and Hill reaction (HRA) were carried out as described previously (Holá *et al.* 2007), *i.e* as the amount of oxygen consumed (or, in case of HRA, formed) by suspensions of isolated chloroplasts [Class II, type C according to Hall (1972) nomenclature] after the addition of artificial electron acceptors or donors: 0.25 mM DCPIP reduced by 10 mM sodium ascorbate, 0.1 mM methylviologen for the PS 1 activity measurements and 7 mM $K_3[Fe(CN)_6]$ for the HRA measurements (irradiance was 170 W m^{-2} of PAR). The content of photosynthetic pigments was determined spectrophotometrically (*Anthelie 2 Advanced*, *Secomam*, Ales, France) after their extraction from 6 leaf discs (diameter 8 mm) with *N,N*-dimethyl formamide (DMF; Wellburn 1994). The specific leaf mass (SLM) was determined as the dry mass of 6 leaf discs (oven-dried at 80 °C for 72 h) to their area ratio. Each genotype/BRs treatment was represented by 3 or 6 (SLM) technical replicates per each experimental replicate and the mean values were used for the statistical evaluation. The data from all four experimental years were pooled together and analyzed by two-way *ANOVA* with genotypes, BRs treatments and their interaction included as the possible sources of variation (*CoStat Version 6.204*, *CoHort* software, Monterey, CA, USA).

The results of statistical analysis revealed that the three maize genotypes significantly differ in all photosynthetic parameters examined (with the exception of Chl *a/b* ratio which did not differ at all, and the HRA measured in samples from leaf 4, where $P = 0.065$) (Table 1). The 2023×CE704 hybrid displayed the highest activity of PS 1 and HRA, as well as the highest content of photosynthetic pigments (Fig. 1), confirming again the previous findings that F1 hybrids of maize show positive heterosis not only for yield and morphology parameters, but for various photosynthetic parameters as well (*e.g.* Baer and Schrader 1985, Mehta *et al.* 1992, Holá *et al.* 1999, 2007, Ahmadzadeh *et al.* 2004, Kočová *et al.* 2009). The inbred line CE704 was characterized by the lowest content of Chls and Cars and usually also by the lowest PS 1 activity and HRA (Fig. 1). As regards SLM, this parameter did not significantly differ among genotypes when recorded for leaf 2, but for both younger leaves its values were lower in the 2023 inbred line compared to the other two genotypes (Fig. 1*C*).

Our treatment of plants with either 24-EPI or AAC had no effect on SLM or the content of Chls or Cars in maize leaves that developed after the application of BRs (Table 1, Fig. 1*D-F*), which agrees with findings of some authors who worked with non-stressed plants of various species (*e.g.* Yu *et al.* 2004, Janeczko *et al.* 2007, Ali *et al.* 2008c, Shahbaz *et al.* 2008, Zhang *et al.* 2008) but disagrees with others (Sairam 1994a,b, Hayat *et al.* 2001, 2007, Fariduddin *et al.* 2003, 2004, 2009a,b, Ali *et al.* 2008a,b). It is interesting to note that with the exception of Ali *et al.* (2008b,c) who worked with *Brassica juncea* or *Vigna radiata*, all authors who observed the positive influence of BRs on the content of Chls worked with 28-homobrassinolide, whereas those that found no effect treated their experimental plants with 24-EPI or with brassinolide. As no other common denominator among these studies (for example the mode of application, the concentration of BR, the age of plants during BRs treatment or during the measurements of Chls content) exists, it would seem that the type of the applied BR is the main determinant in the manifestation of BRs effect on the content of photosynthetic pigments, and plant species is probably the second deciding factor.

Similarly to the content of Chls or Cars, neither the activity of PS 1 nor the HRA were significantly affected by treatment of plants with 24-EPI or AAC, though 24-EPI slightly (but insignificantly) increased the HRA in the F1 hybrid (and, in case of lower leaf insertions, in the 2023 inbred line as well) (Table 1, Fig. 1*A-B*). The activity of PS 1 has not been previously examined in connection with the possible role of BRs in photosynthesis and our study presents the first evidence that the function of this pigment-protein complex is very probably not modified by these phytohormones. The HRA measured with $K_3[Fe(CN)_6]$ as an artificial electron acceptor can to a certain degree be also regarded as a measure of the activity of PS 1 (particularly when chloroplasts with mainly undamaged thylakoid membranes are used for its measurements, as in our case). However, $K_3[Fe(CN)_6]$ also accepts electrones from photosynthetic plastoquinones, *i.e.* our measurements of the HRA can partly represent the PS 2 activity

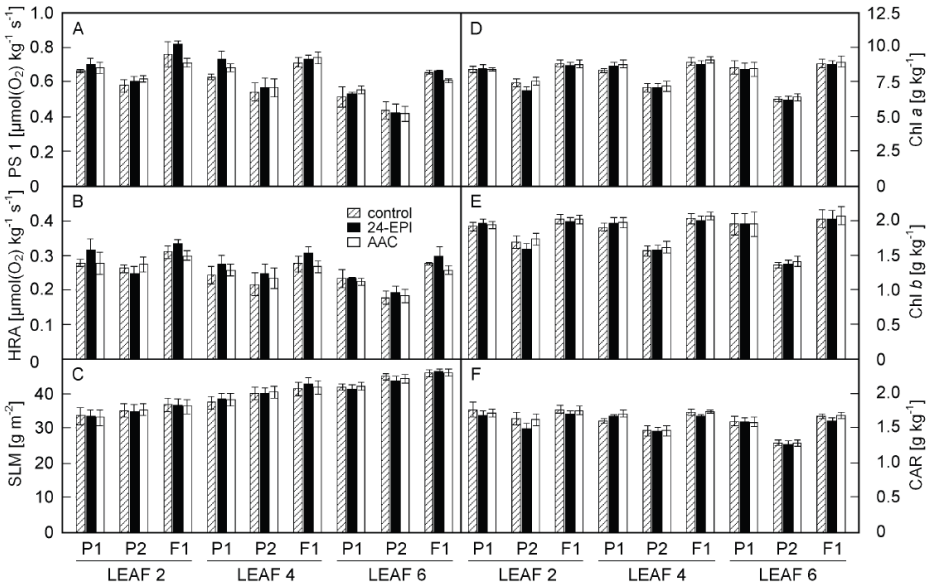


Fig. 1. The photosystem 1 activity (*A*), the Hill reaction activity (*B*), the specific leaf mass (*C*) and the content of chlorophyll *a* (*D*), chlorophyll *b* (*E*) and total carotenoids (*F*) in leaves of maize inbred lines 2023 (P1), CE704 (P2) and their hybrid 2023×CE704 (F1) grown at field conditions and treated with 10^{-12} M aqueous solutions of 24-epibrassinolide (24-EPI), synthetic androstane analogue of castasterone (AAC) or with tap water (control). The means ± SE were calculated from data pooled from four experimental seasons. Leaves are numbered beginning with the last fully developed leaf in the time of brassinosteroid treatment as leaf 0.

Table 1. The results of two-way analysis of variance (*ANOVA*) applied on the the specific leaf mass (SLM) and photosynthetic parameters in leaves of field-grown maize plants of three genotypes (inbred lines 2023 and CE704 and their hybrid 2023×CE704) treated with 10^{-12} M aqueous solutions of 24-epibrassinolide (24-EPI), synthetic androstane analogue of castasterone (AAC) or with tap water (control). Genotypes (G), brassinosteroid treatments (T) and their interaction (G×T) were included as the possible sources of variation for *ANOVA*. The levels of probability (*P*) are shown; the differences between individual genotypes or between 24-EPI, AAC and control treatments were statistically significant only in those cases where $P \leq 0.05$. Leaves are numbered beginning with the last fully developed leaf in the time of brassinosteroid treatment as leaf 0.

	Leaf 2			Leaf 4			Leaf 6		
	G	T	G×T	G	T	G×T	G	T	G×T
SLM	0.168	0.983	0.999	0.036	0.838	0.994	< 0.001	0.900	0.933
PS 1 activity	< 0.001	0.306	0.539	< 0.001	0.318	0.822	< 0.001	0.886	0.745
HRA	0.009	0.552	0.508	0.065	0.327	0.989	< 0.001	0.382	0.821
Chl *a*	< 0.001	0.456	0.655	< 0.001	0.545	0.915	< 0.001	0.885	0.998
Chl *b*	< 0.001	0.666	0.722	< 0.001	0.665	0.987	< 0.001	0.936	0.999
CAR	0.029	0.263	0.974	< 0.001	0.497	0.721	< 0.001	0.740	0.955
Chl *a*/*b*	0.972	0.829	0.991	0.405	0.954	0.958	0.175	0.941	1.000
Chls/CARs	< 0.001	0.277	0.884	< 0.001	0.607	0.697	< 0.001	0.694	0.908

as well. Several authors have examined the photosynthetic efficiency of PS 2 (mainly using Chl fluorescence measurements) in plants treated with BRs and usually either did not observe any effect of these compounds (Yu *et al.* 2004, Ali *et al.* 2008c, Ogweno *et al.* 2008) on the PS 2 activity or the effect was significant only in case these plants were at the same time exposed to some stressful conditions (Shahbaz *et al.* 2008, Zhang *et al.* 2008). Thus, it seems that the positive role of BRs in the regulation of photosynthesis is not associated with their effect on the primary photochemistry but that it concerns some other parts of photosynthetic processes.

References

Ahmadzadeh, A., Lee, E.A., Tollenaar, M.: Heterosis for leaf CO_2 exchange rate during the grain-filling period in maize. - Crop Sci. **44**: 2095-2100, 2004.

Ali, B., Hasan, S.A., Hayat, S., Hayat, Q., Yadav, S., Fariduddin, Q., Ahmad, A.: A role for brassinosteroids in the amelioration of aluminium stress through antioxidant system in mung bean (*Vigna radiata* L. Wilczek). - Environ. exp. Bot. **62**: 153-159, 2008a.

Ali, B., Hayat, S., Fariduddin, Q., Ahmad, A.: 24-epibrassinolide protects against the stress generated by salinity and nickel in *Brassica juncea*. - Chemosphere **72**: 1387-1392, 2008b.

Ali, Q., Athar, H.R., Ashraf, M.: Modulation of growth, photosynthetic capacity and water relations in salt stressed wheat plants by exogenously applied 24-epibrassinolide. - Plant Growth Regul. **56**: 107-116, 2008c.

Baer, G.R., Schrader, L.E.: Inheritance of DNA concentration, and cellular contents of soluble protein, chlorophyll, ribulose bisphosphate carboxylase, and pyruvate Pi dikinase activity in maize leaves. - Crop Sci. **25**: 916-923, 1985.

Bajguz, A., Hayat, S.: Effect of brassinosteroids on the plant responses to environmental stresses. - Plant Physiol. Biochem. **47**: 1-8, 2009.

Fariduddin, Q., Ahmad, A., Hayat, S.: Photosynthetic response of *Vigna radiata* to pre-sowing seed treatment with 28-homobrassinolide. - Photosynthetica **41**: 307-310, 2003.

Fariduddin, Q., Ahmad, A., Hayat, S.: Responses of *Vigna radiata* to foliar application of 28-homobrassinolide and kinetin. - Biol. Plant. **48**: 465-468, 2004.

Fariduddin, Q., Khanam, S., Hasan, S.A., Ali, B., Hayat, S., Ahmad, A.: Effect of 28-homobrassinolide on the drought stress-induced changes in photosynthesis and antioxidant system of *Brassica juncea* L. - Acta Physiol. Plant. **31**: 889-897, 2009a.

Fariduddin, Q., Yusuf, M., Hayat, S., Ahmad, A.: Effect of 28-homobrassinolide on antioxidant capacity and photosynthesis in *Brassica juncea* plants exposed to different levels of copper. - Environ. exp. Bot. **66**: 418-424, 2009b.

Farooq, M., Wahid, A., Basra, S.M.A., Din, I.: Improving water relations and gas exchange with brassinosteroids in rice under drought stress. - J. Agron. Crop Sci. **195**: 262-269, 2009.

Grove, M.D., Spencer, G.F., Rohwedder, W.K., Mandava, N., Worley, J.F., Warthen, J.D., Jr., Steffens, G.L., Flippen-Anderson, J.L., Cook, J.C., Jr.: Brassinolide, a plant growth-promoting steroid isolated from *Brassica napus* pollen. - Nature **281**: 216-217, 1979.

Hall, D.O.: Nomenclature for different types of isolated chloroplasts. - Nature New Biol. **235**: 125-126, 1972.

Haubrick, L.L., Assmann, S.M.: Brassinosteroids and plant function: some clues, more puzzles. - Plant Cell Environ. **29**: 446-457, 2006.

Hayat, S., Ahmad, A., Mobin, M., Fariduddin, Q., Azam, Z.M.: Carbonic anhydrase, photosynthesis, and seed yield in mustard plants treated with phytohormones. - Photosynthetica **39**: 111-114, 2001.

Hayat, S., Ahmad, A., Mobin, M., Hussain, A., Fariduddin, Q.: Photosynthetic rate, growth and yield of mustard plants sprayed with 28-homobrassinolide. - Photosynthetica **38**: 469-471, 2000.

Hayat, S., Ali, B., Hasan, S.A., Ahmad, A.: Brassinosteroid enhanced the level of antioxidants under cadmium stress in *Brassica juncea*. - Environ. exp. Bot. **60**: 33-41, 2007.

Holá, D., Kočová, M., Körnerová, M., Sofrová, D., Sopko, B.: Genetically based differences in photochemical activities of isolated maize (*Zea mays* L.) mesophyll chloroplasts. - Photosynthetica **36**: 187-197, 1999.

Holá, D., Kočová, M., Rothová, O., Wilhelmová, N., Benešová, M.: Recovery of maize (*Zea mays* L.) inbreds and hybrids from chilling stress of various duration: Photosynthesis and antioxidant enzymes. - J. Plant Physiol. **164**: 868-877, 2007.

Janeczko, A., Gullner, G., Skoczowski, A., Dubert, F., Barna, B.: Effects of brassinosteroid infiltration prior to cold treatment on ion leakage and pigment contents in rape leaves. - Biol. Plant. **51**: 355-358, 2007.

Kočová, M., Holá, D., Wilhelmová, N., Rothová, O.: The influence of low-temperature on the photochemical activity of chloroplasts and activity of antioxidant enzymes in maize leaves. - Biol. Plant. **53**: 475-483, 2009.

Kohout, L.: New method for preparation of brassinosteroids. - Coll. Czech Chem. Commun. **59**: 457-460, 1994.

Mehta, H., Sarkar, K.R., Sharma, S.K.: Genetic analysis of photosynthesis and productivity in corn. - Theor. appl. Genet. **84**: 242-255, 1992.

Ogweno, J.O., Song, X.S., Shi, K., Hu, W.H., Mao, W.H., Zhou, Y.H., Yu, J.Q., Nogués, S.: Brassinosteroids alleviate heat-induced inhibition of photosynthesis by increasing carboxylation efficiency and enhancing antioxidant systems in *Lycopersicon esculentum*. - J. Plant Growth Regul. **27**: 49-57, 2008.

Sairam, R.K.: Effect of homobrassinolide application on metabolic activity and grain yield of wheat under normal and water-stress condition. - J. Agron. Crop Sci. **173**: 11-16, 1994a.

Sairam, R.K.: Effect of homobrassinolide application on plant metabolism and grain yield under irrigated and moisture-stress conditions of two wheat varieties. - Plant Growth Regul. **14**: 173-181, 1994b.

Shahbaz, M., Ashraf, M., Athar, H.R.: Does exogenous application of 24-epibrassinolide ameliorate salt induced growth inhibition in wheat (*Triticum aestivum* L.)? - Plant Growth Regul. **55**: 51-64, 2008.

Singh, I., Shono, M.: Physiological and molecular effects of 24-epibrassinolide, a brassinosteroid on thermotolerance of tomato. - Plant Growth Regul. **47**: 111-119, 2005.

Wang, Z.Y., He, J.X.: Brassinosteroid signal transduction – choices of signals and receptors. - Trends Plant Sci. **9**: 91-96, 2004.

Wellburn, A.R.: The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. - Plant Physiol. **144**: 307-313, 1994.

Xia, X.J., Huang, Y.Y., Wang, L., Huang, L.F., Yu, Y.L., Zhou, Y.H., Yu, J.Q.: Pesticides-induced depression of photosynthesis was alleviated by 24-epibrassinolide pretreatment in *Cucumis sativus* L. - Pest. Biochem. Physiol. **86**: 42-48, 2006.

Yu, J.Q., Huang, L.F., Hu, W.H., Zhou, Y.H., Mao, W.H., Ye, S.F., Nogués, S.: A role for brassinosteroids in the regulation of photosynthesis in *Cucumis sativus*. - J. exp. Bot. **55**: 1135-1143, 2004.

Zhang, M., Zhai, Z., Tian, X., Duan, L., Li, Z.: Brassinolide alleviated the adverse effect of water deficits on photosynthesis and the antioxidant of soybean (*Glycine max* L.). - Plant Growth Regul. **56**: 257-264, 2008.

5.2. Ovlivňují brassinosteroidy reakci rostlin na abiotické stresory?

Tato problematika byla zpracována v publikaci

Honnerová J., Rothová O., Holá D., Kočová M., Kohout L., Kvasnica M. (2010): The Exogenous Application of Brassinosteroids to *Zea mays* (L.) Stressed by long-term chilling does not affect the activities of Photosystem 1 or 2. *Journal of Plant Growth Regulation* **61**: 500-505. IF 1,990

Tato práce navazuje na širší výzkum probíhající v naší laboratoři, který se zabývá vlivem abiotických stresorů na vnitrodruhovou genetickou variabilitu rostlin. V této studii jsme mladé rostliny kukuřice (inbrední linie 2023), pěstované ve skleníku, ošetřili postřikem vodných roztoků o čtyřech různých koncentracích (10^{-14}, 10^{-12}, 10^{-10} a 10^{-8}M) 24E a AAC a poté je vystavili dlouhodobému chladovému stresu. Kontrolní rostliny byly dvojího typu, jednak rostliny neošetřené BRs a rovněž vystavené chladu, a dále ošetřené a neošetřené rostliny ponechané v optimálních teplotních podmínkách. Velmi nízká (10^{-14} M) koncentrace AAC aplikovaná na nestresované rostliny způsobila statisticky průkazné zvětšení délky 4. až 7. dospělého listu i zvýšení obsahu všech sledovaných fotosyntetických pigmentů (chlorofyl *a*, chlorofyl *b* i celkové karotenoidy) v listech. U rostlin rostoucích v chladu však byla reakce na ošetření odlišná, pozitivní vliv se projevil vždy pouze při ošetření vyššími koncentracemi BRs (10^{-12}, 10^{-10} a 10^{-8} M) a pouze u obsahů chlorofylu. Nepodařilo se nám zjistit statisticky průkazný vliv BRs na primární fotosyntetické procesy (aktivitu Hillovy reakce ani aktivitu PS1).

V současné době se zabývám také vlivem ošetření BR na rostliny, které jsou stresovány vodním deficitem. Tato problematika zatím nebyla zpracována do impaktovaných článků, průběžné výsledky byly prezentovány pouze ve článcích ve sbornících a formou plakátových sdělení na konferencích.

J Plant Growth Regul (2010) 29:500–505
DOI 10.1007/s00344-010-9153-0

SHORT COMMUNICATIONS

The Exogenous Application of Brassinosteroids to *Zea mays* (L.) Stressed by Long-Term Chilling Does Not Affect the Activities of Photosystem 1 or 2

Jana Honnerová · Olga Rothová · Dana Holá · Marie Kočová · Ladislav Kohout · Miroslav Kvasnica

Received: 24 August 2009 / Accepted: 7 April 2010 / Published online: 11 May 2010

Abstract The effect of various concentrations of exogenously applied 24-epibrassinolide (E) and 2α,3α,17β-trihydroxy-5α-androstan-6-one (A) on the activities of Photosystem 1 and the Hill reaction, the contents of photosynthetic pigments, and the growth of plants was examined in young maize (*Zea mays* L.) plants subjected to long-term chilling stress or grown in normal-temperature conditions. Neither the activity of Photosystem 1 nor the Hill reaction activity of plants was in any way affected by the treatment with brassinosteroids (BRs), which suggests that the photosynthetic complexes of thylakoid membranes are not the primary site of the influence of BRs on photosynthesis. An extremely low (10^{-14} M) concentration of A applied to the nonstressed plants significantly increased the length of their 4th to the 7th leaves and their height, as well as the contents of chlorophylls *a* and *b* and total carotenoids. However, under chilling conditions, this positive effect was significant for the chlorophyll content only and higher concentrations of BRs (10^{-12}, 10^{-10}, 10^{-8} M) usually had no effect at all.

Keywords Brassinosteroids · Carotenoids · Chlorophylls · Growth · Hill reaction · Low-temperature stress · Photosynthesis · Photosystem 1 · *Zea mays*

J. Honnerová · O. Rothová · D. Holá (✉) · M. Kočová
Faculty of Science, Department of Genetics and Microbiology, Charles University in Prague, Viničná 5, 128 43 Prague, Czech Republic
e-mail: danahola@natur.cuni.cz

L. Kohout · M. Kvasnica
Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i., Flemingovo sq. 2, 166 10 Prague, Czech Republic

Introduction

Brassinosteroids (BRs) are polyhydroxylated steroids that are able to act at extremely low concentrations and are pleiotropic with respect to their effect on plant development, morphology, and physiology (Rao and others 2002). In addition to their role in promoting plant growth and development, BRs possess the ability to protect plants against various biotic and abiotic stressors and together with other plant hormones have been shown to participate in regulation of stress responses (recently reviewed by Bajguz and Hayat 2009).

The positive effect of BRs on stressed plants can result from various changes in plant cells, with one of these changes being the modification of the efficiency of photosynthetic processes. The exogenous application of BRs has been shown to ameliorate a stress-induced decrease in the net photosynthetic rate (P_N) in several plant species, for example, *Triticum aestivum* (Sairam 1994; Ali and others 2008c; Shahbaz and others 2008), *Brassica juncea* (Hayat and others 2007; Ali and others 2008b; Fariduddin and others 2009a, b), *Vigna radiata* (Ali and others 2008a), *Lycopersicon esculentum* (Ogweno and others 2008), or *Glycine max* (Zhang and others 2008). However, the exact causes of this phenomenon are far from clear. BRs could prevent loss of photosynthetic pigments, for example, by activating enzymes that participate in chlorophyll biosynthesis (or an induction of their synthesis), as suggested by Hayat and others (2007), Ali and others (2008a, b), or Fariduddin and others (2009a, b). Another possibility is that BRs improve the efficiency of photosynthetic carbon fixation, for example, by overcoming stomatal limitations and, thus, increasing the internal concentration of CO_2 available for photosynthetic enzymes (Ali and others 2008b), although Shahbaz and others (2008) reported that

the BR-induced increase or decrease in stomatal conductance in salt-stressed wheat plants was not related to any significant changes in substomatal CO_2 concentration. BRs could also induce synthesis and/or activation of carbonic anhydrase that catalyzes the interconversion of CO_2 and HCO_3^- (Hayat and others 2007), increases the activation state of ribulose-1,5-carboxylase/oxygenase (Rubisco) (Ali and others 2008c; Ogweno and others 2008), or protects enzymes involved in the regeneration of ribulose-1,5-bisphosphate (Ogweno and others 2008).

The possible role of BRs in improved efficiency of primary photosynthetic processes in plants subjected to unfavorable conditions is yet more obscure, as some authors have described a positive response of the primary photochemistry of stressed plants to exogenously applied BRs (Janeczko and others 2005; Ogweno and others 2008; Zhang and others 2008), whereas others did not observe any such phenomenon (Ali and others 2008c; Shahbaz and others 2008). Moreover, all these studies have addressed only the question of the effects of BRs on the Photosystem (PS) 2 complex; as far as we know, no one has yet attempted to study the possible effect of BRs on PS 1. We have thus decided to examine the response of this photosynthetic complex to BRs and to compare it with the effect of these hormones on Hill reaction activity (HRA), the content of photosynthetic pigments, and selected morphological and growth parameters in maize plants subjected to optimum or suboptimum (long-term chilling) conditions.

Materials and Methods

Kernels of maize (*Zea mays* L., inbred line 2023) were sown into containers (35 cm × 15 cm × 6 cm) filled with garden soil (10 plants per container, the total number of plants = 2000) and allowed to germinate in the greenhouse at 21–25/17–22°C day/night for 10 days. At day 11, seedlings with fully developed 1st leaves were divided into two groups. One group (approximately half of the seedlings) was transferred into the cold greenhouse (chill stress), the other half of the seedlings were transferred into the warm greenhouse (control), and the plants were allowed to develop for another 50 days. The temperature in the cold greenhouse was maintained at 8°C during night and early morning, gradually increased to 23°C from 9:00 to 15:00 (central European time), and then decreased again to 8°C from 15:00 to 22:00. Similarly, in the warm greenhouse, the night temperature was 17°C, increased to 29°C at 15:00, and again decreased in the afternoon and early evening until 22:00. Plants were grown at natural irradiance conditions and watered daily as necessary, and the relative humidity was maintained at 60/80% day/night in both greenhouses.

The stock solutions of 24-epibrassinolide (E) and 2α,3α,17β-trihydroxy-5α-androstan-6-one (A) were prepared by dissolving these BRs in 1 cm^3 ethanol, adding 1 cm^3 dimethyl sulfoxide, and diluting with distilled water to 10^{-4} M concentration. This stock solution was then diluted with distilled water to 10^{-8}, 10^{-10}, 10^{-12}, and 10^{-14} M concentrations that were used for the treatment of plants. Foliar spray with the appropriate aqueous solution of BRs or with distilled water was applied to plants at the time of their transfer into the cold or warm greenhouses (that is, day 11) and again at day 25. The experiments were in four replicates with a completely randomized design, and each temperature/BR treatment combination was represented by at least 25 plants per replicate. At day 39 (counting from the date of sowing, DAS), the majority of the photosynthetic measurements were made, whereas the evaluation of morphological and growth parameters was done both before and after this day until the plants were 60 days old.

At 39 DAS, the 3rd leaves were cut off from 12 plants in each temperature/BR treatment replicate and mesophyll chloroplasts were isolated from the middle part of the leaf blade as described by Holá and others (2003). Chloroplast suspensions were stored at 0°C in the dark and used for polarographic measurements of the activities of PS 1 and HRA (Clark-type oxygen electrode, Theta′90, Czech Republic) as described by Holá and others (2003), that is, as the amount of oxygen consumed (PS1) or produced (HRA) by irradiated chloroplast suspensions after the addition of artificial electron acceptors and/or donors. The contents of chlorophylls (Chl) *a* and *b* and the content of total carotenoids (Car) were determined spectrophotometrically (Wellburn 1994) and the ratios of Chl *a*/*b* and total Chl/Car were calculated.

The heights of whole plants (that is, the distance from the ground to the ligule of the youngest fully developed leaf) and the numbers and lengths of fully developed leaves were recorded in regular intervals (7 days) starting 1 week after the initial BR treatment (that is, 18 DAS). The lengths of the 4th to 7th leaves were measured in the control plants only as these leaves were not yet fully developed in the chill-stressed plants by the end of the experiments. These measurements were made on six randomly selected plants in each temperature/BR treatment replicate.

The data from all measurements were first subjected to two-way ANOVA with interactions, and then data from the control and the chill-stressed plants were analyzed individually by one-way ANOVA. The statistical significance of the differences among individual BR treatments was ascertained using the least significant difference (LSD) test. All statistical evaluations were made with the CoStat v6.204 statistical software (CoHort Software, Monterey, CA, USA).

Results and Discussion

Both PS1 activity and HRA were negatively affected by the exposure of plants to long-term chilling (Tables 1, 2), which is in good agreement with previously published results (for example, Kudoh and Sonoike 2002; Zhang and Scheller 2004; Holá and others 2003). The contents of Chls *a* and *b*, as well as the total Car content, also significantly decreased in leaves of the chill-stressed plants, with carotenoids less affected than Chls (Tables 1, 2). The degradation of photosynthetic pigments, particularly Chls, is another symptom frequently associated with chilling-induced stress (Haldimann 1998). With respect to plant morphology, the long-term chilling significantly decreased both the length of the 2nd and 3rd leaves (Tables 1, 3) and the plant height (Fig. 1; Table 1), which agrees with our previously published results (for example, Holá and others 2003).

The treatment of plants with BRs did not significantly change the values of the PS1 or the HRA activity in either the stressed plants or the controls (Tables 1, 2). This finding is, in our opinion, the most important result of our study. The effect of BRs on the activity of PS1 has not been previously analyzed, although this photosystem is just as important to the proper functioning of photosynthetic processes as PS2 (in some plants subjected to the combination of chilling and light, the activity of PS1 can be the limiting factor for the efficiency of primary photochemistry; Zhang and Scheller 2004). The HRA parameter is, to a certain degree, a measure of the activity of PS2. The possible influence of BRs on the activity of this photosynthetic complex has been studied more, but the results are often conflicting. The majority of authors who examined the possible effect of BRs on primary photochemistry (usually by analysis of chlorophyll fluorescence) did not find any significant effect when plants were grown under optimum conditions (for example, Ali and others 2008c; Ogweno and others 2008; Shahbaz and others 2008), but in some cases the efficiency of PS2 was improved by treatment with BRs in plants stressed, for example, by drought (Zhang and others 2008), salinity (Shahbaz and others 2008), or the presence of cadmium (Janeczko and others 2005). As far as we know, no data exist as yet on the effects of BRs on the PS2 photochemistry in plants stressed by low temperature, and our results do not seem to point to the possibility that the efficiency of this photosystem can be improved by BR treatment in such environmental conditions (at least not in maize). We thus strongly support the view of Yu and others (2004) that the photosynthetic complexes of thylakoid membranes indeed are not the primary site of BR influence

Table 1 Results of the analysis of variance (ANOVA) of the data for selected photosynthetic and morphological parameters of chill-stressed (stress) or nonstressed (control) maize plants

Parameter	Two-way ANOVA			One-way ANOVA	
	T	BRs	T × BRs	Control BRs	Stress BRs
Photosystem 1 activity	0	0.173	0.979	0.711	0.178
Hill reaction activity	0	0.853	0.962	0.903	0.924
Chlorophyll *a* content	0	0	0.009	0	0
Chlorophyll *b* content	0	0	0.002	0	0.001
Carotenoids content	0	0.002	0.495	0.043	0.083
Chlorophyll *a*/*b* ratio	0.074	0.570	0.463	0.524	0.517
Chlorophyll/carotenoids ratio	0	0	0.035	0	0.001
Plant height (18-day-old plants)	0	0	0	0.001	0
Plant height (25-day-old plants)	0	0	0	0.001	0
Plant height (32-day-old plants)	0	0.001	0	0.001	0.004
Plant height (39-day-old plants)	0	0.001	0	0.001	0.330
Plant height (46-day-old plants)	0	0.002	0.003	0.011	0.245
Plant height (53-day-old plants)	0	0.024	0.021	0.045	0.740
Plant height (60-day-old plants)	0	0.030	0.010	0.024	0.500
Final length of the 1st leaf	0.607	0.270	0.235	0.512	0.043
Final length of the 2nd leaf	0	0.594	0.013	0.219	0.012
Final length of the 3rd leaf	0	0.721	0.001	0.083	0.015

Temperature conditions (T) or brassinosteroid treatments (BRs) and their interaction were included as the possible sources of variation for two-way ANOVA. One-way ANOVA was then individually applied to the data from either stressed or control plants. The levels of statistical significance (*P*) are shown

on photosynthesis and that any eventual changes in the parameters associated with the efficiency of photosynthetic electron transport are probably more of a secondary nature and caused indirectly by, for example, the increased efficiency of the Calvin cycle that results in an increased demand for ATP and NADPH production.

The effect of BRs on the content of photosynthetic carotenoids has also not been examined much. Our results seem to indicate that these pigments are affected by BRs to a lesser extent than Chls (Table 2), which is in good agreement with the results published by other authors (Janeczko and others 2005; Cevahir and others 2008).

The application of 10^{-14} M A significantly increased the contents of Chls *a* and *b* in plants grown in both normal and suboptimal temperature conditions, and the same was true for 10^{-12} or 10^{-10} M solution of A or 10^{-14} M E solution sprayed on the control plants (Table 2). The plants stressed by long-term chilling and treated with other concentrations of BRs were not significantly affected by this treatment compared to the plants treated with water only, and in no case (even with the use of 10^{-14} M A) did the treatment of the chill-stressed plants with BRs increase the content of Chls to the level observed in the control plants. Similar dependence of the BR effect on the concentration of BR used was found for plant morphology, where the control plants sprayed with 10^{-14} M solution of either E or A showed significantly greater lengths of the 5th, 6th, and 7th leaves and greater height by the end of the monitoring period compared to the untreated plants (Fig. 1d; Table 3). On the other hand, the application of 10^{-8} M A or E decreased the lengths of the 4th to the 7th leaves and the height of the control plants (Fig. 1; Table 3). The lengths of the individual leaves on the plants subjected to suboptimal temperatures were not significantly affected by BR treatment (with the exception of the application of 10^{-12} M E, which decreased the length of the 3rd leaf), and the effect of BRs on the height of these plants was significant only where the 10^{-14} M solution of E was used (or, during the first week after BR application, the same concentration of A) (Fig. 1; Table 3). It was shown previously that the effective concentration range for any BR can be very narrow, differ quite substantially among plant species, and depend on both the type of BR applied and the mode of its application (for example, Cevahir and others 2008; Fariduddin and others 2009b).

We can thus conclude that although the exogenously applied BRs can perhaps somehow alleviate the negative effect of long-term chilling stress on photosynthesis in maize plants by diminishing the degradation of chlorophylls (and even in this case, the positive effect of BRs on the contents of these photosynthetic pigments manifests itself only when extremely low concentrations of BRs are used and strongly depends on the type of BR), they do not

Table 2 Effect of 24-epibrassinolide (E) and 2α,3α,17β-trihydroxy-5α-androstan-6-one (A) on the activity of Photosystem 1, Hill reaction activity, contents of chlorophylls *a* and *b*, and total carotenoids in leaves of chill-stressed (stress) or nonstressed (control) maize plants

Treatment	Photosystem 1 activity (μmol O_2 kg^{-1} DM s^{-1})		Hill reaction activity (μmol O_2 kg^{-1} DM s^{-1})		Chlorophyll *a* content (mg kg^{-1} DM)		Chlorophyll *b* content (mg kg^{-1} DM)		Carotenoids content (mg kg^{-1} DM)	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Water	0.91 ± 0.03^{b}	0.62 ± 0.01^{c}	0.31 ± 0.01^{a}	0.18 ± 0.01^{d}	13.98 ± 0.40^{e}	8.14 ± 0.12^{e}	4.06 ± 0.10^{cd}	2.26 ± 0.05^{f}	2.50 ± 0.05^{b}	1.97 ± 0.02^{e}
10^{-8} M E	0.94 ± 0.05^{ab}	0.65 ± 0.04^{c}	0.28 ± 0.02^{abc}	0.20 ± 0.01^{cd}	14.10 ± 0.42^{bc}	8.95 ± 0.35^{de}	4.03 ± 0.15^{cd}	2.45 ± 0.08^{ef}	2.49 ± 0.11^{b}	2.13 ± 0.11^{c}
10^{-10} M E	0.94 ± 0.07^{ab}	0.65 ± 0.01^{c}	0.34 ± 0.06^{a}	0.22 ± 0.05^{bcd}	14.10 ± 0.78^{bc}	8.82 ± 0.18^{e}	4.02 ± 0.14^{cd}	2.44 ± 0.11^{ef}	2.67 ± 0.13^{b}	2.13 ± 0.06^{c}
10^{-12} M E	0.91 ± 0.02^{ab}	0.59 ± 0.05^{c}	0.31 ± 0.01^{a}	0.18 ± 0.01^{d}	14.11 ± 0.35^{bc}	7.39 ± 0.23^{e}	4.02 ± 0.08^{cd}	2.23 ± 0.07^{f}	2.51 ± 0.06^{b}	1.94 ± 0.04^{e}
10^{-14} M E	0.94 ± 0.05^{ab}	0.65 ± 0.04^{c}	0.28 ± 0.02^{abc}	0.20 ± 0.01^{cd}	15.52 ± 0.62^{b}	8.35 ± 0.43^{e}	4.59 ± 0.10^{b}	2.30 ± 0.10^{f}	2.57 ± 0.09^{b}	1.98 ± 0.12^{e}
10^{-8} M A	0.86 ± 0.04^{b}	0.56 ± 0.02^{c}	0.30 ± 0.01^{ab}	0.18 ± 0.01^{d}	13.71 ± 0.23^{c}	8.10 ± 0.40^{e}	3.95 ± 0.06^{d}	2.24 ± 0.08^{f}	2.52 ± 0.03^{b}	1.94 ± 0.06^{e}
10^{-10} M A	1.02 ± 0.08^{a}	0.64 ± 0.03^{c}	0.33 ± 0.07^{a}	0.20 ± 0.06^{cd}	14.95 ± 0.91^{bc}	8.25 ± 0.42^{e}	4.67 ± 0.17^{b}	2.32 ± 0.21^{f}	2.60 ± 0.12^{b}	2.02 ± 0.02^{e}
10^{-12} M A	0.91 ± 0.09^{ab}	0.58 ± 0.04^{c}	0.32 ± 0.02^{a}	0.18 ± 0.02^{d}	15.44 ± 1.14^{b}	8.00 ± 0.36^{e}	4.44 ± 0.40^{bc}	2.20 ± 0.11^{f}	2.75 ± 0.20^{ab}	2.07 ± 0.09^{e}
10^{-14} M A	0.86 ± 0.04^{b}	0.56 ± 0.02^{c}	0.30 ± 0.01^{ab}	0.18 ± 0.01^{d}	19.77 ± 0.92^{a}	10.45 ± 0.19^{d}	5.80 ± 0.42^{a}	2.92 ± 0.05^{e}	2.98 ± 0.13^{a}	2.16 ± 0.05^{c}
LSD ($P \leq 0.05$)	0.13		0.09		1.63		0.49		0.26	

DM dry matter

Plants were sprayed with aqueous solutions of brassinosteroids (concentrations 10^{-8} to 10^{-14} M) or with distilled water. The mean values ± SEM ($n = 6$) are given with the LSD values. Values followed by different letters significantly differ at $P \leq 0.05$

Table 3 Effect of 24-epibrassinolide (E) and 2α,3α,17β-trihydroxy-5α-androstan-6-one (A) on the final lengths (mm) of the 2nd to the 7th leaves of chill-stressed (stress) or nonstressed (control) maize plants

Treatment	Leaf 2		Leaf 3		Leaf 4	Leaf 5	Leaf 6	Leaf 7
	Control	Stress	Control	Stress	Control	Control	Control	Control
Water	153 ± 5^{abcd}	134 ± 3^{defgh}	269 ± 8^{abc}	191 ± 5^{fg}	383 ± 7^{a}	450 ± 7^{bcd}	439 ± 8^{bc}	391 ± 5^{b}
10^{-8} M E	154 ± 9^{abcd}	118 ± 4^{h}	285 ± 14^{ab}	161 ± 4^{gh}	398 ± 8^{a}	441 ± 5^{cde}	407 ± 6^{cd}	339 ± 7^{c}
10^{-10} M E	145 ± 9^{cdefg}	144 ± 7^{cdefg}	253 ± 12^{bcd}	212 ± 9^{ef}	360 ± 8^{ab}	427 ± 6^{de}	430 ± 6^{bc}	376 ± 9^{bc}
10^{-12} M E	173 ± 7^{a}	132 ± 6^{defgh}	296 ± 7^{a}	147 ± 42^{h}	399 ± 14^{a}	476 ± 14^{ab}	ND	ND
10^{-14} M E	157 ± 5^{abc}	132 ± 10^{efgh}	269 ± 6^{abc}	210 ± 13^{ef}	387 ± 6^{a}	485 ± 7^{a}	475 ± 9^{ab}	446 ± 10^{a}
10^{-8} M A	142 ± 4^{cdefg}	129 ± 6^{fgh}	241 ± 10^{cde}	192 ± 9^{fg}	328 ± 30^{b}	419 ± 2^{e}	366 ± 38^{d}	321 ± 19^{c}
10^{-10} M A	141 ± 6^{cdefg}	150 ± 3^{bcdef}	248 ± 9^{bcde}	219 ± 5^{def}	357 ± 9^{ab}	436 ± 12^{cde}	446 ± 8^{abc}	402 ± 9^{b}
10^{-12} M A	167 ± 17^{ab}	124 ± 6^{gh}	295 ± 31^{a}	187 ± 10^{fgh}	402 ± 26^{a}	469 ± 13^{abc}	ND	ND
10^{-14} M A	151 ± 4^{abcde}	133 ± 4^{defgh}	269 ± 7^{abc}	195 ± 8^{fg}	402 ± 14^{a}	486 ± 18^{a}	494 ± 29^{a}	454 ± 25^{a}
LSD ($P \leq 0.05$)	27		48		45	38	67	71

ND leaves were not yet fully developed at the end of the measurements

Plants were sprayed with aqueous solutions of brassinosteroids (concentrations 10^{-8} to 10^{-14} M) or with distilled water. The mean values ± SEM ($n = 6$) are given with the LSD values. Values followed by different letters significantly differ at $P \leq 0.05$

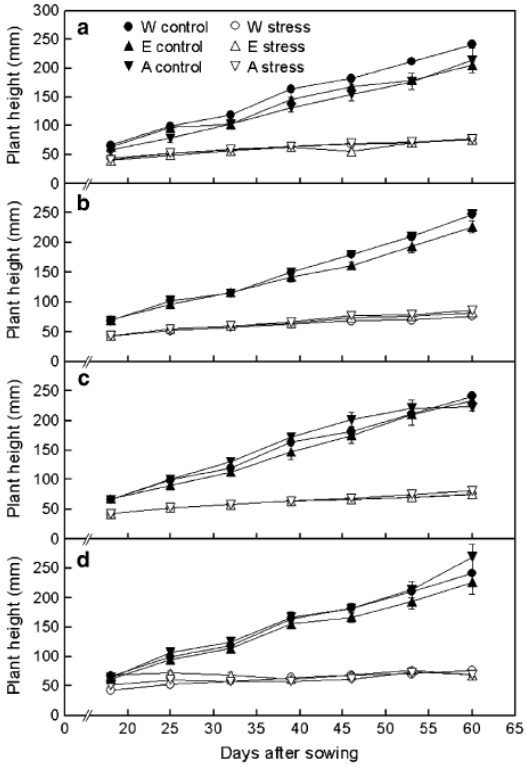


Fig. 1 The effect of brassinosteroids on the height of chill-stressed (stress) or nonstressed (control) maize plants. Plants were sprayed with 10^{-8} M (**a**), 10^{-10} M (**b**), 10^{-12} M (**c**), or 10^{-14} M (**d**) aqueous solutions of 24-epibrassinolide (*E*), 2α,3α,17β-trihydroxy-5α-androstan-6-one (*A*), or distilled water (*W*). The mean values ± SEM ($n = 6$) are shown

improve the efficiency of primary photosynthetic processes and the activities of Photosystem 1 or 2.

Acknowledgments The authors are grateful to Monika Benešová for her help with the measurements of photosynthetic and morphological parameters, and to Dr. Jaroslav Poruba from the CEZEA Breeding Station in Čejč, Czech Republic, for the supply of maize kernels. This study was supported by grant KJB601110611 from the Grant Agency of the Academy of Sciences of the Czech Republic and grant MSM0021620858 from the Ministry of Education, Youth and Sports of the Czech Republic.

References

Ali B, Hasan SA, Hayat S, Hayat Q, Yadav S, Fariduddin Q, Ahmad A (2008a) A role for brassinosteroids in the amelioration of aluminium stress through antioxidant system in mung bean (*Vigna radiata* L. Wilczek). Environ Exp Bot 62:153–159

Ali B, Hayat S, Fariduddin Q, Ahmad A (2008b) 24-Epibrassinolide protects against the stress generated by salinity and nickel in *Brassica juncea*. Chemosphere 72:1387–1392

Ali Q, Athar HR, Ashraf M (2008c) Modulation of growth, photosynthetic capacity and water relations in salt stressed wheat plants by exogenously applied 24-epibrassinolide. Plant Growth Regul 56:107–116

Bajguz A, Hayat S (2009) Effect of brassinosteroids on the plant responses to environmental stresses. Plant Physiol Biochem 47:1–8

Cevahir G, Yentür S, Eryilmaz F, Yilmazer N (2008) Influence of brassinosteroids on pigment content of *Glycine max* L. (soybean) grown in dark and light. J Appl Biol Sci 2:23–28

Fariduddin Q, Khanam S, Hasan SA, Ali B, Hayat S, Ahmad A (2009a) Effect of 28-homobrassinolide on the drought stress-induced changes in photosynthesis and antioxidant system of *Brassica juncea* L. Acta Physiol Plant 31:889–897

Fariduddin Q, Yusuf M, Hayat S, Ahmad A (2009b) Effect of 28-homobrassinolide on antioxidant capacity and photosynthesis in *Brassica juncea* plants exposed to different levels of copper. Environ Exp Bot 66:418–424

Haldimann P (1998) Low growth temperature-induced changes to pigment composition and photosynthesis in *Zea mays* genotypes differing in chilling sensitivity. Plant Cell Environ 21:200–208

Hayat S, Ali B, Hasan SA, Ahmad A (2007) Brassinosteroid enhanced the level of antioxidants under cadmium stress in *Brassica juncea*. Environ Exp Bot 60:33–41

Holá D, Langrová K, Kočová M, Rothová O (2003) Photosynthetic parameters of maize (*Zea mays* L.) inbred lines and F1 hybrids: their different response to, and recovery from rapid or gradual onset of low-temperature stress. Photosynthetica 41:429–442

Janeczko A, Koşcielniak J, Pilipowicz M, Szarek-Łukaszewska G, Skoczowski A (2005) Protection of winter rape photosystem 2 by 24-epibrassinolide under cadmium stress. Photosynthetica 43:293–298

Kudoh H, Sonoike K (2002) Irreversible damage to photosystem I by chilling in the light: cause of the degradation of chlorophyll after returning to normal growth temperature. Planta 215:541–548

Ogweno JO, Song XS, Shi K, Hu WH, Mao WH, Zhou YH, Yu JQ, Nogués S (2008) Brassinosteroids alleviate heat-induced inhibition of photosynthesis by increasing carboxylation efficiency and enhancing antioxidant systems in *Lycopersicon esculentum*. J Plant Growth Regul 27:49–57

Rao SSR, Vardhini BV, Sujatha E, Anuradha S (2002) Brassinosteroids—a new class of phytohormones. Curr Sci 82:1239–1245

Sairam RK (1994) Effects of homobrassinolide application on plant metabolism and grain yield under irrigated and moisture-stress conditions of two wheat varieties. Plant Growth Regul 14:173–181

Shahbaz M, Ashraf M, Athar HR (2008) Does exogenous application of 24-epibrassinolide ameliorate salt induced growth inhibition in wheat (*Triticum aestivum* L.)? Plant Growth Regul 55:51–64

Wellburn AR (1994) The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. Plant Physiol 144:307–313

Yu JQ, Huang LF, Hu WH, Zhou YH, Mao WH, Ye SF, Nogués S (2004) A role for brassinosteroids in the regulation of photosynthesis in *Cucumis sativus*. J Exp Bot 55:1135–1143

Zhang S, Scheller HV (2004) Photoinhibition of Photosystem I at chilling temperature and subsequent recovery in *Arabidopsis thaliana*. Plant Cell Physiol 45:1595–1602

Zhang M, Zhai Z, Tian X, Duan L, Li Z (2008) Brassinolide alleviated the adverse effect of water deficits on photosynthesis and the antioxidant of soybean (*Glycine max* L.). Plant Growth Regul 56:257–264

5.3. Může vnější aplikace i jiné steroidní látky – 20-hydroxyekdysonu – ovlivňovat fotosyntetické procesy?

Tato problematika byla řešena v následující publikaci:

Holá D., Kočová M., Rothová O., Tůmová L., Kamlar M., Macek T. (2013): Exogenously applied 20-hydroxyecdysone increases the net photosynthetic rate but does not affect the photosynthetic electron transport or the content of photosynthetic pigments in *Tetragonia tetragonioides* L. *Acta Physiologia Plantarum* **35**: 3489-3495. IF 1,305

V předložené publikaci byla řešena problematika možného vlivu dalších steroidních látek, ekdysteroidů, běžně se vyskytujících se v rostlinách, na fotosyntetické procesy. V rostlinách byla přítomnost těchto látek mnohokrát prokázána, o jejich fyziologické roli je však dosud minimum údajů. V publikaci je zpracováno naše prioritní zjištění, kdy po ošetření listů pokusných rostlin novozélandského špenátu (*Tetragonia tetragonioides* L.) 2 mM roztokem 20E statisticky průkazně vzrostly hodnoty P_N. Toto zvýšení jsme pozorovali mezi čtvrtou a šestou hodinou po ošetření listu, v delším intervalu již pozorováno nebylo. Žádný pozitivní vliv však nebyl pozorován u většiny parametrů fotosyntetického elektronového transportu (s výjimkou komplexu produkujícího kyslík - OEC) a ani se nezměnily obsahy fotosyntetických pigmentů. Tyto naše výsledky avízují novou potencionální biologickou funkci ekdysteroidů - regulaci fotosyntézy, a vedly nás k zahájení dalších experimentů na toto téma.

Acta Physiol Plant (2013) 35:3489–3495
DOI 10.1007/s11738-013-1379-6

SHORT COMMUNICATION

Exogenously applied 20-hydroxyecdysone increases the net photosynthetic rate but does not affect the photosynthetic electron transport or the content of photosynthetic pigments in *Tetragonia tetragonioides* L.

Dana Holá · Marie Kočová · Olga Rothová · Lenka Tůmová · Marek Kamlar · Tomáš Macek

Received: 1 April 2013 / Revised: 17 June 2013 / Accepted: 30 August 2013 / Published online: 11 September 2013

Abstract Phytoecdysteroids are steroid compounds present in many plant species (sometimes in rather large amounts), but their biological role is still far from being clear. We have found that the exogenous application of 20-hydroxyecdysone (20E) to leaves of *Tetragonia tetragonioides* L. causes stimulation of its net photosynthetic rate (P_N) but does not positively affect the photosynthetic electron transport or the content of photosynthetic pigments. The increase in P_N was observed shortly after 20E treatment and was statistically significant during the 4th and 6th hours after treatment but not later, which could be perhaps caused by a strictly short-term window of opportunity for ecdysteroids to significantly affect photosynthetic processes. To our knowledge, these results are the first to suggest a new potential biological function of phytoecdysteroids—regulation of photosynthesis.

Keywords Ecdysteroids · Photosynthesis · Chlorophylls · Carotenoids · Chlorophyll fluorescence · OJIP analysis · *Tetragonia tetragonioides* L.

Communicated by M. Garstka.

Electronic supplementary material The online version of this article (doi:10.1007/s11738-013-1379-6) contains supplementary material, which is available to authorized users.

D. Holá (✉) · M. Kočová · O. Rothová · L. Tůmová
Department of Genetics and Microbiology, Faculty of Science, Charles University in Prague, Viničná 5, 12844 Prague 2, Czech Republic
e-mail: danahola@natur.cuni.cz

M. Kamlar · T. Macek
Department of Biochemistry and Microbiology, Faculty of Food and Biochemical Technology, Institute of Chemical Technology Prague, Technická 5, 16628 Prague 6, Czech Republic

Abbreviations

20E	20-hydroxyecdysone
Car	Carotenoids
Chl	Chlorophyll
P_N	Net photosynthetic rate
Rubisco	Ribulose 1,5-bisphosphate carboxylase/oxygenase

Introduction

Many species of higher plants contain analogs of arthropod steroid hormones—phytoecdysteroids—in their leaves, roots and seeds (and sometimes also in other organs such as stems, flowers or bulbs) (Dinan et al. 2001). Their concentration can be up to 100-fold higher than ecdysteroid concentration in arthropods, but strongly varies with plant species, organ and developmental stage (Dinan 2009; Bakrim et al. 2008). More than 460 different phytoecdysteroids are currently known to exist (Lafont et al. 2013) and this number steadily increases, particularly as new minor ecdysteroids are being isolated from plant species that have not been previously examined for their presence. The most common phytoecdysteroid in plants is probably 20-hydroxyecdysone (20E) followed by polypodine B (Dinan et al. 2009).

The function of phytoecdysteroids in plants is not clear. The prevailing hypothesis supported by the most evidence is that they act as protective compounds against phytophagous insects and nematodes causing either developmental/reproduction abnormalities or showing antifeedant and/or

deterrent activities (reviewed, e.g., in Dinan 2001, 2009; Festucci-Buselli et al. 2008). The question whether phytoecdysteroids fulfill other biological function(s) in plants remains still open. Dreier and Towers (1988) as well as Macháčková et al. (1995) reported almost no biological activity of 20E as assessed by commonly used plant phytohormone bioassays. However, exogenously applied ecdysteroids have been also shown to stimulate coleoptile or seedlings growth in several plant species (Golovatskaya 2004; Bakrim et al. 2007), to positively affect germination efficiency of tomato seeds (Bakrim et al. 2007), increase α-amylase activity in aleurone layer of barley kernels (Golovatskaya 2004) and enhance DNA, RNA and protein content as well as the content of organic and inorganic phosphorus and saccharides in *Chlorella vulgaris* Beyerinck (Bajguz and Koronka 2001). This seems to point to the possibility of their role in the regulation of plant growth and development and biosynthesis/degradation processes in cells. Clearly, the biological function of phytoecdysteroids in plants (and algae) is far from being elucidated and could be a multiple one similarly to that fulfilled by the other widely represented group of plant sterols, i.e., brassinosteroids.

As affinity chromatography studies made by some of us have revealed that phytoecdysteroids can specifically bind to some photosynthetic proteins (Uhlík et al. 2008; Kamlar et al. 2010a, b) and as the structurally similar brassinosteroids are known to positively affect photosynthetic processes (recently reviewed, e.g, by Holá 2011), we have decided to examine whether the treatment of plants with phytoecdysteroids would also result in an increase of photosynthetic efficiency.

Materials and methods

Plant material, growth conditions and ecdysteroid treatment

Seeds of *Tetragonia tetragonioides* L. (New Zealand spinach) plants were first sown in low dishes filled with garden soil and placed in a naturally lit greenhouse of the Faculty of Science, Charles University in Prague (54°04′ N, 14°25′ E) under semi-controlled conditions (air temperature 25 ± 2/20 ± 2 °C, relative air humidity 50 ± 5/70 ± 5 % day/night). After approximately 40 days, seedlings were transplanted into pots (volume 0.5 dm^3, 1 plant per pot) filled with garden soil and their cultivation in the same greenhouse continued for additional 50 days. Either distilled water (control) or aqueous solution (small amount of Tween® 20 was added in both cases) of 2 mM 20E (this concentration was chosen on the basis of the work of Uhlík et al. 2008) was then applied (using a small soft brush) to both abaxial and adaxial side of the fully developed non-senescent leaves, which were then used for the measurements of the net photosynthetic rate (P_N), the chlorophyll (Chl) *a* fluorescence parameters and the sampling necessary for the determination of the content of photosynthetic pigments, which were made at 1, 2, 4, 6, 24, 48 and 72 h after treatment.

Measurements of the net photosynthetic rate

Net photosynthetic rate was measured between 9:00 a.m. and 1:00 p.m. (Central European Time) with a Clark-type gas-phase leaf disc oxygen electrode (LD2/2, Hansatech, King's Lynn, UK) at 25 °C in CO_2-enriched air (CO_2 was generated in the measurement chamber by the addition of 2 M bicarbonate buffer) as described by Walker (1988). Actinic illumination was provided by a Björkman lamp (LS2, Hansatech, King's Lynn, UK) and its intensity was adjusted using neutral optical filter to 240 μmol m^{-2} s^{-1}. During measurements, leaf disc (3.5 cm diameter, 1 leaf per plant) was initially left for 5 min in the dark and then the lamp was switched on for a further 8 min. The oxygen production recorded for the last 3 min of the light period was used for the calculations of P_N (during this time, the rate of oxygen production was linear).

Chlorophyll fluorescence measurements

Measurements of the polyphasic rise of Chl *a* fluorescence transient (O-J-I-P) were made on the upper surface of dark-adapted (30 min) leaves (1 leaf per plant, 2 technical replicates of each measurement) in situ with the portable fluorometer FluorPen FP100max (Photon System Instruments, Brno, Czech Republic). Saturating pulse (blue light, 455 nm) was set at 3,000 μmol m^{-2} s^{-1}. All fluorescence transients were recorded with a time scan from 10 μs to 2 s with the data acquisition rate of 1 reading per 10 μs for the first 600 μs, 1 reading per 100 μs till $t = 14$ ms, 1 reading per 1 ms till $t = 90$ ms and 1 reading per 10 ms for the rest of the recording period. Various parameters of the JIP test as well as relative variable fluorescence and difference kinetics were calculated from these measurements based on the theory of energy flow in photosynthetic electron-transport chain according to Strasser et al. (2000), Yusuf et al. (2010) and Stirbet and Govindjee (2011).

Determination of the content of photosynthetic pigments

Six leaf discs (0.5 cm diameter) were cut from each leaf (one leaf per plant), placed in 5 cm^3 of *N*,*N*-dimethylformamide and stored in a refrigerator for 7 days with occasional stirring of the extracts. Chl *a*, Chl *b* and total

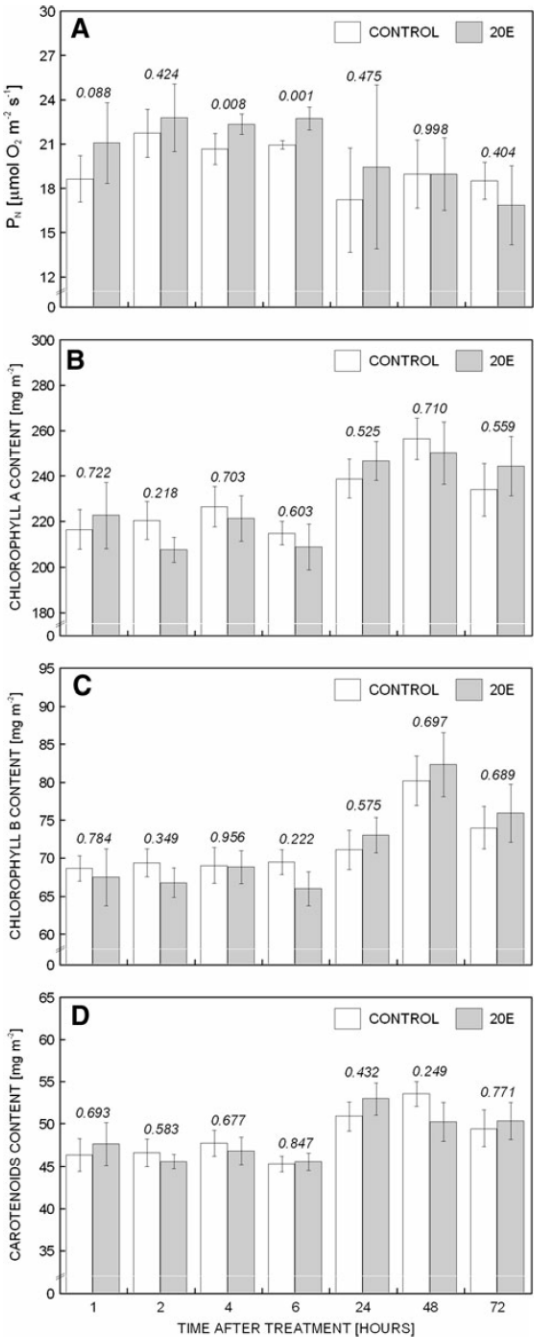


Fig. 1 The net photosynthetic rate (P_N) (**a**) and the content of chlorophylls *a* (**b**), *b* (**c**) and total carotenoids (**d**) in leaves of *Tetragonia tetragonioides* L. measured at various times (1, 2, 4, 6, 24, 48 or 72 h) after treatment of plants with 2 mM aqueous solution of 20-hydroxyecdysone (20E) or water (control). Mean values ± SEM ($n = 6$ for P_N, $n = 8$ for the contents of photosynthetic pigments) are shown. Numbers above the individual column pairs indicate the statistical probability levels obtained from the evaluation of differences between control and 20E treatments using Student′s independent two-sample tests

carotenoids (Car) contents in the extracts were then determined spectrophotometrically (Porra et al. 1989; Wellburn 1994).

Statistical analysis

Each experimental variant (treatment/time) was represented by six to eight biological replicates. Statistical differences between the corresponding control and 20E treatments were determined using Student′s independent two-sample test (CoStat version 6.204, CoHort Software, Monterey, CA, USA).

Results and discussion

The treatment of plants with 20E increased the rate of oxygen evolution from leaf discs during the first 6 and 24 h after treatment (Fig. 1a) with statistically significant differences at 0.05 probability level found for 4 and 6 h after treatment (the other times were not statistically significant due to the larger variability of the samples). This indicates that phytoecdysteroids could play a role not only in the plant defense against herbivorous insects and (possibly) in the regulation of plant elongation and development, as suggested by other authors (Bakrim et al. 2007; Dinan 2001, 2009; Festucci-Buselli et al. 2008; Golovatskaya 2004), but also in the regulation of physiological processes such as photosynthesis.

The exact cause of the 20E-induced stimulation of photosynthesis is at this time point unknown. Although Golovatskaya (2004) has described 20E-caused retardation of leaf senescence assayed as the chlorophyll content in the detached leaves of kidney bean, and Bajguz and Koronka (2001) have also demonstrated the increase of chlorophyll content in *C. vulgaris* cells due to the exogenously applied ecdysone, we did not observe any such effect either for Chls or Car (Fig. 1b–d) which means that the positive effect on photosynthetic pigments (either an increased biosynthesis or a decreased degradation) is probably not the reason for the enhancement of photosynthesis in our case. Brassinosteroids, which are structurally similar to ecdysteroids, have been suggested to regulate the efficiency of photosynthetic electron transport, particularly in plants stressed by various abiotic stressors (e.g., Yu et al. 2004; Janeczko et al. 2005, 2011; Jiang et al. 2012a, b, c; Xia et al. 2009a, b, 2011) and it was thus possible that ecdysteroids could function in a similar manner. The results of

Table 1 The photosynthetic parameters calculated from JIP test based on the chlorophyll *a* fluorescence measurements in leaves of *Tetragonia tetragonioides* L. made at various times (1, 2, 4, 6, 24, 48 or 72 h) after treatment of plants with 2 mM aqueous solution of 20-hydroxyecdysone (20E) or water (C)

Parameter	Biological meaning of the parameter	1 h		2 h		4 h		6 h		24 h		48 h		72 h	
		C	20E	C	20E	C	20E	C	20E	C	20E	C	20E	C	20E
Φ_{P0}	Maximum quantum yield of primary PSII photochemistry	0.79 ± 0.01	0.80 ± 0.01	0.80 ± 0.00	0.78 ± 0.01	0.80 ± 0.01	0.80 ± 0.01	0.79 ± 0.00	0.78 ± 0.01	0.81 ± 0.00	0.80 ± 0.00	**0.80 ± 0.00**	**0.79 ± 0.00**	0.79 ± 0.01	0.80 ± 0.01
Φ_{E0}	Quantum yield of electron-transport flux from Q_A to Q_B	0.43 ± 0.01	0.43 ± 0.01	0.43 ± 0.01	0.41 ± 0.01	0.43 ± 0.01	0.43 ± 0.01	0.41 ± 0.01	0.41 ± 0.02	**0.47 ± 0.01**	**0.45 ± 0.01**	0.46 ± 0.01	0.44 ± 0.01	0.43 ± 0.01	0.44 ± 0.01
Φ_{RE0}	Quantum yield of electron-transport flux until the PSI electron acceptors	0.16 ± 0.02	0.17 ± 0.01	**0.15 ± 0.01**	**0.12 ± 0.01**	0.15 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.18 ± 0.01	0.17 ± 0.01	0.19 ± 0.01	0.20 ± 0.00	0.16 ± 0.01	0.17 ± 0.01
Φ_{D0}	Quantum yield of energy dissipation	0.21 ± 0.01	0.20 ± 0.01	0.20 ± 0.00	0.22 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.21 ± 0.00	0.22 ± 0.01	0.19 ± 0.00	0.20 ± 0.00	**0.20 ± 0.00**	**0.21 ± 0.00**	0.21 ± 0.01	0.20 ± 0.01
ψ_0	Efficiency/ probability with which a PSII trapped electron is transferred from Q_A to Q_B	0.54 ± 0.01	0.54 ± 0.01	0.54 ± 0.01	0.53 ± 0.01	0.54 ± 0.01	0.54 ± 0.01	0.52 ± 0.01	0.53 ± 0.02	**0.58 ± 0.01**	**0.56 ± 0.01**	0.57 ± 0.01	0.56 ± 0.01	0.55 ± 0.01	0.55 ± 0.01
ψ_{R0}	Efficiency/ probability with which a PSII trapped electron is transferred until PSI acceptors	0.20 ± 0.02	0.21 ± 0.01	**0.19 ± 0.01**	**0.15 ± 0.01**	0.19 ± 0.01	0.18 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.23 ± 0.01	0.21 ± 0.01	0.24 ± 0.01	0.25 ± 0.00	0.20 ± 0.01	0.21 ± 0.01
Δ_{R0}	Efficiency/ probability with which an electron from Q_B is transferred until PSI acceptors	0.36 ± 0.03	0.39 ± 0.02	**0.35 ± 0.02**	**0.29 ± 0.02**	0.36 ± 0.01	0.32 ± 0.02	0.31 ± 0.01	0.32 ± 0.01	0.39 ± 0.01	0.38 ± 0.01	**0.41 ± 0.01**	**0.44 ± 0.01**	0.37 ± 0.02	0.38 ± 0.02
γRC	Probability that a PSII chlorophyll functions as RC	0.28 ± 0.00	0.28 ± 0.00	0.29 ± 0.00	0.28 ± 0.00	0.28 ± 0.00	0.28 ± 0.00	0.28 ± 0.00	0.27 ± 0.01	**0.29 ± 0.00**	**0.28 ± 0.00**	0.28 ± 0.00	0.29 ± 0.00	0.28 ± 0.00	0.28 ± 0.00
ABS/RC	Average absorbed photon flux per PSII RC (apparent antenna size of an active PSII)	2.58 ± 0.06	2.52 ± 0.03	2.49 ± 0.05	2.63 ± 0.06	2.57 ± 0.05	2.58 ± 0.06	2.63 ± 0.04	2.72 ± 0.12	**2.51 ± 0.04**	**2.63 ± 0.03**	2.55 ± 0.03	2.45 ± 0.05	2.60 ± 0.04	2.54 ± 0.05
TP_0/RC	Maximum trapped exciton flux per PSII	2.04 ± 0.03	2.01 ± 0.02	1.98 ± 0.03	2.06 ± 0.04	2.05 ± 0.02	2.05 ± 0.04	2.08 ± 0.03	2.11 ± 0.05	**2.03 ± 0.02**	**2.11 ± 0.02**	2.04 ± 0.02	1.94 ± 0.04	2.05 ± 0.02	2.02 ± 0.03
ET_0/RC	Electron-transport flux from Q_A to Q_B per PSII	1.09 ± 0.02	1.09 ± 0.02	1.06 ± 0.02	1.08 ± 0.02	1.10 ± 0.02	1.11 ± 0.01	1.09 ± 0.02	1.10 ± 0.02	1.18 ± 0.01	1.18 ± 0.01	**1.17 ± 0.01**	**1.09 ± 0.03**	1.12 ± 0.02	1.12 ± 0.01
RE_0/RC	Electron-transport flux until PSI acceptors per PSII	0.40 ± 0.04	0.43 ± 0.03	**0.37 ± 0.02**	**0.31 ± 0.02**	0.39 ± 0.02	0.36 ± 0.02	0.33 ± 0.01	0.36 ± 0.02	0.46 ± 0.02	0.44 ± 0.01	0.48 ± 0.01	0.48 ± 0.01	0.41 ± 0.03	0.42 ± 0.03
DI_0/RC	Dissipated energy flux per PSII	0.54 ± 0.03	0.51 ± 0.02	0.50 ± 0.02	0.57 ± 0.03	0.52 ± 0.02	0.53 ± 0.02	0.55 ± 0.02	0.61 ± 0.07	0.49 ± 0.01	0.52 ± 0.01	0.50 ± 0.01	0.51 ± 0.01	0.55 ± 0.02	0.52 ± 0.02

Table 1 continued

Parameter	Biological meaning of the parameter	1 h		2 h		4 h		6 h		24 h		48 h		72 h	
		C	20E	C	20E	C	20E	C	20E	C	20E	C	20E	C	20E
PI_{ABS}	Performance index for energy conservation from photons absorbed by PSII antenna, to the reduction of Q_B	1.78 ± 0.18	1.89 ± 0.16	1.87 ± 0.14	1.60 ± 0.16	1.87 ± 0.19	1.85 ± 0.17	1.60 ± 0.09	1.62 ± 0.21	**2.36 ± 0.12**	**2.00 ± 0.08**	2.18 ± 0.11	2.03 ± 0.11	1.78 ± 0.12	2.00 ± 0.19
PI_{TOTAL}	Performance index for energy conservation from photons absorbed by PSII antenna, until the reduction of PSI acceptors	1.12 ± 0.22	1.27 ± 0.20	**1.05 ± 0.11**	**0.68 ± 0.12**	1.07 ± 0.15	0.93 ± 0.14	0.71 ± 0.05	0.79 ± 0.12	1.55 ± 0.15	1.21 ± 0.09	1.54 ± 0.13	1.62 ± 0.11	1.07 ± 0.14	1.27 ± 0.19

Mean values ± SEM ($n = 8$) are shown (values are given in arbitrary units). Statistically significant differences ($p \leq 0.05$) between control and 20E treatments revealed by Student's independent two-sample tests are shown in bold type. Biological meanings of the individual parameters are based on Strasser et al. (2000), Yusuf et al. (2010) and Stirbet and Govindjee (2011); for exact formulae for their calculation see these references

PS Photosystem

our analysis of parameters calculated from O-J-I-P Chl *a* fluorescence transients did not much support this hypothesis, as the majority of the differences between control and 20E treatments in these parameters were not statistically significant. Those differences that were significant usually demonstrated that control plants had higher values of parameters representing the quantum yields of individual energy fluxes or the efficiencies/probabilities of electron transport compared to the 20E-treated ones (Table 1). However, we also observed the negative value of the K-band (which is visible in the time range of 50 μs to 2 ms at O-J-I-P difference kinetics curves; Strasser 1997) for all examined time intervals with the exception of 72 h (Supplementary Fig. 1). The magnitude of this K-band corresponded rather well with the observed changes in P_N, so this suggests that there could perhaps be a better activation of the oxygen-evolving complex of Photosystem II (OEC) in the 20E-treated plants compared to the control ones. Moreover, one of the OEC proteins was identified as a direct target protein for ecdysteroid ligands by some of us (unpublished data), similarly to ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), which was found by Uhlík et al. (2008) to be another photosynthetic protein specifically binding to 20E in New Zealand spinach. In vitro assay made by these authors showed that various ecdysteroids are able to increase Rubisco carboxylase activity when their concentration is at least tenfold higher than that of Rubisco.Thus, the positive effect of ecdysteroids on photosynthesis could perhaps reflect the regulation of the activity of various photosynthetic proteins by direct ecdysteroid binding. However, at this time point this is purely a speculation which needs to be further supported by, e.g., co-crystallization studies of steroid molecule and its putative target or by computational modeling.

The short-term duration of the 20E-caused stimulation of P_N is not particularly surprising. Yu et al. (2004), as well as Jiang et al. (2012b), who analyzed the effect of exogenously applied 24-epibrassinolide on primary photosynthetic processes, content and/or activity of some enzymes of carbon metabolism, saccharides and photosynthetic pigments and the carboxylation efficiency in *Cucumis sativus* L., have demonstrated that it changes during time and that the stimulation is usually the highest shortly (3–6 h to 24 h) after treatment and starts dropping after 3 days. Xia et al. (2009a, b) have described a somewhat similar effect of this brassinosteroid, although they did use a time range of days rather than hours. As regards ecdysteroids, studies of this type are severely lacking; however, Bakrim et al. (2007) have described the stimulation of tomato shoot elongation by 20E during the 3rd and 4th day after germination, but a rather strong inhibition during the 5th day. In our case, the positive effect of 20E on P_N in leaves of New Zealand spinach was indeed a

rather short-term one, but the situation could be different in other plant species. Our preliminary data from the experiments made with some other plants suggest that this is indeed the case. We think that the window of opportunity for ecdysteroids to significantly affect photosynthetic processes (and probably other physiological processes as well) is only temporal, strictly a short-term one, and strongly depends on the plant species.

As far as we know, our study is the first one to demonstrate that this type of plant steroid compounds can enhance the net photosynthetic rate directly in vivo. It offers an interesting possibility of a new potential biological function of ecdysteroids in higher plants, which should be, in our opinion, examined further.

Author contribution D. Holá designed and conducted the experiments, statistically analyzed data and wrote the manuscript. M. Kočová and O. Rothová designed and conducted the experiments. L. Tůmová conducted the experiments. M. Kamlar and T. Macek supplied the 20-hydroxyecdysone and corrected the manuscript. All authors have read and approved the final version of the manuscript.

Acknowledgments This study was supported by the Czech Science Foundation (Grant 501/11/1650), the Charles University in Prague (Grant SVV-2013-267205) and the Grant Agency of the Charles University in Prague (Grant B/BIO/612612).

References

Bajguz A, Koronka A (2001) Effect of ecdysone application on the growth and biochemical changes in *Chlorella vulgaris* cells. Plant Physiol Biochem 39:707–715. doi:10.1016/S0981-9428(01)01287-6

Bakrim A, Lamhamdi M, Sayah F, Chibi F (2007) Effect of plant hormones and 20-hydroxyecdysone on tomato (*Lycopersicon esculentum*) seed germination and seedlings growth. Afr J Biotechnol 6:2792–2802

Bakrim A, Maria A, Sayah F, Lafont R, Takvorian N (2008) Ecdysteroids in spinach (*Spinacia oleracea* L.): biosynthesis, transport and regulation of levels. Plant Physiol Biochem 46:844–854. doi:10.1016/j.plaphy.2008.06.002

Dinan L (2001) Phytoecdysteroids: biological aspects. Phytochemistry 57:325–339. doi:10.1016/S0031-9422(01)00078-4

Dinan L (2009) The Karlson lecture. Phytoecdysteroids: what use are they? Arch Insect Biochem Physiol 72:126–141. doi:10.1002/arch.20334

Dinan L, Savchenko T, Whiting P (2001) On the distribution of phytoecdysteroids in plants. Cell Mol Life Sci 58:1121–1132. doi:10.1007/PL00000926

Dinan L, Harmatha J, Volodin V, Lafont R (2009) Phytoecdysteroids: diversity, biosynthesis and distribution. In: Smagghe G (ed) Ecdysone: structures and functions. Springer, Dordrecht, pp. 3-45. doi:10.1007/978-1-4020-9112-4_1

Dreier SI, Towers GHN (1988) Activity of ecdysone in selected plant growth bioassays. J Plant Physiol 132:502–512. doi:10.1016/S0176-1617(88)80073-7

Festucci-Buselli RA, Contim LAS, Barbosa LCA, Stuart J, Otoni WC (2008) Biosynthesis and potential functions of the ecdysteroid 20-hydroxyecdysone—a review. Botany 86:978–987. doi:10.1139/B08-049

Golovatskaya IF (2004) Effect of ecdysterone on morphological and physiological processes in plants. Russ J Plant Physiol 51:452–458. doi:10.1023/B:RUPP.0000028689.97402.d5

Holá D (2011) Brassinosteroids and photosynthesis. In: Hayat S, Ahmad A (eds) Brassinosteroids: a class of plant hormone. Springer, Dordrecht, pp. 143-192. doi:10.1007/978-94-007-0189-2_6

Janeczko A, Kościelniak J, Pilipowicz M, Szarek-Łukaszewska G, Skoczowski A (2005) Protection of winter rape photosystem 2 by 24-epibrassinolide under cadmium stress. Photosynthetica 43:293–298. doi:10.1007/s11099-005-0048-4

Janeczko A, Oklešťková J, Pociecha E, Kościelniak J, Mirek M (2011) Physiological effects and transport of 24-epibrassinolide in heat-stressed barley. Acta Physiol Plant 33:1249–1259. doi:10.1007/s11738-010-0655-y

Jiang Y, Cheng F, Zhou Y, Xia X, Mao W, Shi K, Chen Z, Yu J (2012a) Brassinosteroid-induced CO_2 assimilation is associated with increased stability of redox-sensitive photosynthetic enzymes in the chloroplasts in cucumber plants. Biochem Biophys Res Commun 426:390–394. doi:10.1016/j.bbrc.2012.08.100

Jiang Y, Cheng F, Zhou Y, Xia X, Mao W, Shi K, Chen Z, Yu J (2012b) Hydrogen peroxide functions as a secondary messenger for brassinosteroids-induced CO_2 assimilation and carbohydrate metabolism in *Cucumis sativus*. J Zheijiang Univ-Sci B (Biomed Biotechnol) 13:811–823. doi:10.1631/jzus.B1200130

Jiang YP, Cheng F, Zhou YH, Xia XJ, Shi K, Yu JQ (2012c) Interactive effects of CO_2 enrichment and brassinosteroid on CO_2 assimilation and photosynthetic electron transport in *Cucumis sativus*. Environ Exp Bot 75:98–106. doi:10.1016/j.envexpbot.2011.09.002

Kamlar M, Uhlík O, Kohout L, Šanda M, Macek T (2010a) Affinity chromatography as the method for brassinosteroid-binding protein isolation. J Biotechnol 150:S490

Kamlar M, Uhlík O, Chlubnová I, Kohout L, Harmatha J, Ježek R, Šanda M, Pišvejcová A, Macek T (2010b) Affinity chromatography as a method of studying the mechanism of action of plant oxysterols. Chem Listy 104:215–222 (In Czech)

Lafont R, Harmatha J, Marion-Poll F, Dinan L, Wilson ID: The ecdysone handbook, 3rd edn, on-line, http://ecdybase.org. Accessed 25 March 2013

Macháčková I, Vágner M, Sláma K (1995) Comparison between the effects of 20-hydroxyecdysone and phytohormones on growth and development in plants. Eur J Entomol 92:309–316

Porra RJ, Thompson WA, Kriedemann PE (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochim Biophys Acta 975:384–394. doi:10.1016/S0005-2728(89)80347-0

Stirbet A, Govindjee (2011) On the relation between the Kautsky effect (chlorophyll *a* fluorescence induction) and photosystem II: basics and applications of the OJIP fluorescence transient. J Photochem Photobiol B Biol 104:236–257. doi:10.1016/j.jphotobiol.2010.12.010

Strasser BJ (1997) Donor side capacity of photosystem II probed by chlorophyll *a* fluorescence analysis. Photosynth Res 52:147–155. doi:10.1023/A:1005896029778

Strasser RJ, Srivastava A, Tsimilli-Michael M (2000) The fluorescence transient as a tool to characterize and screen photosynthetic samples. In: Yunus M, Pathre U, Mohanty P (eds) Probing

photosynthesis: mechanism, regulation and adaptation. Taylor & Francis, London, pp 445–483

Uhlík O, Kamlar M, Kohout L, Ježek R, Harmatha J, Macek T (2008) Affinity chromatography reveals RuBisCO as an ecdysteroid-binding protein. Steroids 73:1433–1440. doi:10.1016/j.steroids.2008.07.009

Walker D (1988) The use of the oxygen electrode and fluorescence probes in simple measurements of photosynthesis, 2nd edn. Oxygraphics, Sheffield

Wellburn AR (1994) The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. Plant Physiol 144:307–313. doi:10.1016/S0176-1617(11)81192-2

Xia XJ, Huang LF, Zhou YH, Mao WH, Shi K, Wu JX, Asami T, Chen Z, Yu JQ (2009a) Brassinosteroids promote photosynthesis and growth by enhancing activation of Rubisco and expression of photosynthetic genes in *Cucumis sativus*. Planta 230: 1185–1196. doi:10.1007/s00425-009-1016-1

Xia XJ, Zhang Y, Wu JX, Wang JT, Zhou YH, Shi K, Yu YL, Yu JQ (2009b) Brassinosteroids promote metabolism of pesticides in cucumber. J Agric Food Chem 57:8406–8413. doi:10.1021/jf901915a

Xia XJ, Zhou YH, Ding J, Shi K, Asami T, Cheng Z, Yu JQ (2011) Induction of systemic stress tolerance by brassinosteroid in *Cucumis sativus*. New Phytol 191:706–720. doi:10.1111/j.1469-8137.2011.03745.x

Yu JQ, Huang LF, Hu WH, Zhou YH, Mao WH, Ye SF, Nogués S (2004) A role for brassinosteroids in the regulation of photosynthesis in *Cucumis sativus*. J Exp Bot 55:1135–1143. doi:10.1093/jxb/erh124

Yusuf MA, Kumar D, Rajwanshi R, Strasser RJ, Tsimilli-Michael M, Govindjee, Sarin NB (2010) Overexpression of γ-tocopherol methyl transferase gene in transgenic *Brassica juncea* plants alleviates abiotic stress: physiological and chlorophyll a fluorescence measurements. Biochim Biophys Acta 1797: 1428–1438. doi:10.1016/j.bbabio.2010.02.002

Supplementary Figure 1 The difference kinetics $\Delta W_{OJ}=(W_{OJ\ 20E} - W_{OJ\ CONTROL})$ revealing the K-band and calculated from the relative variable fluorescence $W_{OJ}=(F_t-F_0)/(F_J-F_0)$, where F_t represents the fluorescence intensity measured at any time during the recording period, F_J the fluorescence intensity at the J-step, and F_0 the initial fluorescence intensity. Measurements were made in leaves of *Tetragonia tetragonioides* L. at various times (1, 2, 4, 6, 24, 48 or 72 hours) after treatment of plants with 2 mM aqueous solution of 20-hydroxyecdysone (20E) or water (Control). Mean values (n=8) are shown for each time point.

5.4. Jaký je vliv 24-epibrassinolidu a 20-hydroxyekdysonu na fotosyntetické procesy u kukuřice a u špenátu?

Tato problematika byla zpracována v následující publikaci:

Rothová O., Holá D., Kočová M., Tůmová L., Hnilička F., Hniličková H., Kamlar M., Macek T. (2014): 24-Epibrassinolide and 20-hydroxyecdysone affect photosynthesis differently in maize and spinach. *Steroids* **85**: 44-57. IF 2,803

Zabývali jsme se vlivem postřiků 24E a 20E na mladé rostliny kukuřice a špenátu, a to na jejich mladé nevyvinuté listy a na listy vyvinuté, dospělé. Tyto dva rostlinné druhy se odlišují přirozeným obsahem 20E v rostlině, u kukuřice nebyla akumulace oxysterolů prokázána, u špenátu ano. Jednotlivé charakteristiky byly měřeny 1 hodinu po postřiku a potom 1 týden po postřiku 10^{-8} M vodnými roztoky steroidů. *In vivo* jsme měřili parametry fluorescence chlorofylu *a,* a dále P_N, rychlost transpirace, vodivost průduchů a intercelulární koncentraci CO_2. Dále jsme se soustředili na měření aktivity PS2 v izolovaných chloroplastech a obsahů fotosyntetických pigmentů po jejich extrakci z listů.

Rostliny, které byly ošetřené steroidy obou druhů, reagovaly snížením efektivity celého fotosyntetického elektron-transportního řetězce, ale zjistili jsme, že odpověď PS2 na ošetření byla u jednotlivých druhů různá. U kukuřice byl zjištěn pozitivní vliv ošetření na aktivitu OEC a mírně vyšší energetická konektivita mezi jednotlivými PS, u špenátu byla odpověď na ošetření 24E nebo 20E opačná. Samostatné ošetření rostlin jednotlivými látkami působilo na obsah fotosyntetických pigmentů pozitivně pouze u kukuřice. U špenátu jsme zjistili pokles hodnot charakteristik výměny plynů v důsledku aplikace 24E nebo 20E mezi listy a prostředím, tento výsledek jsme však nezaznamenali u kukuřice. Při společném ošetření rostlin oběma látkami jsme žádné rozdíly oproti kontrolním rostlinám nezjistili. Prokázali jsme tedy, že ošetření rostlin nízkými koncentracemi 20E ovlivňuje účinnost různých částí fotosyntetických procesů obdobně jako 24E. Je možné, že může docházet ke kompetitivnímu účinku těchto příbuzných látek. Podle výsledků našich experimentů BRs neregulují pouze aktivitu PS2, ale i některých dalších částí

fotosyntetického transportního řetězce, k tomu však nemusí docházet stejným způsobem. Zaznamenali jsme také výrazné mezidruhové rozdíly ve fyziologické odpovědi na ošetření rostlin a zároveň i rozdíly, závislé na vývojovém stádiu jednotlivých rostlin.

Steroids 85 (2014) 44–57

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

24-Epibrassinolide and 20-hydroxyecdysone affect photosynthesis differently in maize and spinach

Olga Rothová [a,1], Dana Holá [a,*,1], Marie Kočová [a], Lenka Tůmová [a], František Hnilička [b], Helena Hniličková [b], Marek Kamlar [c], Tomáš Macek [c]

[a] Charles University in Prague, Faculty of Science, Department of Genetics and Microbiology, Viničná 5, 128 43 Prague 2, Czech Republic
[b] Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Botany and Plant Physiology, Kamýcká 129, 165 21 Prague 6, Czech Republic
[c] Institute of Chemical Technology Prague, Faculty of Food and Biochemical Technology, Department of Biochemistry and Microbiology, Technická 5, 166 28 Prague 6, Czech Republic

ARTICLE INFO

Article history:
Received 2 September 2013
Received in revised form 1 April 2014
Accepted 11 April 2014
Available online 23 April 2014

Keywords:
Ecdysteroids
Brassinosteroids
Chlorophyll
Gas exchange
OJIP analysis
Photosynthetic electron transport

ABSTRACT

The aim of the work was to examine the effect of brassinosteroid (24-epibrassinolide; 24E) and ecdysteroid (20-hydroxyecdysone; 20E) on various parts of primary photosynthetic processes in maize and spinach. Additionally, the effect of steroids on gaseous exchange, pigment content and biomass accumulation was studied. The efficiency of the photosynthetic whole electron-transport chain responded negatively to the 24E or 20E treatment in both species, but there were interspecific differences regarding Photosystem (PS) II response. A positive effect on its oxygen-evolving complex and a slightly better energetical connectivity between PSII units were observed in maize whereas the opposite was true for spinach. The size of the pool of the PSI end electron acceptors was usually diminished due to 24E or 20E treatment. The treatment of plants with 24E or 20E applied individually positively influenced the content of photosynthetic pigments in maize (not in spinach). On the other hand, it did not affect gaseous exchange in maize but resulted in its reduction in spinach. Plants treated with combination of both steroids mostly did not significantly differ from the control plants. We have demonstrated for the first time that 20E applied in low (10 nM) concentration can affect various parts of photosynthetic processes similarly to 24E and that brassinosteroids regulate not only PSII but also other parts of the photosynthetic electron transport chain – but not necessarily in the same way.

1. Introduction

Ecdysteroids (ECs) and brassinosteroids (BRs) are two groups of plant steroids that are chemically somewhat similar (both are polyhydroxysteroids with C_{27}, C_{28} or C_{29} skeleton and carbonyl function at C6) but differ in details of their structures. They also differ in various other aspects such as their biosynthesis, levels, distribution within plant kingdom as well as the distribution in various plant organs, their biological functions, etc. [1]. Our knowledge on BRs' synthesis, perception, signaling, interaction with other phytohormones, mechanisms of their function and mechanisms of the inactivation of unnecessary BRs in plant cells has rather significantly advanced during the last decade (see *e.g.* recent reviews [2–9]; also the last monography on this topic edited by Hayat and Ahmad [10]). On the other hand, information on ECs is much more scarce, although these compounds are currently becoming objects of interest in connection with their possible medicinal applications (see *e.g.* reviews [1,11,12]).

Even though the percentage of higher plant species containing ECs in amounts detectable by current analytic methods is estimated to be only about 5–6%, the number of species tested for their presence is still very small (approx. 2%). Interestingly, it is thought that most plants do contain genes coding enzymes necessary for EC biosynthesis but this biosynthesis is actively repressed or down-regulated [1]. The amount of ECs in species that are known to contain them does not usually exceed 0.01–1% of plant dry mass and consists of 1–2 major ECs and a number of minor structural analogues [13,14]. However, ECs' levels in plants considerably vary

Abbreviations: BRs, brassinosteroids; Car, carotenoids; CF, chlorophyll fluorescence; Chl, chlorophyll; ci, intercellular CO_2 concentration; E, transpiration rate; ECs, ecdysteroids; g_s, stomatal conductance; OEC, oxygen-evolving complex; P_N, net photosynthetic rate; PS, photosystem; SLM, specific leaf mass; 20E, 20-hydroxyecdysone; 24E, 24-epibrassinolide.

* Corresponding author. Tel.: +420 2 21951200; fax: +420 2 21951724.
E-mail address: danahola@natur.cuni.cz (D. Holá).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.steroids.2014.04.006
0039-128X/

among species, varieties/accessions, individual plants and organs and also depend on developmental stage [15–20]. ECs are most often detected in leaves (particularly the young ones), roots or seeds but sometimes in other organs (stems, flowers etc.) as well [17]. Bakrim and others [18] have examined in certain detail the developmental changes of ECs' distribution in various organs of spinach (which is known to accumulate rather high levels of ECs). They concluded that ECs' biosynthesis in this species takes place mostly in older leaves and ECs are then transferred into young leaves which by themselves do not synthesize ECs but act only as sinks for these compounds. When this ability to transport ECs from older leaves is prevented, negative feedback control of ECs' biosynthesis takes place [18].

The function of ECs in plants is not exactly clear. The prevailing opinion supported by the most evidence is that they participate in plant defense against non-adapted herbivorous insects, nematodes or crustaceans, either as simple deterrents, compounds with antifeedant activity or inductors of developmental defects [1,14,16]. In addition, a hormonal role similar to BRs has also been suggested, although the results of standard bioassays used for the determination of phytohormone activity in plants available up to this time have mostly been negative [21,22]. However, slight gibberellin-like activity of ECs was described in rice by Macháčková and others [22]. Golovatskaya [23] reported an elongation of wheat coleoptiles caused by 20-hydroxyecdysone applied in 10^{-10} to 10^{-7} M concentrations and intensified by additional application of auxin. Other study made with tomato showed that 10^{-4} or 10^{-5} M concentration of this steroid affects germination percentage, shoot and root elongation, protein and proline content in seedlings of this species grown from seeds soaked with its solution [24]. However, whether the effect was stimulating or inhibiting depended both on the concentration and time elapsed from germination and could have changed rather quickly [24]. Bajguz and Koronka [25] found a significant stimulating effect of ecdysone applied in the range of 10^{-15} to 10^{-7} M to cell suspension of *Chlorella vulgaris* on cell growth and contents of nucleic acids, proteins, saccharides, chlorophyll (Chl) and phosphorus, as well as an inhibiting effect on protein secretion by cells after the 9th day of their cultivation with 10^{-7} M ecdysone. The ecdysone-caused increase in Chl content (particularly Chl *b*) in kidney bean leaves was reported by Golovatskaya [23]. These results suggest that naturally occurring ECs could fulfill roles additional to their supposed protective function against phytophagous organisms.

The exploitation of a bioaffinity chromatography using polymeric carriers with ECs and BRs bound by the oriented immobilisation and free ligands for the competitive elution identified some plant proteins with high affinity to these compounds. Among them were osmotin [26], ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) [27,28], glutathione *S*-transferase and one protein of photosynthetic oxygen-evolving complex (OEC) (Macek, unpublished results). Moreover, the *in vitro* testing of the Rubisco carboxylase activity [27] and the oxygen production in isolated chloroplasts [29] has shown that the addition of ECs to reaction mixtures in both cases significantly increased yields of these reactions. This opens an interesting possibility of a direct involvement of ECs in the regulation of photosynthesis which would be an entirely new potential function for these steroids.

On the other hand, BRs' involvement in the regulation of photosynthetic processes is a well accepted phenomenon and the enhancement of the photosynthetic rate by the exogenous application of these phytohormones has widely been demonstrated in various plant species (reviewed *e.g.* by Holá [30]). However, it is still not clear what exactly causes BR-associated improvements in photosynthetic function (the most favoured hypothesis is that BRs somehow enhance the efficiency of the photosynthetic carbon reduction cycle). Actually, only the Zheijiang University group made some of their studies [31–34] with the examination of various aspects of the functional relationship between BRs and photosynthesis as their primary aim. The majority of studies dealing with the BRs' role in plants included the analysis of photosynthetic processes only as an ancillary method for evaluation of general BRs' effects on plants examined for various other reasons. The results of some of these studies seem to point out that BRs can either directly or indirectly affect not only the photosynthetic fixation of CO_2 but the photosynthetic electron transport chain as well. The measurements of the slow Chl fluorescence (CF) induction kinetics are usually the method of choice for the examination of primary photosynthetic processes in BR-treated plants. The most commonly evaluated parameters are Φ_{PSII} (the effective quantum yield of photochemical energy conversion in Photosystem (PS) II), F_v/F_m (the maximum quantum yield of PSII in dark-adapted leaves), F_v'/F_m' (the maximum quantum yield of PSII in light-adapted leaves), q_P (photochemical CF quenching) or NPQ (non-photochemical CF quenching). This can certainly bring useful information; however, for even more detailed dissection of the photosynthetic electron transport, a multiparametric analysis of the fast CF induction kinetics (the OJIP part of CF curve) is indicated as perhaps even more powerful tool [35–37]. Curiously enough, only Janeczko and others [38,39] have ever attempted to use this method of CF analysis for the examination of BRs effects on plants. We have decided to apply this approach to examine the effect of BRs (represented by 24-epibrassinolide; 24E) and ECs (represented by 20-hydroxyecdysone; 20E) on various parts of primary photosynthetic processes in maize and spinach. Additionally, the effect of steroids on gaseous exchange, pigment content and biomass accumulation was studied. Steroids were applied both separately and jointly to find out whether these two types of compounds act synergistically or antagonistically. Maize and spinach were selected for this analysis based on their known difference in ECs' accumulation. Maize does not naturally accumulate non-conjugated ECs [40] but BRs (mainly brassinolide, castasterone, typhasterol, teasterone and dolichosterone) were found in its pollen and primary roots [41]. Non-conjugated ECs are present in spinach shoot and seeds (levels range from cca 0.5 to cca 1.2 $\mu g\ mg^{-1}$ dry mass) [18,19]. The presence of BRs in this species, according to our knowledge, has not been examined.

2. Experimental

2.1. Plant material, growth conditions and steroid treatments

Maize (*Zea mays* L., the inbred line "2023" supplied by the CEZEA Maize Breeding Station, *Čejč, Czech Republic*) and common spinach (*Spinacia oleracea* L., var. "Matador", SEMPRA PRAHA, *Praha, Czech Republic*) were used as the experimental material. Maize kernels were sown directly into pots (12 cm diameter, 13 cm depth, filled with garden soil), spinach seeds were first sown into low dishes filled with the same type of soil and individual seedlings transplanted into pots after approx. 40 days. Each pot containing 1 plant was placed in a naturally-lit greenhouse of the Faculty of Science, Charles University in Prague (54°04′ N, 14°25′ E) under semi-controlled conditions (air temperature 25 ± 2/20 ± 2 °C, relative air humidity 50 ± 5/70 ± 5% day/night) and watered daily with tap water as necessary.

After 32 d for maize and 115 d for spinach (counting the date of sowing as day 1), plants were sprayed with 10^{-8} M aqueous solutions of 24E [(22*R*,23*R*,24*R*)-2α,3α,22,23-tetrahydroxy-24-methyl-7-oxa-7-homo-5α-cholestan-6-one], 20E (2β,3β,14,20*R*,22*R*,25-hexahydroxy-5β-cholest-7-en-6-one), both (24/20E) or the control solution. This concentration was selected on the basis of our previous experiments made with 24E-treated maize [42].

Chemical structures of both steroids are shown in Fig. 1. Steroid solutions were prepared by the dilution of 10^{-3} M stock solutions with distilled water and adding 1 cm^3 of Tween® 20 per 1 dm^3; the stock solutions were made by dissolving the necessary amount of the respective steroid in 1 cm^3 of 96% ethanol and adding 9 cm^3 of distilled water. The control solution was prepared in the same way without any steroid. Approx. 0.5 cm^3 of the respective solution was used per each plant and was applied in three sprays from a hand-held sprayer: two aimed at the adaxial surface of the leaves chosen for the subsequent measurements and the third one directed at the top whorl of leaves. The leaves used for the measurements of photosynthetic parameters were always those that were mature (M, fully developed) and young (Y, not fully developed) at the time of the treatment. At the time of the treatment, maize plants had 2 fully developed leaves and spinach plants had 10–13 leaves. The 2nd leaf was chosen as the "M" one and the 3rd leaf as the "Y" one in maize, whereas the lower level, ovate leaf represented the "M" one and the upper level, triangular-based leaf was considered as the "Y" one in spinach. All measurements and samplings were performed 1 day and 1 week after the treatment; this also meant that at the latter date of measurements, the Y leaves reached the stage of full development and the M leaves just started to show first signs of senescence (drying at their extreme tips in maize, very slight yellowing in spinach). The experiments were made in 4 biological replicates, each experimental treatment/time of measurements was represented by the total number of 44 plants of each species. Twelve plants were used for the analysis of leaf gas exchange, the CF measurements, the determination of photosynthetic pigments' content and plant biomass and the remaining plants were used for the isolation of photochemically active chloroplasts and the subsequent PSII activity measurements. The CF measurements and the samplings of leaves for the chloroplast isolation and the determination of photosynthetic pigments' content took place between 8:00 and 10:00 a.m., Central European Time. The gas exchange measurements were conducted between 8:30 and 11:30 a.m., Central European Time.

2.2. Chlorophyll fluorescence measurements

The measurements of the polyphasic rise of CF transient (OJIP) were made on the upper surface of dark-adapted (30 min) leaves (the middle part of the leaf blade) *in situ* with the portable fluorometer FluorPen FP100max (*Photon System Instruments, Brno, Czech Republic*). The intensity of the saturating pulse (blue light, 455 nm) was 3000 μmol m^{-2} s^{-1}. All CF transients were recorded with a time scan from 10 μs to 2 ms with the data acquisition rate of 1 reading per 10 μs for the first 600 μs, 1 reading per 100 μs till t = 14 ms, 1 reading per 1 ms till t = 90 ms and 1 reading per 10 ms for the rest of the recording period. Fluorescence values recorded at 40 μs (F_0, initial fluorescence intensity), 300 μs (F_K, fluorescence intensity at K-step), 2 ms (F_J, fluorescence intensity at J-step), 30 ms (F_I, fluorescence intensity at I-step), and $F_M = F_P$ (maximum fluorescence intensity) were used for the calculations of various parameters of the JIP test shown in Table 1, based on the theory of energy flow in photosynthetic electron-transport chain according to Strasser and others [35] and Stirbet and Govindjee [37]. The calculations of the relative variable fluorescence (*i.e.* normalizations of whole fluorescence transients) as described by Yusuf and others [42] were used to obtain further information on primary photosynthetic processes: $W_{OI} = (F_t - F_0)/(F_I - F_0)$, $W_{OJ} = (F_t - F_0)/(F_J - F_0)$, $W_{OK} = (F_t - F_0)/(F_K - F_0)$ and $W_{IP} = (F_t - F_I)/(F_P - F_I)$, where F_t represents the fluorescence intensity measured at any time during the recording period. To compare the steroid-treated plants with the control plants and to better reveal K- and L-bands, the calculations of the difference kinetics $\Delta W_{OJ} = (W_{OJ\ Steroid} - W_{OJ\ Control})$ and $\Delta W_{OK} = (W_{OK\ Steroid} - W_{OK\ Control})$ were also made [43].

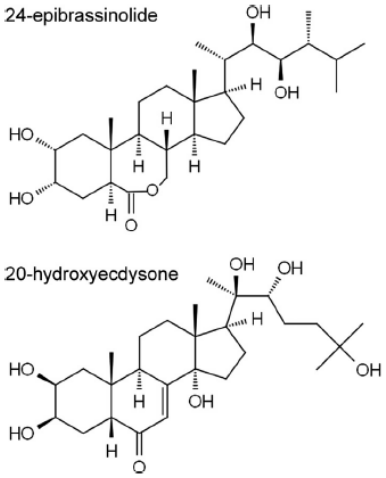


Fig. 1. Chemical structures of 24-epibrassinolide [(22*R*,23*R*,24*R*)-2α,3α,22,23-tetrahydroxy-24-methyl-7-oxa-7α-homo-5α-cholestan-6-one] and 20-hydroxyecdysone [2β,3β,14α,20*R*,22*R*,25-hexahydroxy-5β-cholest-7-en-6-one].

2.3. PSII activity measurements in isolated chloroplasts

Approx. 1–2 g (fresh mass) of leaf tissue (without the midrib) was cut into small pieces, placed in 50 cm^3 of pre-cooled (0–4 °C) isolation buffer (0.4 M sucrose, 50 mM $MgCl_2$, 50 mM Tris–HCl, pH 7.0) and homogenized for 18 s at 15,000 rpm using the OV5 homogenizer (*Velp Scientifica, Milano, Italy*) with the VSS2CCR2 dispersing tool. The homogenate was filtered through 8 layers of cheesecloth and centrifuged at 1000×*g* and 0 °C for 10 min. The pellet containing photochemically active broken chloroplasts of the II class (type C) according to Hall nomenclature [44] was resuspended in 0.75 cm^3 of pre-cooled storage buffer (0.4 M sucrose, 6 mM $MgCl_2$, 40% glycerol, 50 mM Tris–HCl, pH 7.0) and suspensions were stored at 0 °C in dark until the PSII activity measurements. Chl content in suspensions was determined spectrophotometrically (*Anthelie Advanced 2, Secomam, Lyon, France*) in 80% aqueous acetone [45] with 1:100 (v:v) chloroplast:acetone dilution.

The PSII activity was measured polarographically as described in [46] using a Clark-type oxygen electrode (*Theta' 90, Prague, Czech Republic*) in a measurement chamber constructed after [47]. A constant temperature of 25 °C was maintained during the measurements and the reaction mixtures were constantly stirred with a magnetic stirrer. Each mixture contained 5 cm^3 of the isolation buffer, the volume of chloroplast suspension corresponding to 7 μg of Chl, and 2 mM potassium ferricyanide together with 1 mM 2,6-dimethylbenzoquinone as the artificial electron acceptors. The mixtures were irradiated by white light (850 μmol m^{-2} s^{-1}).

2.4. Determination of the content of photosynthetic pigments

Four leaf discs (0.5 cm diameter) were cut from the middle part of the leaf blade of the respective leaves, placed in 5 cm^3 of *N,N*-dimethylformamide and stored in a refrigerator for 7 days with an occasional stirring of the extracts. Chl *a*, Chl *b* and total carotenoids (Car) contents in the extracts were then determined spectrophotometrically (*Anthelie Advanced 2, Secomam, Lyon, France*) and calculated using the equations given in [45,48].

Table 2
Selected photosynthetic parameters of the JIP test measured in maize (*Zea mays* L.) leaves treated with 24-epibrassinolide (24E), 20-hydroxyecdysone (20E), both (24/20E) or distilled water (Control). Young and mature refer to the state of the leaves at the date of their treatment. For explanation of various parameters see Table 1 in the article. Different letters (a–c) indicate significant ($p \leqslant 0.05$) differences between average values according to Fisher's LSD tests (data from 1 day and 1 week after the treatment were analyzed separately).

Parameter (a.u.)	1 day after the treatment				1 week after the treatment			
	Control	24E	20E	24/20E	Control	24E	20E	24/20E
Young leaf								
V_J	0.47^{a}	0.49^{a}	0.49^{a}	0.47^{a}	0.50^{a}	0.48^{a}	0.49^{a}	0.49^{a}
V_I	0.74^{b}	0.77^{a}	0.77^{a}	0.76^{a}	0.77^{c}	0.79^{a}	0.79^{ab}	0.77^{bc}
M_0	1.33^{a}	1.37^{a}	1.37^{a}	1.35^{a}	1.44^{a}	1.30^{b}	1.31^{ab}	1.41^{ab}
S_M	424.45^{a}	449.31^{a}	439.99^{a}	413.16^{a}	509.56^{a}	482.92^{ab}	481.36^{ab}	449.58^{b}
S_S	0.35^{a}	0.36^{a}	0.36^{a}	0.35^{a}	0.35^{b}	0.37^{a}	0.37^{a}	0.35^{b}
φ_{PO}	0.74^{a}	0.75^{a}	0.75^{a}	0.75^{a}	0.74^{a}	0.76^{a}	0.76^{a}	0.74^{a}
φ_{EO}	0.39^{a}	0.39^{a}	0.38^{a}	0.40^{a}	0.37^{a}	0.39^{a}	0.39^{a}	0.38^{a}
φ_{REO}	0.19^{a}	0.17^{b}	0.17^{b}	0.18^{ab}	0.17^{a}	0.16^{a}	0.16^{a}	0.17^{a}
φ_{DO}	0.26^{a}	0.25^{a}	0.25^{a}	0.25^{a}	0.26^{a}	0.24^{a}	0.24^{a}	0.26^{a}
ψ_{EO}	0.53^{a}	0.51^{a}	0.51^{a}	0.53^{a}	0.50^{a}	0.52^{a}	0.52^{a}	0.51^{a}
ψ_{REO}	0.26^{a}	0.23^{b}	0.23^{b}	0.24^{b}	0.24^{a}	0.21^{c}	0.21^{bc}	0.23^{ab}
δ_{RO}	0.48^{a}	0.45^{ab}	0.43^{b}	0.46^{a}	0.48^{a}	0.45^{ab}	0.43^{b}	0.46^{a}
γ_{RC}	0.21^{a}	0.21^{a}	0.21^{a}	0.21^{a}	0.20^{b}	0.22^{a}	0.22^{a}	0.21^{b}
ABS/RC	3.86^{a}	3.75^{a}	3.72^{a}	3.84^{a}	3.93^{a}	3.56^{b}	3.56^{b}	3.89^{a}
TP_0/RC	2.85^{a}	2.80^{a}	2.78^{a}	2.87^{a}	2.89^{a}	2.69^{b}	2.70^{b}	2.87^{a}
ET_0/RC	1.52^{a}	1.44^{ab}	1.40^{b}	1.52^{a}	1.45^{ab}	1.39^{c}	1.39^{bc}	1.46^{a}
RE_0/RC	1.01^{a}	0.95^{c}	0.94^{c}	0.97^{b}	0.68^{a}	0.57^{b}	0.57^{b}	0.65^{a}
DI_0/RC	0.74^{a}	0.64^{a}	0.64^{a}	0.69^{a}	1.04^{a}	0.87^{a}	0.86^{a}	1.03^{a}
PI_{ABS}	0.85^{a}	0.93^{a}	0.87^{a}	0.88^{a}	0.79^{a}	0.95^{a}	0.94^{a}	0.79^{a}
PI_{TOTAL}	0.78^{a}	0.77^{a}	0.65^{a}	0.74^{a}	0.73^{a}	0.80^{a}	0.70^{a}	0.67^{a}
Mature leaf								
V_J	0.46^{c}	0.48^{ab}	0.49^{a}	0.47^{bc}	0.51^{ab}	0.49^{ab}	0.48^{b}	0.54^{a}
V_I	0.74^{c}	0.76^{b}	0.78^{a}	0.76^{bc}	0.76^{a}	0.76^{a}	0.76^{a}	0.76^{a}
M_0	1.33^{a}	1.32^{a}	1.34^{a}	1.31^{a}	1.47^{ab}	1.37^{b}	1.34^{b}	1.58^{a}
S_M	433.88^{a}	454.60^{a}	443.27^{a}	430.52^{a}	419.32^{b}	463.95^{a}	471.69^{a}	392.20^{b}
S_S	0.35^{b}	0.36^{a}	0.36^{a}	0.36^{ab}	0.35^{ab}	0.36^{a}	0.36^{a}	0.34^{b}
φ_{PO}	0.74^{a}	0.75^{a}	0.75^{a}	0.75^{a}	0.72^{b}	0.74^{ab}	0.75^{a}	0.72^{b}
φ_{EO}	0.40^{ab}	0.39^{ab}	0.38^{b}	0.40^{a}	0.35^{ab}	0.38^{ab}	0.39^{a}	0.33^{b}
φ_{REO}	0.19^{a}	0.18^{a}	0.16^{b}	0.18^{a}	0.17^{a}	0.18^{a}	0.18^{a}	0.17^{a}
φ_{DO}	0.26^{a}	0.25^{a}	0.25^{a}	0.25^{a}	0.28^{a}	0.26^{ab}	0.25^{b}	0.28^{a}
ψ_{EO}	0.54^{a}	0.52^{bc}	0.51^{c}	0.53^{ab}	0.49^{ab}	0.51^{ab}	0.52^{a}	0.46^{b}
ψ_{REO}	0.26^{a}	0.24^{b}	0.22^{c}	0.24^{ab}	0.24^{a}	0.24^{a}	0.24^{a}	0.24^{a}
δ_{RO}	0.48^{a}	0.45^{ab}	0.43^{b}	0.46^{a}	0.48^{a}	0.45^{ab}	0.43^{b}	0.46^{a}
γ_{RC}	0.21^{b}	0.21^{a}	0.21^{a}	0.21^{ab}	0.20^{bc}	0.21^{ab}	0.21^{a}	0.20^{c}
ABS/RC	3.88^{a}	3.66^{b}	3.66^{b}	3.73^{ab}	4.01^{ab}	3.77^{bc}	3.74^{c}	4.10^{a}
TP_0/RC	2.86^{a}	2.76^{b}	2.75^{b}	2.80^{ab}	2.88^{ab}	2.78^{b}	2.80^{b}	2.93^{a}
ET_0/RC	1.53^{a}	1.44^{bc}	1.41^{c}	1.49^{ab}	1.41^{a}	1.42^{a}	1.46^{a}	1.35^{a}
RE_0/RC	1.01^{a}	0.91^{b}	0.91^{c}	0.93^{b}	0.69^{a}	0.66^{a}	0.67^{a}	0.69^{a}
DI_0/RC	0.73^{a}	0.65^{b}	0.60^{b}	0.68^{b}	1.12^{ab}	0.99^{ab}	0.94^{b}	1.17^{a}
PI_{ABS}	0.86^{a}	0.91^{a}	0.87^{a}	0.93^{a}	0.66^{bc}	0.80^{ab}	0.88^{a}	0.60^{c}
PI_{TOTAL}	0.79^{a}	0.75^{ab}	0.65^{b}	0.79^{a}	0.62^{a}	0.67^{a}	0.66^{a}	0.50^{a}

spinach (Table 3) to approx. 90% of control values. The *in vitro* measurements of the PSII activity did not reveal any significant differences among various treatments in either species (Tables 4 and 5).

The K-band revealed by the difference kinetics ΔW_{OJ} (Fig. 2C and D) was within a negative range for both 24E and 20E individual treatments of maize which suggested the better function of OEC in these plants compared to the control ones. The combination of both steroids did not have such effect and the K-band was much less pronounced. For spinach, the situation was reverse with the K-band in the positive range. The effect of the individual 24E or 20E treatments was again more marked than the effect of the combination of both steroids (Fig. 3C and D).

The L-band which is observable when plotting the difference kinetics ΔW_{OK} against time and which informs on the energetical connectivity between individual PSII units was not particularly pronounced in maize suggesting that the steroid-treated plants of this species did not much differ in this aspect from the control ones (Fig. 2E and F). On the other hand, treatment of spinach with the individually applied 24E or 20E (not their combination) resulted in the positive L-band (with almost tenfold amplitude compared to maize) suggesting the poorer energetical connectivity between the PSII units. This was slightly more pronounced for 20E than for 24E (Fig. 3E and F).

As regards the whole photosynthetic electron-transport chain (*i.e.* until the PSI electron acceptors), treatment with 24E or 20E applied individually (not in combination) to both species negatively affected the respective JIP test parameters (*e.g.* φ_{REO}, ψ_{REO}, δ_{REO} and RE_0/RC). In maize, this was seen particularly in the Y leaves (Table 2). The curves of the relative variable fluorescence W_{IP} in maize mostly overlapped (Fig. 2G and H, main graphs) indicating that the rates of the reduction of the end electron acceptors in the steroid-treated plants were similar to those in the control plants. However, the individual application of either steroid resulted in the decrease of the size of the pool of PSI end electron acceptors in the Y leaves, as seen from the lower position of the W_{OI} curves (Fig. 2G, inset graph). In spinach, both types of leaves were affected similarly and the decrease of the values of the respective JIP test parameters was in some cases rather strong (down to 75% of control) (Table 3). Slower reduction of the PSI electron acceptors was observed in plants treated with the individual application of 24E or 20E compared to the control plants or plants treated with the combination of both steroids (as seen from the

Table 3
Selected photosynthetic parameters of the JIP test measured in spinach (*Spinacia oleracea* L.) leaves treated with 10^{-8} M 24-epibrassinolide (24E), 10^{-8} M 20-hydroxyecdysone (20E), both (24/20E) or distilled water (Control). Young and mature refer to the state of the leaves at the date of their treatment. For explanation of various parameters see Table 1 in the article. Different letters (a–c) indicate significant ($p \leqslant 0.05$) differences between average values according to Fisher's LSD tests (data from 1 day and 1 week after the treatment were analyzed separately).

Parameter (a.u.)	1 day after the treatment				1 week after the treatment			
	Control	24E	20E	24/20E	Control	24E	20E	24/20E
Young leaf								
V_J	0.44^{a}	0.44^{a}	0.44^{a}	0.45^{a}	0.40^{b}	0.44^{a}	0.45^{a}	0.41^{b}
V_I	0.80^{ab}	0.80^{a}	0.78^{b}	0.80^{ab}	0.75^{b}	0.80^{a}	0.82^{a}	0.76^{b}
M_0	1.08^{a}	1.14^{a}	1.11^{a}	1.12^{a}	0.94^{b}	1.18^{a}	1.20^{a}	1.02^{b}
S_M	641.78^{a}	597.79^{a}	577.31^{a}	622.15^{a}	615.20^{a}	595.17^{a}	549.18^{a}	616.67^{a}
S_S	0.41^{a}	0.39^{b}	0.40^{ab}	0.40^{a}	0.43^{a}	0.38^{c}	0.38^{c}	0.40^{b}
φ_{PO}	0.80^{a}	0.78^{b}	0.78^{b}	0.80^{a}	0.81^{a}	0.77^{b}	0.76^{b}	0.80^{a}
φ_{EO}	0.45^{a}	0.43^{a}	0.44^{a}	0.44^{a}	0.49^{a}	0.43^{b}	0.42^{b}	0.48^{a}
φ_{REO}	0.16^{ab}	0.15^{b}	0.17^{a}	0.16^{ab}	0.20^{a}	0.15^{b}	0.14^{b}	0.19^{a}
φ_{DO}	0.20^{b}	0.22^{a}	0.22^{a}	0.20^{b}	0.19^{b}	0.23^{a}	0.24^{a}	0.20^{b}
ψ_{EO}	0.56^{a}	0.56^{a}	0.56^{a}	0.55^{a}	0.60^{a}	0.56^{b}	0.55^{b}	0.59^{a}
ψ_{REO}	0.20^{ab}	0.20^{b}	0.22^{a}	0.20^{ab}	0.25^{a}	0.20^{b}	0.18^{b}	0.24^{a}
δ_{RO}	0.36^{b}	0.35^{b}	0.39^{a}	0.37^{ab}	0.42^{a}	0.35^{b}	0.33^{b}	0.40^{a}
γ_{RC}	0.25^{a}	0.23^{b}	0.24^{ab}	0.24^{a}	0.26^{a}	0.23^{c}	0.22^{c}	0.25^{b}
ABS/RC	3.08^{b}	3.30^{a}	3.20^{ab}	3.11^{b}	2.88^{b}	3.44^{a}	3.52^{a}	3.09^{b}
TP_0/RC	2.45^{b}	2.57^{a}	2.50^{ab}	2.48^{b}	2.33^{c}	2.66^{a}	2.67^{a}	2.49^{b}
ET_0/RC	1.38^{b}	1.43^{a}	1.40^{ab}	1.35^{b}	1.40^{b}	1.48^{a}	1.47^{a}	1.47^{ab}
RE_0/RC	0.63^{b}	0.73^{a}	0.70^{ab}	0.64^{b}	0.55^{b}	0.78^{a}	0.84^{a}	0.61^{b}
DI_0/RC	0.50^{b}	0.51^{b}	0.55^{a}	0.50^{b}	0.58^{a}	0.52^{b}	0.49^{b}	0.58^{a}
PI_{ABS}	1.65^{a}	1.37^{b}	1.44^{ab}	1.54^{ab}	2.22^{a}	1.26^{c}	1.17^{c}	1.95^{b}
PI_{TOTAL}	0.97^{a}	0.76^{a}	0.94^{a}	0.91^{a}	1.60^{a}	0.70^{c}	0.60^{c}	1.32^{b}
Mature leaf								
V_J	0.44^{b}	0.44^{ab}	0.45^{ab}	0.45^{a}	0.40^{b}	0.44^{a}	0.44^{a}	0.41^{b}
V_I	0.78^{a}	0.78^{a}	0.80^{a}	0.80^{a}	0.75^{c}	0.80^{a}	0.81^{a}	0.77^{b}
M_0	1.06^{b}	1.10^{ab}	1.17^{a}	1.10^{ab}	0.91^{c}	1.17^{a}	1.19^{a}	1.00^{b}
S_M	577.66^{a}	526.02^{a}	575.40^{a}	603.88^{a}	687.43^{a}	567.94^{a}	613.17^{a}	581.06^{a}
S_S	0.41^{a}	0.40^{ab}	0.39^{b}	0.41^{a}	0.43^{a}	0.38^{c}	0.37^{c}	0.41^{b}
φ_{PO}	0.80^{a}	0.78^{bc}	0.77^{c}	0.80^{ab}	0.80^{a}	0.78^{b}	0.76^{c}	0.80^{a}
φ_{EO}	0.45^{a}	0.44^{ab}	0.42^{b}	0.43^{ab}	0.49^{a}	0.43^{b}	0.42^{b}	0.47^{a}
φ_{REO}	0.18^{a}	0.17^{ab}	0.15^{b}	0.16^{ab}	0.20^{a}	0.15^{c}	0.15^{c}	0.18^{b}
φ_{DO}	0.20^{c}	0.22^{ab}	0.23^{a}	0.20^{bc}	0.20^{c}	0.22^{b}	0.24^{a}	0.20^{c}
ψ_{EO}	0.56^{a}	0.56^{ab}	0.55^{ab}	0.55^{b}	0.60^{a}	0.56^{b}	0.56^{b}	0.59^{a}
ψ_{REO}	0.22^{a}	0.22^{a}	0.20^{a}	0.20^{a}	0.25^{a}	0.20^{c}	0.19^{c}	0.23^{b}
δ_{RO}	0.39^{a}	0.39^{a}	0.36^{a}	0.37^{a}	0.41^{a}	0.35^{c}	0.35^{c}	0.38^{b}
γ_{RC}	0.25^{a}	0.24^{ab}	0.23^{b}	0.25^{a}	0.26^{a}	0.23^{c}	0.22^{c}	0.25^{b}
ABS/RC	3.03^{b}	3.22^{ab}	3.38^{a}	3.03^{b}	2.87^{c}	3.39^{a}	3.52^{a}	3.05^{b}
TP_0/RC	2.42^{b}	2.52^{ab}	2.59^{a}	2.41^{b}	2.31^{c}	2.63^{a}	2.68^{a}	2.45^{b}
ET_0/RC	1.37^{b}	1.41^{ab}	1.42^{a}	1.32^{c}	1.39^{b}	1.46^{a}	1.49^{a}	1.44^{ab}
RE_0/RC	0.61^{a}	0.71^{ab}	0.79^{a}	0.62^{bc}	0.56^{b}	0.76^{a}	0.84^{a}	0.60^{b}
DI_0/RC	0.54^{a}	0.55^{a}	0.52^{ab}	0.49^{b}	0.57^{a}	0.52^{b}	0.52^{b}	0.55^{ab}
PI_{ABS}	1.71^{a}	1.47^{ab}	1.23^{b}	1.57^{a}	2.21^{a}	1.28^{b}	1.17^{b}	1.96^{a}
PI_{TOTAL}	1.11^{a}	0.98^{ab}	0.72^{b}	0.95^{ab}	1.57^{a}	0.71^{c}	0.63^{c}	1.22^{b}

shift of the W_{IP} curves to the right, Fig. 3G and H, main graphs). In addition, the size of the pool of these end electron acceptors was also diminished in these plants (Fig. 3G and H, inset graphs).

The above described interspecific differences and the differences among the response of the individual parts of photosynthetic electron-transport chain were reflected in the parameters PI_{ABS} and PI_{TOTAL}, which characterize overall performance of photosynthetic apparatus from the absorption of photons by light-harvesting antenna to the reduction of either Q_B (*i.e.* mostly PSII performance) or the PSI end electron acceptors (*i.e.* the whole photosynthetic electron-transport chain performance). The values of these parameters either did not change or increased (in case of PI_{ABS}) in maize but strongly decreased (down to approx. 40–50% of control) in spinach (Tables 2 and 3).

The contents of photosynthetic pigments in spinach leaves were not significantly affected by steroid treatment (Table 5). On the other hand, the treatment of maize plants with steroids (particularly 20E) increased Chl *a*, Chl *b* and Car contents (Table 4).

The individual application of both steroids decreased P_N, g_S and E of spinach leaves but due to a somewhat large variability among individual plants, these differences were statistically significant only exceptionally, mostly for the M leaves of 24E-treated plants (in this case, the decrease was down to approx. 80% of control for P_N, 50% of control for E and 40% of control for g_S). Contrary to this, the intercellular CO_2 concentration increased up to 130% of the control; again, these differences were not always statistically significant (Table 5). Neither of these parameters significantly changed after steroid treatment in maize (Table 4). SLM was mostly not affected by steroid application in either species (Tables 4 and 5), nor were any significant differences between steroid-treated and control plants observed for the dry mass of shoot or roots (data not shown).

4. Discussion

Any examination of a possible biological function of some compound that naturally occurs in a plant organism has to be based either on the work with loss- or gain-of-function mutants with changes in its biosynthesis/degradation/transport/signaling, or with plants subjected to its exogenous application. In case of BRs, mutants are still not as abundant as we would like to and are

Table 4
Gas exchange parameters, photosynthetic pigment contents and biomass accumulation measured in maize (*Zea mays* L.) leaves treated with 24-epibrassinolide (24E), 20-hydroxyecdysone (20E), both (24/20E) or distilled water (Control). Young and mature refer to the state of the leaves at the date of their treatment. Different letters (a–c) indicate significant ($p \leqslant 0.05$) differences between average values according to Fisher's LSD tests (data from 1 day and 1 week after the treatment were analyzed separately).

Parameter	1 day after the treatment				1 week after the treatment			
	Control	24E	20E	24/20E	Control	24E	20E	24/20E
Young leaf								
Net photosynthetic rate [μmol CO_2 m^{-2} (leaf area) s^{-1}]	8.59^{a}	8.78^{a}	8.29^{a}	8.42^{a}	9.40^{a}	9.17^{a}	9.83^{a}	10.09^{a}
Transpiration rate [mmol H_2O m^{-2} (leaf area) s^{-1}]	1.01^{a}	1.17^{a}	1.13^{a}	1.12^{a}	1.16^{a}	1.13^{a}	1.17^{a}	1.23^{a}
Stomatal conductance [mol H_2O m^{-2} (leaf area) s^{-1}]	0.05^{a}	0.06^{a}	0.05^{a}	0.05^{a}	0.06^{a}	0.06^{a}	0.06^{a}	0.07^{a}
Intercellular CO_2 concentration [μmol mol^{-1}]	188.90^{a}	226.88^{a}	283.31^{a}	241.18^{a}	202.78^{a}	212.48^{a}	192.86^{a}	205.82^{a}
Photosystem II activity [mmol O_2 kg^{-1} (chlorophyll) s^{-1}]	27.77^{a}	26.37^{a}	27.71^{a}	28.06^{a}	27.51^{ab}	26.64^{b}	28.52^{ab}	29.61^{a}
Chlorophyll *a* content [g kg^{-1} (dry mass)]	13.90^{a}	14.22^{a}	14.30^{a}	14.60^{a}	14.23^{a}	17.10^{a}	17.16^{a}	14.21^{a}
Chlorophyll *b* content [g kg^{-1} (dry mass)]	3.81^{a}	3.82^{a}	3.72^{a}	3.98^{a}	4.09^{a}	4.64^{a}	4.94^{a}	4.12^{a}
Total carotenoids content [g kg^{-1} (dry mass)]	2.62^{a}	2.66^{a}	2.76^{a}	2.62^{a}	2.66^{ab}	2.97^{ab}	3.05^{a}	2.59^{b}
Chlorophyll *a*/*b* ratio	3.65^{a}	3.73^{a}	3.91^{a}	3.66^{a}	3.50^{b}	3.69^{a}	3.47^{b}	3.47^{b}
Chlorophyll (*a*+*b*)/total carotenoids ratio	6.75^{ab}	6.78^{ab}	6.42^{b}	7.09^{a}	6.73^{a}	7.26^{a}	7.25^{a}	7.01^{a}
Specific leaf mass [g m^{-2} (leaf area)]	10.81^{a}	10.59^{a}	10.53^{a}	10.81^{a}	10.45^{a}	10.43^{a}	10.75^{a}	10.62^{a}
Mature leaf								
Net photosynthetic rate [μmol CO_2 m^{-2} (leaf area) s^{-1}]	8.99^{a}	9.26^{a}	8.76^{a}	7.71^{a}	7.80^{a}	7.28^{a}	8.10^{a}	8.12^{a}
Transpiration rate [mmol H_2O m^{-2} (leaf area) s^{-1}]	1.05^{a}	1.08^{a}	1.10^{a}	1.07^{a}	0.97^{a}	0.88^{a}	1.02^{a}	1.04^{a}
Stomatal conductance [mol H_2O m^{-2} (leaf area) s^{-1}]	0.05^{a}	0.05^{a}	0.05^{a}	0.05^{a}	0.05^{a}	0.04^{a}	0.05^{a}	0.06^{a}
Intercellular CO_2 concentration [μmol mol^{-1}]	209.65^{a}	186.56^{a}	215.10^{a}	211.65^{a}	218.31^{a}	197.71^{a}	212.12^{a}	221.29^{a}
Photosystem II activity [mmol O_2 kg^{-1} (chlorophyll) s^{-1}]	30.88^{a}	29.38^{a}	31.56^{a}	31.79^{a}	30.25^{a}	29.16^{a}	29.10^{a}	30.49^{a}
Chlorophyll *a* content [g kg^{-1} (dry mass)]	10.25^{c}	12.82^{ab}	13.46^{a}	11.46^{bc}	12.23^{a}	12.30^{a}	13.85^{a}	11.80^{a}
Chlorophyll *b* content [g kg^{-1} (dry mass)]	3.07^{c}	3.76^{ab}	3.97^{a}	3.35^{bc}	3.64^{a}	3.50^{a}	4.11^{a}	3.55^{a}
Total carotenoids content [g kg^{-1} (dry mass)]	2.09^{b}	2.39^{ab}	2.55^{a}	2.23^{b}	2.27^{a}	2.23^{a}	2.46^{a}	2.20^{a}
Chlorophyll *a*/*b* ratio	3.34^{a}	3.40^{a}	3.37^{a}	3.42^{a}	3.38^{b}	3.51^{a}	3.37^{b}	3.33^{b}
Chlorophyll (*a*+*b*)/total carotenoids ratio	6.36^{c}	6.94^{a}	6.81^{ab}	6.62^{b}	6.91^{a}	7.06^{a}	7.30^{a}	6.94^{a}
Specific leaf mass [g m^{-2} (leaf area)]	12.95^{ab}	12.28^{b}	13.26^{a}	12.71^{ab}	12.26^{ab}	12.50^{ab}	12.88^{a}	11.80^{b}

Table 5
Gas exchange parameters, photosynthetic pigment contents and biomass accumulation measured in spinach (*Spinacia oleracea* L.) leaves treated with 24-epibrassinolide (24E), 20-hydroxyecdysone (20E), both (24/20E) or distilled water (Control). Young and mature refer to the state of the leaves at the date of their treatment. Different letters (a–c) indicate significant ($p \leqslant 0.05$) differences between average values according to Fisher's LSD tests (data from 1 day and 1 week after the treatment were analyzed separately).

Parameter	1 day after the treatment				1 week after the treatment			
	Control	24E	20E	24/20E	Control	24E	20E	24/20E
Young leaf								
Net photosynthetic rate [μmol CO_2 m^{-2} (leaf area) s^{-1}]	18.35^{a}	16.24^{b}	16.59^{ab}	15.16^{b}	16.24^{a}	14.86^{a}	15.59^{a}	15.41^{a}
Transpiration rate [mmol H_2O m^{-2} (leaf area) s^{-1}]	3.41^{a}	2.63^{b}	2.88^{ab}	2.86^{b}	3.70^{a}	2.93^{a}	3.35^{a}	2.91^{a}
Stomatal conductance [mol H_2O m^{-2} (leaf area) s^{-1}]	0.35^{a}	0.20^{b}	0.25^{ab}	0.25^{ab}	0.28^{a}	0.20^{a}	0.25^{a}	0.24^{a}
Intercellular CO_2 concentration [μmol mol^{-1}]	291.30^{a}	291.50^{a}	298.85^{a}	292.76^{a}	244.71^{b}	318.10^{a}	325.12^{a}	269.92^{ab}
Photosystem II activity [mmol O_2 kg^{-1} (chlorophyll) s^{-1}]	10.55^{a}	10.47^{a}	10.35^{a}	10.29^{a}	10.15^{a}	10.45^{a}	10.17^{a}	10.16^{a}
Chlorophyll *a* content [g kg^{-1} (dry mass)]	12.39^{a}	12.41^{a}	12.53^{a}	12.43^{a}	12.02^{a}	12.19^{a}	12.34^{a}	12.20^{a}
Chlorophyll *b* content [g kg^{-1} (dry mass)]	3.87^{a}	3.89^{a}	3.91^{a}	3.85^{a}	4.04^{a}	4.08^{a}	4.07^{a}	4.08^{a}
Total carotenoids content [g kg^{-1} (dry mass)]	2.81^{a}	2.85^{a}	2.84^{a}	2.83^{a}	2.40^{a}	2.43^{a}	2.51^{a}	2.43^{a}
Chlorophyll *a*/*b* ratio	3.20^{a}	3.19^{a}	3.20^{a}	3.23^{a}	2.98^{a}	2.99^{a}	3.03^{a}	2.99^{a}
Chlorophyll (*a*+*b*)/total carotenoids ratio	5.79^{a}	5.72^{a}	5.79^{a}	5.75^{a}	6.68^{ab}	6.70^{ab}	6.54^{b}	6.72^{a}
Specific leaf mass [g m^{-2} (leaf area)]	29.09^{a}	27.02^{a}	27.60^{a}	30.22^{a}	29.00^{a}	29.92^{a}	30.94^{a}	28.47^{a}
Mature leaf								
Net photosynthetic rate [μmol CO_2 m^{-2} (leaf area) s^{-1}]	15.95^{a}	15.48^{a}	14.60^{a}	15.70^{a}	15.81^{a}	12.27^{b}	13.97^{ab}	15.57^{ab}
Transpiration rate [mmol H_2O m^{-2} (leaf area) s^{-1}]	2.81^{a}	2.18^{a}	2.36^{a}	2.62^{a}	2.90^{a}	1.51^{b}	2.21^{ab}	2.64^{a}
Stomatal conductance [mol H_2O m^{-2} (leaf area) s^{-1}]	0.23^{a}	0.15^{b}	0.17^{ab}	0.20^{ab}	0.20^{a}	0.08^{b}	0.13^{ab}	0.19^{a}
Intercellular CO_2 concentration [μmol mol^{-1}]	282.42^{a}	279.99^{a}	287.32^{a}	270.30^{a}	251.15^{b}	250.86^{b}	321.22^{a}	270.39^{b}
Photosystem II activity [mmol O_2 kg^{-1} (chlorophyll) s^{-1}]	10.58^{a}	11.12^{a}	11.09^{a}	11.23^{a}	9.56^{a}	9.69^{a}	9.56^{a}	9.72^{a}
Chlorophyll *a* content [g kg^{-1} (dry mass)]	10.52^{a}	10.94^{a}	10.37^{a}	10.77^{a}	10.32^{a}	10.30^{a}	10.54^{a}	10.34^{a}
Chlorophyll *b* content [g kg^{-1} (dry mass)]	3.54^{a}	3.58^{a}	3.42^{a}	3.61^{a}	3.71^{a}	3.61^{a}	3.64^{a}	3.61^{a}
Total carotenoids content [g kg^{-1} (dry mass)]	2.23^{a}	2.35^{a}	2.25^{a}	2.28^{a}	2.06^{a}	2.06^{a}	2.16^{a}	2.08^{a}
Chlorophyll *a*/*b* ratio	2.98^{a}	3.05^{a}	3.04^{a}	2.99^{a}	2.79^{a}	2.86^{a}	2.89^{a}	2.86^{a}
Chlorophyll (*a*+*b*)/total carotenoids ratio	6.30^{ab}	6.20^{bc}	6.13^{c}	6.32^{a}	6.84^{a}	6.76^{ab}	6.56^{c}	6.72^{b}
Specific leaf mass [g m^{-2} (leaf area)]	28.49^{a}	28.79^{a}	27.28^{a}	28.06^{a}	26.05^{b}	29.75^{a}	28.49^{ab}	26.15^{b}

restricted mostly to *Arabidopsis*, rice, pea and in some cases tomato [7]. As regards the other group of plant steroids examined in our study, *i.e.* ECs, mutant plants with changed ECs' levels are even more rare [1,14]. This means that most analyses of BRs' and ECs' biological function have been made in plants treated with these steroids applied to them exogenously (mostly by foliar application – leaf spraying, or seed/plant soaking). This was our choice for this study as well. The obtained results can be divided into two main groups. The first one brings a more detailed information on the effect BRs have on primary photosynthetic processes than has been available thus far. The second group of results suggests a possibility of an entirely new biological function of ECs in the regulation of photosynthesis and offers a tentative glimpse of the possible functional relationship between these two types of plant steroids.

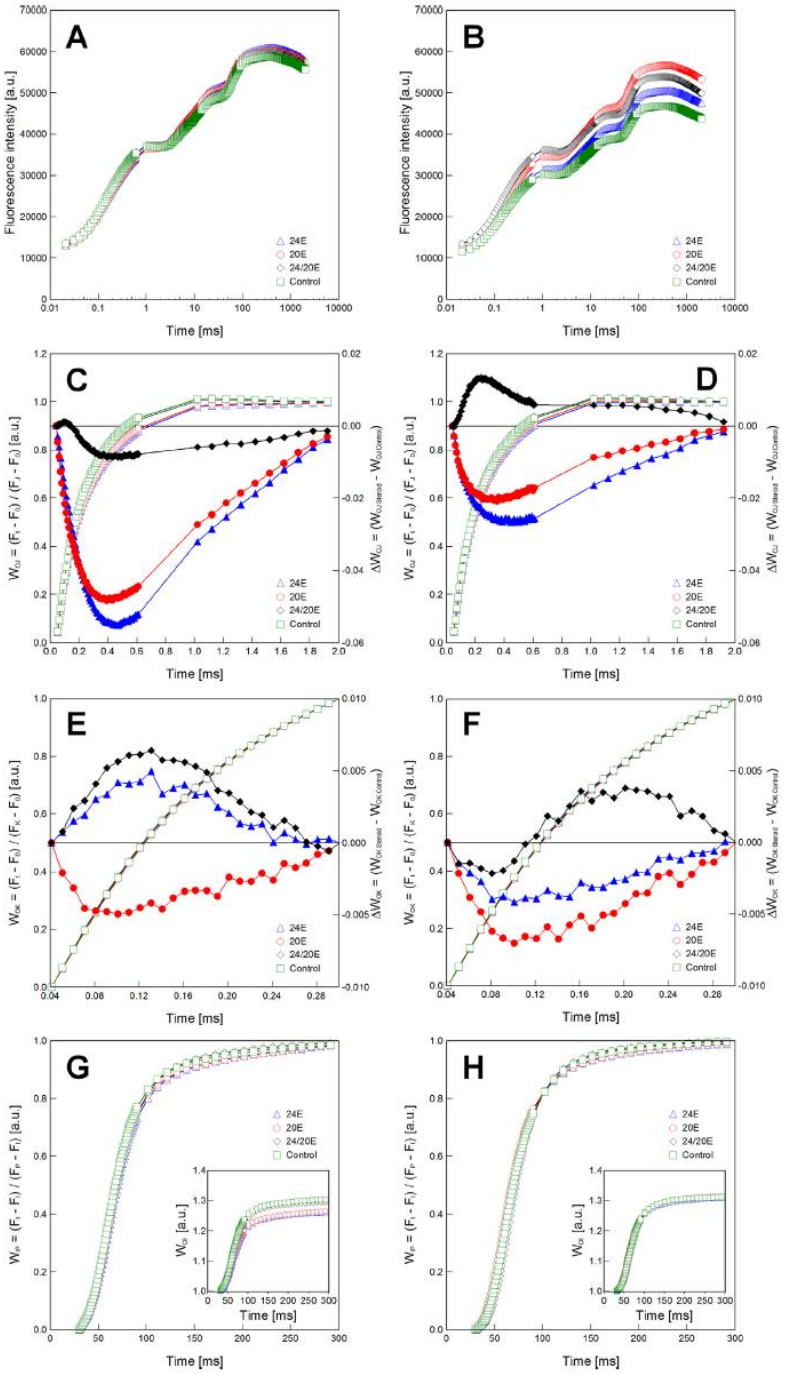


Fig. 2. The polyphasic rise of chlorophyll *a* fluorescence transients (OJIP) [panels A and B], the relative variable fluorescence W_{OJ} [panels C and D], W_{OK} [panels E and F], W_{IP} [panels G and H, main graphs] and W_{OI} [panels G and H, inset graphs] and the difference kinetics revealing the K- and L-bands, ΔW_{OJ} [panels C and D] and ΔW_{OK} [panels E and F] as measured in young [panels A, C, E and G] or mature [panels B, D, F and H] leaves of maize one week after their treatment with 24-epibrassinolide (24E), 20-hydroxyecdysone (20E), both (24/20E) or distilled water (Control). Average values are always shown, the relative variable fluorescences are plotted on left vertical axes and shown in open symbols, the difference kinetics are plotted on right vertical axes and shown in solid symbols. Young and mature refer to the state of leaves at the date of the treatment.

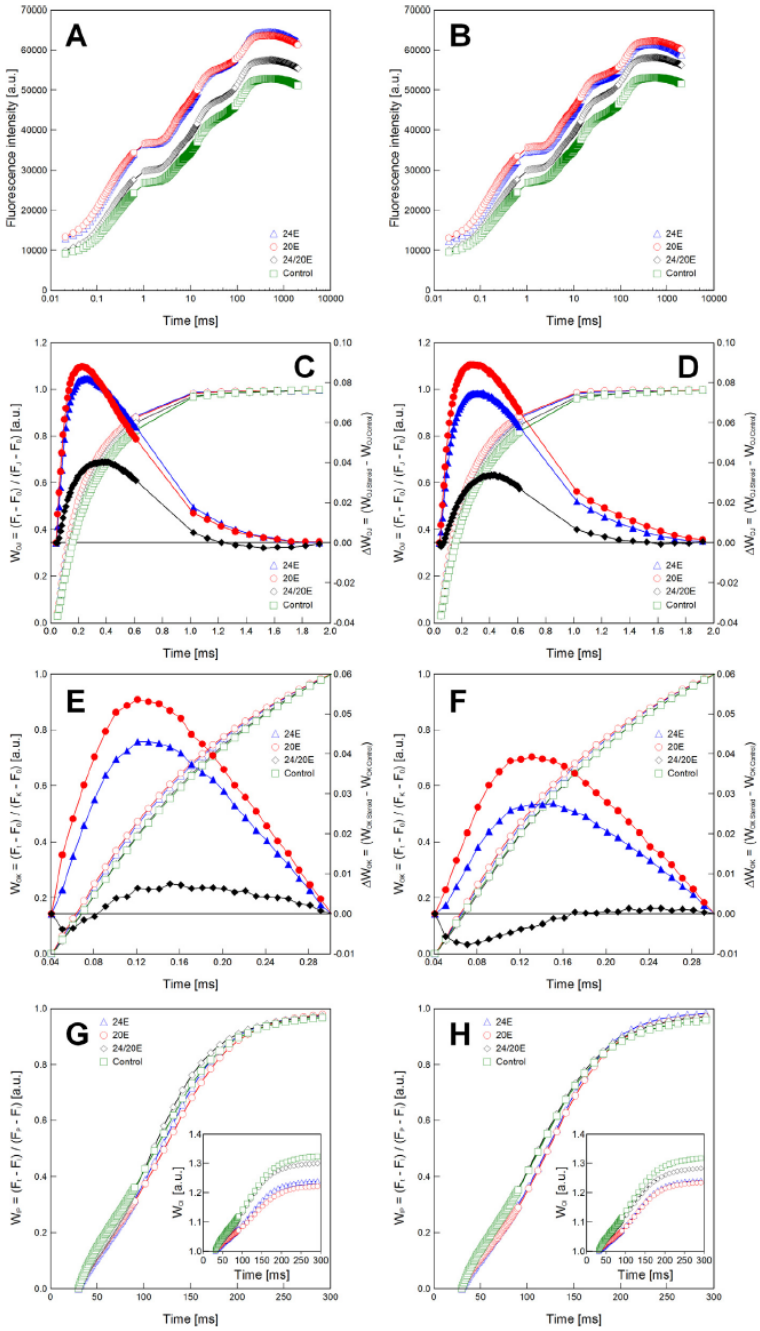


Fig. 3. The polyphasic rise of chlorophyll *a* fluorescence transients (OJIP) [panels A and B], the relative variable fluorescence W_{OJ} [panels C and D], W_{OK} [panels E and F], W_{IP} [panels G and H, main graphs] and W_{OI} [panels G and H, inset graphs] and the difference kinetics revealing the K- and L-bands, ΔW_{OJ} [panels C and D] and ΔW_{OK} [panels E and F] as measured in young [panels A, C, E and G] or mature [panels B, D, F and H] leaves of spinach one week after their treatment with 24-epibrassinolide (24E), 20-hydroxyecdysone (20E), both (24/20E) or distilled water (Control). Average values are always shown, the relative variable fluorescences are plotted on left vertical axes and shown in open symbols, the difference kinetics are plotted on right vertical axes and shown in solid symbols. Young and mature refer to the state of leaves at the date of the treatment.

As stated in the Introduction, a really detailed examination of the photosynthetic electron-transport chain in BR-treated plants has not been made often but at least some parameters associated with primary photochemistry (usually quantum yields of PSII photochemistry) were measured in several plant species. An interesting result of our current study was the finding that primary photosynthetic processes (together with the contents of photosynthetic pigments) in leaves of both examined species responded to the steroid treatment in a totally different way, with spinach mostly negatively influenced by the treatment with individual steroids while maize was either not at all or more-or-less positively affected. The authors who previously worked with wheat [50,51], barley [39], maize [52,53], winter rape [38], tomato [54–56] or pepper [57,58] did not find any significant effect of BRs' application on PSII function when plants were grown under normal conditions. However, others [31–34,59–62] dealing with cucumber plants observed an improvement in Φ_{PSII} in plants treated with 24E. A significant increase in this parameter (as well as in F_v'/F_m' and q_P) was described also for melon plants by Zhang and others [63]. Similarly, some authors who worked with cucumber [64], rapeseed [65] or mungbean [66] plants also reported an 24E- or 28-homobrassinolide-induced increase of F_v/F_m. Thus, all three possible types of the PSII response to BRs (*i.e.* none, positive and – as seen in spinach in our study – negative) have been demonstrated and both the previous analyses and our current study imply that each plant species shows its own distinctive response of PSII complex to these steroids and that – as is often the case in biology – what applies for one species is not necessarily true for another one.

Curiously enough, many studies demonstrated a significant improvement of PSII function in plants (including the species which did not respond to exogenously applied BRs under normal conditions) treated with BRs and afterwards (or previously) exposed to various stressors, *e.g.* cold [67–69], heat [39,63], drought [70–72] or salinity [51,64,65,73,74]; on the other hand, other authors [50,57] did not observe any such phenomenon for BR-treated and salt-stressed plants). Positive effect of BRs on some parameters associated with PSII function was described also in plants stressed by cadmium [38,55], nickel [75], copper [64], the excess of boron [66], polychlorinated biphenyls [56], phenanthrene [76–78], pyrene [77], plants exposed to various herbicides, fungicides or insecticides [59,60,67,79,80] or plants grown in elevated CO_2 conditions [34]. This indicates that the response of this photosynthetic complex to BRs is further affected by the environment plants are exposed to and that the overall change in plant condition caused by unfavourable conditions non-specifically induces BRs' regulation of PSII in all plant species.

Our results also showed that (besides these interspecific differences) BRs can have different effects on various parts of a photosynthetic photon absorption and electron-transport chain. We demonstrated that not only the efficiency of PSII *per se* can be affected by these compounds, but that they can (not always in the same way) change the apparent size of its antenna, the functionality of its OEC or the energetical connectivity between individual PSII units (again, the positive or negative character of such changes depends not only on the plant species but on the developmental state of the leaf and on the time elapsed from BR treatment as well). In their recent study, Krumova and others [81] analyzed the structure of thylakoid membranes and PSII function in *Arabidopsis* mutants with enhanced BR signaling, inactive BR receptors or a deficiency in BR biosynthesis and concluded that BRs affect photosynthesis by regulating the stability of OEC, thus indirectly affecting the assembly of PSII complexes and supercomplexes and their arrangement in thylakoid membranes. Janeczko and others [38] also observed the effect of 24E on OEC in winter rape; interestingly, this effect was positive in plants treated with cadmium but negative (although not significantly) in untreated plants. This is just another example of a strong dependency of BR-induced regulation of the photosynthetic apparatus on an overall state of plants and their environment.

In addition to PSII, other parts of photosynthetic electron-transport chain also clearly respond to BRs and this response can be quite different from the PSII response. For example, in spinach, the negative influence of BRs applied for both PSII and the whole electron-transport chain efficiencies, but the electron transport in PSII in our steroid-treated maize was almost non-affected by BRs whereas the whole electron-transport chain and the size of the pool of the PSI end electron acceptors was affected negatively. The *in vitro* analysis of the PSI activity in suspensions of chloroplasts isolated from BR-treated maize plants [52,53] did not reveal any significant changes of this parameter, so it is possible that the changes in the efficiency of the whole electron-transport chain observed for this species in our current study are associated neither with the intrinsic efficiency of PSII nor with PSI but with some other component of the electron-transport chain; however, this would require a further, more detailed study. The opposite effects BRs can have on various components of photosynthetic apparatus could also probably explain why the P_N did not change with steroid treatment in our maize plants even when the size of the PSI acceptors' pool and the efficiency of the whole electron-transport chain was diminished, as this negative effect could perhaps have somewhat been compensated by the observed increase in the amount of photosynthetic pigments and photon absorption. On the other hand, the negative response of P_N to 24E-treatment in spinach could have perhaps been associated with overall negative effect of this compound on primary photochemistry. Other mechanisms such as a stomatal closure could maybe play a role as well but the absence of a negative effect of 24E on intercellular CO_2 concentration rather indicates that this is not the case. It is also possible that the exogenous application of steroids resulted in the excessive accumulation of these compounds in spinach leaves, thus perhaps crossing the borders of rather narrow concentration range which is probably typical for a promotive effect of exogenous BRs on photosynthetic apparatus [82].

The molecular mechanisms of BR-induced regulation of photosynthesis are mostly unknown. BRs regulate the development of stomata which could indirectly affect photosynthetic efficiency. Interestingly, it seems that these steroids can both repress and promote stomatal formation depending on the respective plant organ (*i.e.* leaves, cotyledon, hypocotyl), and probably also on the environmental conditions [83,84]. A key component of BR signaling pathway, BRASSINOSTEROID INSENSITIVE2 (BIN2) kinase, has been implicated as an essential link between BRs and mitogen-activated protein kinase (MAPK) signaling cascade which regulates stomatal development [85,86]. Another possibility how BRs could regulate photosynthesis is their implication in the maintenance of the cellular/chloroplast redox homeostasis and the synthesis/activation of redox-sensitive photosynthetic enzymes by an H_2O_2-mediated increase in a ratio of reduced to oxidized glutathione [87].There are also some indications that BRs (and other steroids) can directly bind to some photosynthetic proteins (*e.g.* Rubisco, PsbP protein of OEC) and could perhaps function as allosteric regulators of their activity [27,28], Macek, unpublished results].

The expression of genes coding for photosynthetic proteins could also be either direct or indirect target of BR signaling pathway. BRs mostly down-regulate the expression of genes coding for various photosynthetic proteins [30,88,89]. The expression of two GOLDEN 2-LIKE transcription factors (GLK1 and GLK2) which regulate production of nuclear-encoded photosynthetic proteins is inhibited by BRASSINAZOLE RESISTANT1 (BRZ1) and/or PHYTOCHROME-INTERACTING FACTOR4 (PIF4) transcription factors, other essential components of BR-signaling pathway [90]. Transcription of plastid-encoded photosynthetic genes is also affected

by BRs. Efimova and others demonstrated that *Arabidopsis* mutants defective in BR synthesis showed an increased level of transcription of genes encoding some subunits of PSII, PSI or chloroplast ATP-synthase and that exogenous application of 10^{-6} M concentration of 24E to these mutants also activated the transcription of these genes [91,92]. It is possible that a precisely maintained BR concentration is necessary for the repression of photosynthetic genes in normal plants and that any deviation from this balance (either caused by BR deficiency or BR excess in mutants or plants supplied with exogenous BRs) can reverse this process. However, most of these hypotheses need further experimental support and while it is still missing, the exact molecular mechanisms of BR-induced regulation of photosynthesis can be only speculated on.

Our observation that steroid treatment affects photosynthetic processes more strongly in the mature leaves compared to the young, still not completely developed ones and that this applied both for spinach and maize is also rather interesting. We do not think it is related to the differences between the C3 (spinach) and C4 (maize) types of photosynthetic metabolism, as the appearance of C4 photosynthetic system in maize is associated with leaves of higher levels and leaves 1–3 are usually considered to have C3 type of metabolism [93]. Janeczko and others [39] observed similar differences in BR-response between younger and mature leaves of heat-stressed barley plants treated with 24E and they speculated that the younger leaves had as yet an insufficient number of BRs' receptors and that the BR-signaling pathway thus could not be activated as well as in the older, mature leaves. Our results could support this hypothesis as well, although its further verification would again require a more detailed study and a comparison of various developmental stages of leaves in various plant species. Unfortunately, other authors who examined BRs' effects on photosynthesis did usually work only with fully mature leaves from the start (and did not follow the natural course of leaf development), even in studies that analyzed a time-dependent effect of these compounds, such as the works with cucumber [31,32,60,61], tomato [54] or rapeseed [65]. These studies have shown that BRs' effect on various components of the photosynthetic apparatus can be sometimes seen very early (in the range of several hours) and can persist for a longer periods of time (several days or even weeks) which is similar to our results.

Besides a more detailed information on BRs' effects on primary photochemistry, the second (in our opinion even more important) main result our study offers is the discovery that exogenously applied EC can have similar effect on photosynthetic processes as BRs in a similarly low concentration. This introduces a concept that the structural similarity of these compounds could perhaps be somehow reflected in the functional similarity as well, at least in some aspects. The possibility that BRs are not the sole group of plant steroids with function in the regulation of photosynthesis has recently been supported by findings that at least some representants of another class of steroids, mammalian sex hormones (estrogens, androgens and progesterone), not only also naturally occur in plants but can affect various plant biological processes [12,94,95]. Janeczko and others [96] have described a positive effect of androstenedione on P_N and g_S in soybean plants after their recovery from drought exposure, although the efficiency of PSII was not affected in this case. Progesterone treatment was found to lessen the negative effects of the infection of *Arabidopsis* plants with *Pseudomonas syringae* on the PSII efficiency [97]. As regards ECs, with the exception of the work of Golovatskaya [23] who found that ecdysterone applied in 10^{-8} to 10^{-9} M concentrations to detached kidney bean leaves slows their yellowing (*i.e.* maintains the content of Chls), and our own previous *in vitro* or *in vivo* analyses made with higher concentrations of 20E [27–29,98], this study is to our knowledge the first *in vivo* one documenting that these steroids can have a similar role in the regulation of higher plant photosynthesis in similarly low concentration as BRs.

Our results demonstrated that the effects of 24E and 20E on photosynthesis mostly copied each other when these steroids were applied individually, but these effects were much diminished and usually did not significantly differ from the control when the 24/20E combination was used. This applied both for plant species rich in endogenous ECs (spinach) and the species that does not accumulate endogenous ECs (maize). Based on her observations of antagonistic effects of ecdysterone and 24-epibrassinolide in the assay for the elongation of wheat coleoptile segments, Golovatskaya [23] has suggested that ECs and BRs could compete for a common receptor. Some authors have also shown that BRs and ECs can have antagonistic function in insects and proposed that they probably compete at the binding site of the insect hormone receptor [99,100]. However, Voigt and others [101] assessed the effects of various synthetic brassinosteroid/ecdysteroid hybrid molecules in the *Drosophila* B_{II} cells bioassay for EC agonist and antagonist activities and in the rice lamina inclination test for BR activity and concluded that although some of these hybrid molecules possessed the agonist activity in the first bioassay and most of them showed some biological activity in the second bioassay, every small structural difference between castasterone (as the representant of BRs) and 20E had its impact on these activities. They therefore proposed that both the insect ECs' receptor and the plant BRs' receptor have highly specific structural requirements for the ligand binding and that such competition for a common receptor is highly unlikely. However, the possible competition between BRs and ECs does not necessarily have to be at the ligand-binding site of the BR receptor, as it has been shown that both types of these molecules can specifically bind to some other proteins (including those participating in photosynthesis) as well and perhaps act as allosteric activators [26–28]. Without the detailed screening of plant proteome in order to identify all proteins BRs and ECs can bind to, and without the comparison of their respective binding abilities and the effect this binding could have on the conformation and biological function of such proteins, this is of course a pure speculation. It is also possible that exogenous 24E and 20E, when applied individually, could indirectly repress/induce the expression of different photosynthetic genes (which could nevertheless result in similar effects on photosynthetic function) and their joint application could fully or partially restore the fine balance necessary for the normal function of photosynthetic apparatus. Other types of crosstalk between these two types of steroids are also possible, similarly to those known for other groups of plant hormones [102]. Unfortunately, ECs still remain outsiders in the field of scientific interests and we do know practically nothing about their precise fate in the plant cell, their interactions with its components and their possible function in signaling pathways and gene expression regulation.

5. Conclusions

Our study has brought two main results: (i) a demonstration that 20-hydroxyecdysone can affect various parts of photosynthetic processes in low concentration similarly to BRs and that there is probably at least some competitive behaviour between those two groups of plant steroids in this aspect of plant physiology, and (ii) a proof that as regards their influence on primary photosynthetic processes, BRs regulate not only PSII but also other parts of the photosynthetic electron transport chain – but not necessarily in the same way. We have also shown that there are distinctive interspecific and developmental differences regarding this process, and these differences should be in our opinion further examined, *i.e.* across a wide range of plant species, in various stages of plant development and types of environment, and using various

techniques that are currently available for photosynthetic measurements.

6. Competing interest

The authors declare that they have no competing interests.

Acknowledgements

This work was supported by the Czech Science Foundation (Grant 501/11/1650), the Grant Agency of Charles University in Prague (Grants B/BIO/612612, SVV-2013-267205 and SVV-2014-260081) and the Ministry of Education, Youth and Sport of the Czech Republic (Grant MSM0021620858 and S-grant). The authors would also like to thank Tomáš Rýgl from the Czech University of Life Sciences Prague for his help with the gas exchange measurements, and Dr. Jaroslav Poruba from the CEZEA Maize Breeding Station for his supply of maize kernels.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.steroids.2014.04.006.

References

[1] Dinan L. The Karlson lecture. Phytoecdysteroids: what use are they? Arch Insect Biochem Physiol 2009;72:126–41.
[2] Gudesblat GE, Russinova E. Plants grow on brassinosteroids. Curr Opin Plant Biol 2011;14:530–7.
[3] Yang CJ, Zhang C, Lu YN, Jin JQ, Wang XL. The mechanisms of brassinosteroids' action: From signal transduction to plant development. Mol Plant 2011;4:588–600.
[4] Choudhary SP, Yu JQ, Yamaguchi-Shinozaki K, Shinozaki K, Tran LSP. Benefits of brassinosteroid crosstalk. Trends Plant Sci 2012;17:594–605.
[5] Kutschera U, Wang ZY. Brassinosteroid action in flowering plants: a Darwinian perspective. J Exp Bot 2012;63:3511–22.
[6] Vriet C, Russinova E, Reuzeau C. Boosting crop yields with plant steroids. Plant Cell 2012;24:842–57.
[7] Zhao B, Li J. Regulation of brassinosteroid biosynthesis and inactivation. J Integr Plant Biol 2012;54:746–59.
[8] Fridman Y, Savaldi-Goldstein S. Brassinosteroids in growth control: how, when and where. Plant Sci 2013;209:24–31.
[9] Hao J, Yin Y, Fei S. Brassinosteroid signaling network: implications on yield and stress tolerance. Plant Cell Rep 2013;32:1017–30.
[10] Hayat S, Ahmad A, editors. Brassinosteroids: a class of plant hormone. Springer Science+Business Media B.V.: Dordrecht-Heidelberg-London-New York; 2011.
[11] Báthori M, Pongrácz Z. Phytoecdysteroids – from isolation to their effects on humans. Curr Med Chem 2005;12:153–72.
[12] Speranza A. Into the world of steroids. A biochemical "keep in touch" in plants and animals. Plant Signal Behaviour 2010;5:940–3.
[13] Lafont R. Phytoecdysteroids in world flora: diversity, distribution, biosynthesis and evolution. Russ J Plant Physiol 1998;45:276–95.
[14] Dinan L, Harmatha J, Volodin V, Lafont R. Phytoecdysteroids: diversity, biosynthesis and distribution. In: Smagghe G, editor. Ecdysone: structures and functions. Dordrecht-Heidelberg-London-New York: Springer Science+Business Media B.V; 2009. p. 3–45.
[15] Dinan L. Distribution and levels of phytoecdysteroids within individual plants of species of the *Chenopodiaceae*. Eur J Entomol 1995;92:295–300.
[16] Dinan L. Phytoecdysteroids: biological aspects. Phytochemistry 2001;57:325–39.
[17] Dinan L, Savchenko T, Whiting P. On the distribution of phytoecdysteroids in plants. Cell Mol Life Sci 2001;58:1121–32.
[18] Bakrim A, Maria A, Sayah F, Lafont R, Takvorian N. Ecdysteroids in spinach (*Spinacia oleracea* L.): biosynthesis, transport and regulation of levels. Plant Physiol Biochem 2008;46:844–54.
[19] Festucci-Buselli RA, Contim LAS, Barbosa LCA, Stuart J, Otoni WC. Biosynthesis and potential functions of the ecdysteroid 20-hydroxyecdysone – a review. Botany 2008;86:978–87.
[20] Cheng DM, Yousef GG, Lila MA. Variation in phytoecdysteroid accumulation in seeds and shoots of *Spinacia oleracea* L. accessions. Hortic Sci 2010;45:1634–8.
[21] Dreier SI, Towers GHN. Activity of ecdysone in selected plant growth bioassays. J Plant Physiol 1988;132:502–12.
[22] Macháčková I, Vágner M, Sláma K. Comparison between the effects of 20-hydroxyecdysone and phytohormones on growth and development in plants. Eur J Entomol 1995;92:309–16.
[23] Golovatskaya IF. Effect of ecdysterone on morphological and physiological processes in plants. Russ J Plant Physiol 2004;51:452–8.
[24] Bakrim A, Lamhamdi M, Sayah F, Chibi F. Effect of plant hormones and 20-hydroxyecdysone on tomato (*Lycopersicon esculentum*) seed germination and seedlings growth. Afr J Biotechnol 2007;6:2792–802.
[25] Bajguz A, Koronka A. Effect of ecdysone application on the growth and biochemical changes in *Chlorella vulgaris* cells. Plant Physiol Biochem 2001;39:707–15.
[26] Viktorová J, Krásný L, Kamlar M, Nováková M, Macková M, Macek T. Osmotin, a pathogenesis-related protein. Curr Prot Pept Sci 2012;13:672–81.
[27] Uhlík O, Kamlar M, Kohout L, Ježek R, Harmatha J, Macek T. Affinity chromatography reveals RuBisCO as an ecdysteroid-binding protein. Steroids 2008;73:1433–40.
[28] Kamlar M, Uhlík O, Kohout L, Šanda M, Macek T. Affinity chromatography as the method for brassinosteroid-binding protein isolation. J Biotechnol 2010;150:S490.
[29] Holá D, Rothová O, Kočová M, Fridrichová L, Macek T. Phytoecdysteroids together with brassinosteroids stimulate oxygen-evolving activity of photosystem II. Freiburg, Germany: Plant Biology Congress; 30 July–3 August 2012. Book of Abstracts, p. 771.
[30] Holá D. Brassinosteroids and photosynthesis. In: Hayat S, Ahmad A, editors. Brassinosteroids: a class of plant hormone. Dordrecht-Heidelberg-London-New York: Springer Science+Business Media B.V.; 2011. p. 143–92.
[31] Yu JQ, Huang LF, Hu WH, Zhou YH, Mao WH, Ye SF, et al. A role for brassinosteroids in the regulation of photosynthesis in *Cucumis sativus*. J Exp Bot 2004;55:1135–43.
[32] Xia XJ, Huang LF, Zhou YH, Mao WH, Shi K, Wu JX, et al. Brassinosteroids promote photosynthesis and growth by enhancing activation of Rubisco and expression of photosynthetic genes in *Cucumis sativus*. Planta 2009;230:1185–96.
[33] Jiang Y, Cheng F, Zhou Y, Xia X, Mao W, Shi K, et al. Brassinosteroid-induced CO_2 assimilation is associated with increased stability of redox-sensitive photosynthetic enzymes in the chloroplasts in cucumber plants. Biochem Biophys Res Commun 2012;426:390–4.
[34] Jiang YP, Cheng F, Zhou YH, Xia XJ, Shi K, Yu JQ. Interactive effects of CO_2 enrichment and brassinosteroid on CO_2 assimilation and photosynthetic electron transport in *Cucumis sativus*. Environ Exp Bot 2012;75:98–106.
[35] Strasser RJ, Srivastava A, Tsimilli-Michael M. The fluorescence transient as a tool to characterize and screen photosynthetic samples. In: Yunus M, Pathre U, Mohanty P, editors. Probing photosynthesis: mechanism, regulation and adaptation. London: Taylor and Francis; 2000. p. 445–83.
[36] Strasser RJ, Tsimilli-Michael M, Srivastava A. Analysis of the chlorophyll a fluorescence transient. In: Papageorgiou GC, Govindjee, editors. Chlorophyll a fluorescence. A signature of photosynthesis. Dordrecht: Springer; 2004. p. 321–62.
[37] Stirbet A, Govindjee. On the relation between the Kautsky effect (chlorophyll a fluorescence induction) and photosystem II: basics and applications of the OJIP fluorescence transient. J Photochem Photobiol B Biol 2011;104:236–57.
[38] Janeczko A, Kościelniak J, Pilipowicz M, Szarek-Łukaszewska G, Skoczowski A. Protection of winter rape photosystem 2 by 24-epibrassinolide under cadmium stress. Photosynthetica 2005;43:293–8.
[39] Janeczko A, Oklešťková J, Pociecha E, Kościelniak J, Mirek M. Physiological effects and transport of 24-epibrassinolide in heat-stressed barley. Acta Physiol Plant 2011;33:1249–59.
[40] Devarenne TP, Sen-Michael B, Adler JH. Biosynthesis of ecdysteroids in *Zea mays*. Phytochemistry 1995;40:1125–31.
[41] Bajguz A. Brassinosteroids – occurrence and chemical structures in plants. In: Hayat S, Ahmad A, editors. Brassinosteroids: a class of plant hormone. Dordrecht-Heidelberg-London-New York: Springer Science+Business Media B.V.; 2011. p. 1–27.
[42] Fridrichová L, Trubanová N, Kočová M, Holá D, Rothová O, Jelénková I, et al. 24-Epibrassinolide improves the response of young maize and faba been plants during drought stress: joint analysis of plant biochemistry, physiology, morphology and development. Freiburg, Germany: Plant Biology Congress; 30 July–3 August 2012. Book of Abstracts, p. 766.
[43] Yusuf MA, Kumar D, Rajwanshi R, Strasser RJ, Tsimilli-Michael M, Govindjee K, et al. Overexpression of γ-tocopherol methyl transferase gene in transgenic *Brassica juncea* plants alleviates abiotic stress: physiological and chlorophyll *a* fluorescence measurements. Biochim Biophys Acta 2010;177:1428–38.
[44] Hall DO. Nomenclature for isolated chloroplasts. Nature New Biol 1972;235:125–6.
[45] Porra RJ, Thompson WA, Kriedemann PE. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochim Biophys Acta 1989;975:384–94.
[46] Holá D, Kočová M, Rothová O, Hlízová E, Fridrichová L, Lhoták Z, et al. A universal method for the isolation of photochemically active broken chloroplasts from conifer needles and its possible application in photosynthetic studies. Photosynthetica 2012;50:291–304.
[47] Bartoš J, Berková E, Šetlík I. A versatile chamber for gas exchange measurements in suspensions of algae and chloroplasts. Photosynthetica 1975;9:395–406.

[48] Wellburn AR. The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. Plant Physiol 1994;144:307–13.
[49] Benešová M, Holá D, Fischer L, Jedelský PL, Hnilička F, Wilhelmová N, et al. The physiology and proteomics of drought tolerance in maize: early stomatal closure as a cause of lower tolerance to short-term dehydration? PLoS ONE 2012;7:e38017.
[50] Ali Q, Athar HR, Ashraf M. Modulation of growth, photosynthetic capacity and water relations in salt stressed wheat plants by exogenously applied 24-epibrassinolide. Plant Growth Regul 2008;56:107–16.
[51] Shahbaz M, Ashraf M, Athar HR. Does exogenous application of 24-epibrassinolide ameliorate salt induced growth inhibition in wheat (*Triticum aestivum* L.)? Plant Growth Regul 2008;55:51–61.
[52] Honnerová J, Rothová O, Holá D, Kočová M, Kohout L, Kvasnica M. The exogenous application of brassinosteroids to *Zea mays* (L.) stressed by long-term chilling does not affect the activities of photosystems 1 or 2. J. Plant Growth Regul 2010;29:500–5.
[53] Kočová M, Rothová O, Holá D, Kvasnica M, Kohout L. The effect of brassinosteroids on photosynthetic parameters in leaves of two field-grown maize inbred lines or their F1 hybrid. Biol Plant 2012;54:785–8.
[54] Ogweno JO, Song XS, Shi K, Hu WH, Mao WH, Zhou YH, et al. Brassinosteroids alleviate heat-induced inhibition of photosynthesis by increasing carboxylation efficiency and enhancing antioxidant systems in *Lycopersicon esculentum*. J Plant Growth Regul 2008;27:49–57.
[55] Ahammed GJ, Choudhary SP, Chen SC, Xia XJ, Shi K, Zhou YH, et al. Role of brassinosteroids in alleviation of phenanthrene-cadmium co-contamination-induced photosynthetic inhibition and oxidative stress in tomato. J Exp Bot 2013;64:199–213.
[56] Ahammed GJ, Ruan YP, Zhou J, Xia XJ, Shi K, Zhou YH, et al. Brassinosteroid alleviates polychlorinated biphenyls-induced oxidative stress by enhancing antioxidant enzymes activity in tomato. Chemosphere 2013;90:2645–53.
[57] Houimli SM, Denden M, El Hadj SB. Induction of salt tolerance in pepper (*Capsicum annuum*) by 24-epibrassinolide. Eur Asia J Bio Sci 2008;2:83–90.
[58] Ibn Maaouia-Houimli S, Dridi-Mouhandes B, ben Mansour-Gueddes S, Denden M. 24-Epibrassinolide ameliorates the adverse effect of salt stress (NaCl) on pepper (*Capsicum annuum* L.). J Stress Physiol Biochem 2012;8:2332–40.
[59] Xia XJ, Huang YY, Wang L, Huang LF, Yu YL, Zhou YH, et al. Pesticides-induced depression of photosynthesis was alleviated by 24-epibrassinolide pretreatment in *Cucumis sativus* L. Pesticide Biochem Physiol 2006;86:42–8.
[60] Xia XJ, Zhang Y, Wu JX, Wang JT, Zhou YH, Shi K, et al. Brassinosteroids promote metabolism of pesticides in cucumber. J Agric Food Chem 2009;57:8406–13.
[61] Xia XJ, Zhou YH, Ding J, Shi K, Asami T, Cheng Z, et al. Induction of systemic stress tolerance by brassinosteroid in *Cucumis sativus*. New Phytol 2011;191:706–20.
[62] Jiang Y, Cheng F, Zhou Y, Xia X, Mao W, Shi K, et al. Hydrogen peroxide functions as a secondary messenger for brassinosteroids-induced CO_2 assimilation and carbohydrate metabolism in *Cucumis sativus*. J Zheijiang Univ Sci B (Biomed Biotechnol) 2012;13:811–23.
[63] Zhang YP, Zhu XH, Ding HD, Yang SJ, Chen YY. Foliar application of 24-epibrassinolide alleviates high-temperature-induced inhibition of photosynthesis in seedlings of two melon cultivars. Photosynthetica 2013;51:341–9.
[64] Fariduddin Q, Khalil RRAE, Mir BA, Yusuf M, Ahmad A. 24-epibrassinolide regulates photosynthesis, antioxidant enzyme activities and proline content of *Cucumis sativus* under salt and/or copper stress. Environ Monit Assess 2013;185:7845–56.
[65] Hayat S, Maheshwari P, Wani AS, Irfan M, Aleymeni MN, Ahmad A. Comparative effect of 24 homobrassinolide and salicylic acid in the amelioration of NaCl stress in *Brassica juncea* L. Plant Physiol Biochem 2012;53:61–8.
[66] Yusuf M, Fariduddin Q, Ahmad A. 28-homobrassinolide mitigates boron induced toxicity through enhanced antioxidant system in *Vigna radiata* plants. Chemosphere 2011;85:1574–84.
[67] Cui JX, Zhou YH, Ding JG, Xia XJ, Shi K, Chen SC, et al. Role of nitric oxide in hydrogen peroxide-dependent induction of abiotic stress tolerance by brassinosteroids in cucumber. Plant Cell Environ 2011;34:347–58.
[68] Fariduddin Q, Yusuf M, Chalkoo S, Hayat S, Ahmad A. 28-homobrassinolide improves growth and photosynthesis in *Cucumis sativus* L. through an enhanced antioxidant system in the presence of chilling stress. Photosynthetica 2011;49:55–64.
[69] Jiang YP, Huang LF, Cheng F, Zhou YH, Xia XJ, Mao WH, et al. Brassinosteroids accelerate recovery of photosynthetic apparatus from cold stress by balancing the electron partitioning, carboxylation and redox homeostasis in cucumber. Physiol Plant 2012;148:133–45.
[70] Zhang M, Zhai Z, Tian X, Duan L, Li Z. Brassinolide alleviated the adverse effect of water deficits on photosynthesis and the antioxidant of soybean (*Glycine max* L.). Plant Growth Regul 2008;56:257–64.
[71] Li YH, Liu YJ, Xu XL, Jin M, An LZ, Zhang H. Effect of 24-epibrassinolide on drought stress-induced changes in *Chorispora bungeana*. Biol Plant 2012;56:192–6.
[72] Hu W, Yan X, Xiao Y, Zeng J, Qi H, Ogweno JO. 24-epibrassinosteroid alleviate drought-induced inhibition of photosynthesis in *Capsicum annuum*. Sci Hortic 2013;150:232–7.
[73] Wu XX, Ding HD, Zhu ZW, Yang SJ, Zha DS. Effects of 24-epibrassinolide on photosynthesis of eggplant (*Solanum melongena* L.) seedlings under salt stress. Afr J Biotechnol 2012;11:8665–71.
[74] Yuan L, Shu S, Sun J, Guo S, Tezuka T. Effects of 24-epibrassinolide on the photosynthetic characteristics, antioxidant system, and chloroplast ultrastructure in *Cucumis sativus* L. under $Ca(NO_3)_2$ stress. Photosynth Res 2012;112:205–14.
[75] Yusuf M, Fariduddin Q, Hayat S, Hasan SA, Ahmad A. Protective response of 28-homobrassinolide in cultivars of *Triticum aestivum* with different levels of nickel. Arch Environ Contam Toxicol 2011;60:68–76.
[76] Ahammed GJ, Gao CJ, Ogweno JO, Zhou YH, Xia XJ, Mao WH, et al. Brassinosteroids induce plant tolerance against phenanthrene by enhancing degradation and detoxification in *Solanum lycopersicum* L. Ecotoxicol Environ Safety 2012;80:28–36.
[77] Ahammed GJ, Yuan HL, Ogweno JO, Zhou YH, Xia XJ, Mao WH, et al. Brassinosteroid alleviates phenanthrene and pyrene phytotoxicity by increasing detoxification activity and photosynthesis in tomato. Chemosphere 2012;86:546–55.
[78] Ahammed GJ, Zhang S, Shi K, Zhou YH, Yu JQ. Brassinosteroid improves seed germination and early development of tomato seedlings under phenanthrene stress. Plant Growth Regul 2012;68:87–96.
[79] Piñol R, Simón E. Effect of 24-epibrassinolide on chlorophyll fluorescence and photosynthetic CO_2 assimilation in *Vicia faba* plants treated with the photosynthesis-inhibiting herbicide terbutryn. J Plant Growth Regul 2009;28:97–105.
[80] Li P, Chen L, Zhou Y, Xia X, Shi K, Chen Z, et al. Brassinosteroids-induced systemic stress tolerance was associated with increased transcripts of several defence-related genes in the phloem in *Cucumis sativus*. PLoS ONE 2013;8:e66582.
[81] Krumova S, Zhiponova M, Dankov K, Velikova V, Balashev K, Andreeva T, et al. Brassinosteroids regulate the thylakoid membrane architecture and the photosystem II function. J Photochem Photobiol B Biol 2013;126:97–104.
[82] Bajguz A, Asami T. Suppression of *Wolffia arrhiza* growth by brassinazole, an inhibitor of brassinosteroid biosynthesis and its restoration by endogenous 24-epibrassinolide. Phytochemistry 2005;66:1787–96.
[83] Serna L. What causes opposing actions of brassinosteroids on stomatal development? Plant Physiol 2013;162:3–8.
[84] Gudesblat GE, Betti C, Russinova E. Brassinosteroids tailor stomatal production to different environments. Trends Plant Sci 2012;12:685–7.
[85] Kim TW, Michniewicz M, Bergmann DC, Wang ZY. Brassinosteroid regulates stomatal development by GSK3-mediated inhibition of a MAPK pathway. Nature 2012;482:419–23.
[86] Kong X, Pan J, Cai G, Li D. Recent insights into brassinosteroid signaling in plants: its dual control of plant immunity and stomatal development. Mol Plant 2012;5:1179–81.
[87] Jing YP, Cheng F, Zhou YH, Xia XJ, Mao WH, Shi K, Chen Z, Yu JQ. Cellular glutathione redox homeostasis plays an important role in the brassinosteroid-induced increase in CO_2 assimilation in *Cucumis sativus*. New Phytol 2012;194:932–43.
[88] Guo H, Li L, Aluru M, Aluru S, Yin Y. Mechanisms and networks for brassinosteroid regulated gene expression. Curr Opin Plant Biol 2013;16:545–53.
[89] Zhu JY, Sae-Seaw J, Wang ZY. Brassinosteroid signalling. Development 2013;140:1615–20.
[90] Oh E, Zhu JY, Wang ZY. Interaction between BZR1 and PIF4 integrates brassinosteroid and environmental responses. Nature Cell Biol 2012;14:802–11.
[91] Efimova MV, Kusnetsov VV, Kravtsov AK, Karnachuk RA, Khripach VA, Kuznetsov VV. Regulation of the transcription of plastid genes in plants by brassinosteroids. Dokl Biol Sci 2012;445:272–5.
[92] Efimova MV, Kusnetsov VV, Kravtsov AK, Bartashevich DA, Karnachuk RA, Kovtun IS, Kuznetsov VV. Expression of plastid genome and development of *Arabidopsis thaliana* with disturbed synthesis of brassinosteroids. Russ J Plant Physiol 2012;59:28–34.
[93] Crespo HM, Frean M, Cresswell CR, Tew J. The occurrence of both C3 and C4 photosynthetic characteristics in a single *Zea mays* plant. Planta 1979;147:257–63.
[94] Janeczko A, Skoczowski A. Mammalian sex hormones in plants. Folia Histochem Cytobiol 2005;43:71–9.
[95] Janeczko A. The presence and activity of progesterone in the plant kingdom. Steroids 2012;77:169–73.
[96] Janeczko A, Kocurek M, Marcinska I. Mammalian androgen stimulates photosynthesis in drought-stressed soybean. Cent Eur J Biol 2012;7:802–9.
[97] Janeczko A, Tóbiás I, Skoczowski A, Dubert F, Gullner G, Barna B. Progesterone moderates damage in *Arabidopsis thaliana* caused by infection with *Pseudomonas syringae* or *P. fluorescens*. Biol Plant 2013;57:169–73.
[98] Holá D, Kočová M, Rothová O, Tůmová L, Kamlar M, Macek T. Exogenously applied 20-hydroxyecdysone increases the net photosynthetic rate but does not affect the photosynthetic electron transport or the content of photosynthetic pigments in *Tetragonia tetragonioides* L. Acta Physiol Plant 2013;35:3489–95.

[99] Lehmann M, Vorbrodt HM, Adam G, Koolman J. Antiecdysteroid activity of brassinosteroids. Experientia 1988;44:355–6.
[100] Richter K, Koolman J. Antisteroid effects of brassinosteroids in insects. In: Cutler HG, Yokota T, Adam G, editors. Brassinosteroids: chemistry, bioactivity and applications. Washington DC: American Chemical Society; 1991. p. 265–78.
[101] Voigt B, Whiting P, Dinan L. The ecdysteroid agonist/antagonist and brassinosteroid-like activities of synthetic brassinosteroid/ecdysteroid hybrid molecules. Cell Mol Life Sci 2001;58:1133–40.
[102] Depuydt S, Hardtke CS. Hormone signalling crosstalk in plant growth regulation. Curr Biol 2011;21:R365–73.

Supplementary Fig. 1. The polyphasic rise of chlorophyll *a* fluorescence transients (OJIP) [*panels A, B*], the relative variable fluorescence W_{OJ} [*panels C, D*], W_{OK} [*panels E, F*], W_{IP} [*panels G, H, main graphs*] and W_{OI} [*panels G, H, inset graphs*] and the difference kinetics revealing the K- and L- bands, ΔW_{OJ} [*panels C, D*] and ΔW_{OK} [*panels E, F*] as measured in young [*panels A, C, E, G*] or mature [*panels B, D, F, H*] leaves of maize one day after their treatment with 24-epibrassinolide (24E), 20-hydroxyecdysone (20E), both (24/20E) or distilled water (Control). Average values are always shown, the relative variable fluorescences are plotted on left vertical axes and shown in open symbols, the difference kinetics are plotted on right vertical axes and shown in solid symbols. Young and mature refer to the state of leaves at the date of the treatment.

Supplementary Fig. 2. The polyphasic rise of chlorophyll *a* fluorescence transients (OJIP) [*panels A, B*], the relative variable fluorescence W_{OJ} [panels *C, D*], W_{OK} [*panels E, F*], W_{IP} [*panels G, H, main graphs*] and W_{OI} [*panels G, H, inset graphs*] and the difference kinetics revealing the K- and L- bands, ΔW_{OJ} [*panels C, D*] and ΔW_{OK} [*panels E, F*] as measured in young [*panels A, C, E, G*] or mature [*panels B, D, F, H*] leaves of spinach one day after their treatment with 24-epibrassinolide (24E), 20-hydroxyecdysone (20E), both (24/20E) or distilled water (Control). Average values are always shown, the relative variable fluorescences are plotted on left vertical axes and shown in open symbols, the difference kinetics are plotted on right vertical axes and shown in solid symbols. Young and mature refer to the state of leaves at the date of the treatment.

6. Diskuze

6.1. Reakce různých genotypů kukuřice na aplikaci brassinosteroidů v přírodních podmínkách

Vliv ošetření rostlin postřikem nebo máčením semen v roztocích BR u různých druhů rostlin včetně kukuřice byl již před námi často studován, avšak tyto pokusy byly obvykle prováděny v umělých a ne přírodních podmínkách a navíc obvykle u velmi mladých rostlin. Většinou byly také postřiky provedeny jen v jednom vývojovém stádiu rostlin. Amzalag (2002) u čiroku zjistil při časné aplikaci 10^{-8} M 24E průkazný nárůst biomasy i délky listové čepele, při pozdějším postřiku se však tento efekt neprokázal. V našich pokusech se ukázalo, že postřik kukuřice stimuloval nárůst biomasy v obou sledovaných vývojových stádiích, avšak délka listu byla ovlivněna jen při postřiku v dřívějším vývojovém stádiu. Vývojové stádium rostliny v době ošetření je tedy důležité pro případné agronomické využití BR.

Ještě výraznější závislost na době ošetření jsme pozorovali při vývoji samičích květenství kukuřice. Při postřiku různými koncentracemi BR ve stádiu V3/4 jsme pozorovali zpomalení nástupu kvetení a nižší počet samičích květenství na rostlině, obdobně jako Janeczko (2003) a Kęsy *et al.* (2003). Na druhé straně jsme zjistili zkrácení doby vývoje květů při ošetření rostlin ve stádiu V5/6. Jelikož se samičí květy zakládají ve stádiu V3 až V5 a samčí ve V3 (Ritchie *et al.*, 1993), je možné, že aplikace BR před tímto obdobím ovlivňuje kvetení rostlin negativně a pokud jsou rostliny postříkány po tomto období, je vliv BR naopak pozitivní. Efektivní koncentrace BR je pravděpodobně velmi závislá na rostlinném druhu, případně i genotypu. V našich pokusech byl celkový trend pozorovaných změn u jednotlivých genotypů podobný, ale rozdíl 1 až 2 dny mezi jednotlivými genotypy zaznamenán byl.

Interpretace morfologických dat s ohledem na ranou a pozdní aplikaci BRs je obtížnější. Zatímco v našich experimentech jsme rozdíl mezi oběma aplikacemi steroidu zjistili pouze u délky listové čepele (avšak ne u všech listů, které se vyvíjely při nebo po postřiku), Amzallag (2002) u čiroku pozoroval průkazný vliv 24E na 5. až 7. list, ne však na listy níže ani výše položené. Obecně mají BR pozitivní vliv na velikost rostlin či počet listů u různých druhů rostlin (Arora *et al* 2008, Hayat 2001a, Sharma *et* Bhardway 2007,

Vardhini *et* Rao 1998). Na druhou stranu byl popsán i negativní vliv na růst rostlin po působení BR (Takematsu *et* Takeuchi 1989, Őzdemir *et al* 2004, Farooq *et al* 2009, Vlašánková *et al.* 2009). Vlašánková *et al.* (2009), kteří pracovali se stejnými BR jako my v našich experimentech, pozorovali inhibici růstu epikotylů hrachu a semenáčku lnu. Avšak všechny tyto práce byly prováděny na velmi mladých rostlinách v přísně kontrolovaných laboratorních podmínkách a BR byly obvykle přidány do růstového média, nebo v jejich roztocích přímo klíčila semena. Ošetření ve velmi časných stádiích obvykle nevykazuje žádný nebo vykazuje dokonce negativní vliv na růst studovaných rostlin.

Velmi také záleží na genotypu rostliny a typu BR. Ve většině prací je studován pouze jeden rostlinný druh a jen velmi málo autorů analyzujících vliv BR na rostliny zároveň pracovalo s více genotypy. Různé genotypy jednoho druhu v experimentech někdy reagují odlišně (Hnilička *et al* 2007, Torres-Ruiz *et al* 2007). Během našich experimentů se dvěma inbredními liniemi kukuřice a jejich F1 křížencem jsme zjistili, že rozdíly v odpovědi na ošetření BRs mezi oběma inbredními liniemi byly výraznější, než mezi nimi a křížencem, a že reakce obou inbredních genotypů byla často opačná (např. u počtu fertilních palic na rostlinu, váhy palice, váhy 100 semen). Odpověď F1 hybrida byla obvykle podobnější rodiči, který byl donorem pylu, což bylo neočekávané zjištění, které by stálo za detailní rozbor při sledování více parametrů a většího množství rodičů a kříženců, aby se lépe dala popsat případná dědičnost odpovědi na působení BR.

V našich experimentech v polních podmínkách se neprojevil statisticky průkazně vliv ošetření BR na obsahy fotosyntetických pigmentů (chlorofylů a karotenoidů) ani na aktivitu PS2 či PS1. Aktivitu PS2 po ošetření BR zkoumalo více autorů (hlavně prostřednictvím měření fluorescence chlorofylu *a*), avšak u rostlin pěstovaných v optimálních podmínkách prostředí často také nezjistili žádný vliv aplikovaného hormonu na tuto část fotosyntetických procesů (Yu *et al* 2004, Ali *et al* 2008c, Ogweno *et al* 2008). Rozdíly mezi ošetřenými a neošetřenými rostlinami však byly obvykle zjištěny tehdy, pokud rostliny byly vystaveny stresovým podmínkám (Shahbaz *et al* 2008, Zhang *et al* 2008).

6.2. Pomáhají brassinosteroidy rostlině zvládat abiotické stresy?

Rostliny kukuřice, které byly vystaveny dlouhodobému stresu způsobenému chladem, po ošetření BR neměly statisticky průkazně ovlivněné aktivity PS1 ani PS2. Tento výsledek považujeme za velmi důležitý, protože vliv BR na PS1 nebyl před námi dosud studován, ačkoliv je tento fotosystém velmi důležitou složkou primárních fotosyntetických procesů a při stresu chladem může být limitujícím faktorem pro efektivitu primární fotochemie (Zhang *et* Scheller 2004). Aktivity PS2 při ošetření rostlin BR jsou studovány častěji, zejména prostřednictvím analýzy fluorescence chlorofylu *a*, avšak výsledky nejsou jednoznačné. Většina autorů nezaznamenala vliv BRs na PS2 v kontrolních podmínkách, ale například při stresu suchem (Zhang *et al* 2008), zvýšenou salinitou (Shahbaz *et al* 2008, Farridudin *et al* 2013), nebo v přítomnosti kadmia (Janeczko *et al* 2005), mědi (Farridudin *et al* 2013) bóru (Yusuf et al 2011) či různým herbicidů, fungicidů nebo insekticidů (Xia *et al* 2006, 2013, Piñol *et* Simón 2009, Cui *et al* 2011, Li *et al* 2013) se účinnost PS2 po působení BR zlepšila. Data o vlivu BR na primární fotosyntetické procesy při působení chladu jsou vzácnější a naše výsledky neukazují, že by ošetření BR u kukuřice v těchto podmínkách mělo pozitivní vliv.

Z fotosyntetických pigmentů vykazovaly lepší odezvu na aplikaci BR při stresu chladem chlorofyly než karotenoidy, což je výsledek ve shodě se zjištěními dalších autorů (Janeczko *et al* 2005, Cevahir *et al* 2008). Nejlepší ochranný účinek na množství chlorofylu *a* i *b* ve stresovaných rostlinách měly nejnižší koncentrace BR, tj. 10^{-14} M. Nejnižší koncentrace BR ve stresu také nejlépe ochranně působily na délku listů, zatímco nejvyšší použité koncentrace působily inhibičně. Účinné koncentrace BR bývají obvykle velmi nízké, v optimální koncentraci pro ošetření mohou být samozřejmě mezidruhové rozdíly a záleží také na typu aplikovaného BRs a způsobu jeho aplikace (Cevahir *et al* 2008, Farridudin *et al* 2009b).

6.3. Mají ekdysteroidy biologickou funkci v rostlině – regulaci fotosyntetických procesů?

O ekdysteroidech obsažených v rostlinách se předpokládá, že plní ochrannou funkci před napadením rostlin fytofágy a spekuluje se, že se možná podílí i na regulaci elongace a vývoje rostlin (Bakrim *et al* 2007, Dinan 2001, 2009, Festucci-Buselli *et al*. 2008, Golovatskaya, 2004). Naše experimenty s novozélandským špenátem *Tetragonia tetragonioides* L. však prokázaly, že během prvních 24 hodin po postřiku rostlin 20E vzrostly hodnoty P_N. To ukazuje, že další funkcí těchto látek v rostlině by mohla také být regulace fotosyntetických procesů. Tato jejich vlastnost dosud nebyla známa. V literatuře byly pouze ve dvou případech popsány pozitivní změny obsahů fotosyntetických pigmentů v důsledku aplikace ekdysteroidů (Bajguz *et* Koronka 2001, Golovatskaya, 2004), avšak toto zjištění se v našem případě nepotvrdilo, což znamená, že pozitivní vliv na stav fotosyntetických pigmentů není v našem případě důvod růstu fotosyntetické účinnosti. Pomocí O-J-I-P analýzy (Strasser 1997) jsme však zjistili, že by tyto látky možná mohly podporovat aktivitu jedné součásti PS2 – OEC.

Při dalších experimentech zabývajících se tímto tématem jsme jako rostlinný materiál zvolili kukuřici (*Zea mays* L.) a špenát (*Spinacia oleracea* L.), neboť se jedná o druhy, které se liší přirozeným obsahem ekdysteroidů. Zároveň jsme studovali i vliv BR, které jsou fytoekdysteroidům strukturně velmi podobné, na různé části primárních fotosyntetických procesů, opět pomocí O-J-I-P analýzy fluorescence chlorofylu. Tím jsme získali jednak výsledky, které dávají detailnější informace o vlivu BR na fotosyntetický elektrontransportní řetězec, jednak prioritní výsledek ukazující, že exogenně aplikované ekdysteroidy (20E) mohou také ovlivňovat fotosyntetické procesy, a to v podobně nízkých koncentracích, jako je to známo o BR.

Velmi zajímavým výsledkem naší práce bylo zjištění, že primární fotosyntetické procesy obvykle společně s obsahy fotosyntetických pigmentů reagovaly na aplikaci BR a/nebo ekdysteroidů u studovaných rostlinných druhů zcela odlišně. Vliv postřiku na špenát byl převážně negativní, u kukuřice víceméně pozitivní, nicméně poměrně slabý. Autoři, kteří před námi zkoumali vliv postřiku BR na funkci PS2 u pšenice (Ali et al 2008c, Shahbas *et al* 2008), ječmene (Janečko *et al* 2011), rajčat (Ahammed *et al* 2013, Ogweno *et al* 2008) nebo paprice (Houimli *et al* 2008, Ibn Maaouia-Houimli *et al* 2012)

nenašli žádný signifikantní vliv BR na funkci PS2. Pokud však byla pokusným materiálem okurka (Yu *et al*, 2004, Xia *et al* 2009, Jiang *et al* 2012a,b) byl zjištěn průkazný pozitivní vliv na různé parametry primárních fotosyntetických reakcí. Podobné výsledky byly získány také při práci s melounem (Zhang *et al* 2013), řepkou (Hayat *et al* 2012) nebo vignou zlatou (Yusuf *et al* 2011). Vliv BR na PS2 může být tedy pozitivní, žádný nebo negativní v závislosti na druhu rostliny – co platí pro jeden druh, nemusí platit pro jiný.

Naše výsledky dále ukazují, že BRs mohou mít odlišný vliv na jednotlivé části fotosyntetického elektron-transportního řetězce a nejedná se tedy pouze o ovlivnění efektivity PS2. V této souvislost k zajímavým výsledkům dospěla Krumová se spolupracovníky (2014) která studovala tylakoidní membrány u BR-mutant *Arabidopsis thaliana* a dospěla k závěru, že BR ovlivňují fotosyntézu prostřednictvím ovlivnění stability OEC a tím pak mají vliv i na další fotosyntetické komplexy.

Vliv 20E a 24E v našich experimentech byl často obdobný při samostatné aplikaci, pokud jsme je aplikovali společně, nezaznamenali jsme žádný rozdíl od kontrolních rostlin ošetřených pouze vodou. Antagonistický vliv 24E a 20E na segmenty koleoptile u pšenice popsala Golovatskaya (2004), která se domnívá, že tyto látky v rostlině mohou soutěžit o společný receptor. Antagonistická funkce obou typů látek byla popsána rovněž u hmyzu, kde autoři předpokládají rovněž kompetici o stejný receptor (Lehmann *et al.*, 1988). Oba typy látek však nemusí nutně soutěžit jen o ligandové místo BR-receptoru, neboť se ukazuje, že oba typy steroidů se mohou specificky vázat k dalším proteinům, z nichž některé se účastní i fotosyntézy, pravděpodobně ve funkci alosterických aktivátorů (Uhlík *et al.*, 2008, Viktorová *et al*., 2012). Konečné objasnění této problematiky však vyžaduje velké množství dalších experimentů.

7. Závěry

- Exogenní aplikace brassinosteroidů má statisticky průkazný vliv nejen na celkový růst, morfologii a výnos rostlin kukuřice, pěstovaných v polních podmínkách, ale i na tvorbu či rychlost vývoje samčích a samičích květenství. Tento vliv závisí na typu použitého brassinosteroidu a jeho koncentraci, na genotypu ošetřených rostlin i na stáří rostlin v době postřiku.
- Statisticky průkazný vliv brassinosteroidů na efektivitu fotosyntetického elektron-transportního řetězce ani na obsah chlorofylů ani karotenoidů nebyl u rostlin kukuřice pěstovaných v polních podmínkách zjištěn.
- U velmi mladých rostlin kukuřice pěstovaných v optimálních podmínkách ve skleníku exogenní aplikace brassinosteroidu pozitivně ovlivnila některé morfologické parametry a obsah chlorofylů a karotenoidů. U rostlin vystavených chladu byl statisticky průkazný pouze vliv na obsah chlorofylu, a to při postřiku rostlin roztokem o jiné koncentrací brassinosteroidu než v kontrolních podmínkách.
- Exogenní aplikace ekdysteroidu na listy novozélandského špenátu krátkodobě pozitivně stimulovala rychlost čisté fotosyntézy, neměla však pozitivní vliv na fotosyntetický elektronový transport ani na obsah fotosyntetických pigmentů. Tento výsledek jako první naznačil, že ekdysteroidy mohou mít *in vivo* nějakou biologickou funkci v regulaci fotosyntézy.
- Ekdysteroidy mohou ovlivňovat různé části primárních fotosyntetických procesů při aplikaci v obdobně nízkých koncentracích jako brassinosteroidy. Mezi těmito steroidními látkami pravděpodobně může docházet ke kompetici na nějaké úrovni genové expese, buněčné signalizace či metabolizmu.
- Brassinosteroidy i ekdysteroidy nemají vliv pouze na fotosystém 2, ale i na další části fotosyntetického elektron-transportního řetězce. Ty však nemusí reagovat stejným způsobem.
- Vliv brassinosteroidů a ekdysteroidů na fotosyntetický aparát rostlin tedy výrazně závisí na rostlinném druhu, na vývojovém stádiu rostliny i listů a na podmínkách vnějšího prostředí, kterým je rostlina vystavena.

8. Seznam použité literatury

Abe H., Morishita T., Uchiyama M., Marumo S., Munakata K., Takatsuto S, Ikekawa S. (1982) Identification of brassinolide-like substances in Chinese cabbage. *Agricultur Biol Chemistry* **46**: 2609-2611

Ahammed G.J., Zhou Y.H., Xia X.J., Mao W.H., Shi K., Yuş J.Q. (2013) Brassinosteroid regulates secondary metabolism in tomato towards enhanced tolerance to phenanthrene. *Biol Plant 57*:154-158

Amzallag G.N. (2002) Brassinosteroids as metahormones: evidence for their specific influence during the critical period in Sorghum development. *Plant Biol* **4**:656-663.

Ali B., Hasan S.A., Hayat S., Hayat Q., Yadav S., Fariduddin Q., Ahmad A. (2008a) A role for brassinosteroids in the amelioration of aluminium stress through antioxidant system in mung bean (*Vigna radiata* L. Wilczek). *Environ Exp Bot* **62**: 153-159

Ali B., Hayat S., Fariduddin Q., Ahmad A. (2008b) 24-epibrassinolide protects against the stress generated by salinity and nickel in *Brassica juncea*. *Chemosphere* **72**:1387-1392

Ali Q., Athar H.R., Ashraf M. (2008c) Modulation of growth, photosynthetic capacity and water relations in salt stressed wheat plants by exogenously applied 24-epibrassinolide. *Plant Growth Regul* **56**:107-116

Arora N., Bhardwaj R., Sharma P., Arora H.K. (2008) Effect of 28-homobrassinolide on growth, lipid peroxidation and antioxidative enzyme activities in seedlings of *Zea mays* L. under salinity stress. *Acta Physiol Plant* **30**:833-839

Bajguz A., Hayat S. (2009) Effects of brassinosteroids on the plant responses to environmental stresses. *Plant Physiol Biochem* **47**:1-8

Bajguz A., Koronka A. (2001) Effect of ecdysone application on the growth and biochemical changes in Chlorella vulgaris cells. *Plant Physiol Biochem* **39**:707-715

Bajguz A., Tretyn A. (2003) The chemical characterisctics and distribution of brassinosteroids in plants. *Phytochemistry* **62**:1027-1046

Bakrim A., Lamhamdi M., Sayah F., Chibi F. (2007) Effect of plant hormones and 20-hydroxyecdysone on tomato (*Lycopersicon esculentum*) seed germination and seedlings growth. *Afr J Biotechnol* **6**:2792-2802

Bakrim A., Maria A., Sayah F., Lafont R., Takvorian N. (2008) Ecdysteroids in spinach (Spinacia oleracea L.): biosynthesis, transport and regulation of levels. *Plant Physiol Biochem* **46**:844-854

Cao S., Xu Q., Cao Y., Qian K., An K., Zhu Y., Binzeng H., Zhao H., Kuai B. (2005) Loss-of-function mutations in *DET2* gene lead to an enhanced resistance to oxidative stress in Arabidopsis. *Physiol Plant* **123**: 57-66

Cerrana R, Lado P, Anastasia M, Ciuffreda P, Allevi P (1984) Regulating effects of brassinosteroids and of sterols on growth and H^+ secretion in maize roots. *J Plant Physiol* 114:221-225

Cevahir G., Yentűr S., Eryilmaz F., Yilmazer N. (2008) Influence of brassinosteroids on pigment content of *Glycine max* L. (soybean) grown in dark and light. *J Appl Biol Sci* **2**:23-28

Clouse S. D., Sasse J. M. (1998) Brassinosteroids: essential regulators of plant growth and development, *Annu Rev Plant Physiol Plant Mol Biol* **49**:427-451

Cui JX, Zhou YH, Ding JG, Xia XJ, Shi K, Chen SC, Asami T., Chen Z., Yu J.-Q. (2011) Role of nitric oxide in hydrogen peroxide-dependent induction of abiotic stress tolerance by brassinosteroids in cucumber. *Plant Cell Environ* **34**:347-58

Dinan L. (1995) Distribution and levels of phytoecdysteroids within individual plants of species of the *Chenopodiaceae*. *Eur J Entomol* **92:**295-300

Dinan L. (2001) Phytoecdysteroids: biological aspects. *Phytochemistry* **57**:325-339

Dinan L., Savchenko T., Whiting P. (2001) On the distribution of phytoecdysteroids in plants. *Cell Mol Life Sci* **58**:1121-1132

Dinan L. (2009) The Karlson lecture. Phytoecdysteroids: what use are they? *Arch Insect Biochem Physiol* **72**:126-141

Dinan L., Harmatha J., Volodin V., Lafont R. (2009) Phytoecdysteroids: diversity, biosynthesis and distribution. In: Smagghe G. (ed.) Ecdysone: structures and functions. Springer, Dordrecht, pp. 3-45

Divi U.K., Krishna P. (2009) Brassinosteroid: a biotechnological target for enhancing crop yield and stress tolerance. *New Biotechnol* **26**:131-136

Divi U.K., Rahman T., Krishna P. (2010) Brassinosteroid-mediated stress tolerance in *Arabidopsis* shows interactions with abscisic acid, ethylene and salycilic acid pathways. *BCM Plant Biology* **10** (151):1-14

Dreier S.I., Towers G.H.N. (1988) Activity of ecdysone in selected plant growth bioassays. *J Plant Physiol* **132**:502-512

Fariduddin Q., Ahmad A., Hayat S. (2003) Photosynthetic response of *Vigna radiata* to pre-sowing seed treatment with 28-homobrassinolide. *Photosynthetica* **41**:307-310

Fariduddin Q., Ahmad A., Hayat S. (2004) Responses of *Vigna radiata* to foliar application of 28-homobrassinolide and kinetin. *Biol Plant* **48**:465-468

Fariduddin Q., Hasan S.A.S., Ali B., Hayat S., Ahmad A. (2008) Effect of modes of application of 28-homobrassinolide on mung bean. *Turk J Biol* **32**:17-21

Fariduddin Q., Khanam S., Hasan S.A., Ali B., Hayat S., Ahmad A. (2009a) Effect of 28-homobrassinolide on the drought stress-induced changes in photosynthesis and antioxidant system of Brassica juncea L. *Acta Physiol. Plant.* **31**:889-897

Fariduddin, Q., Yusuf, M., Hayat, S., Ahmad, A. (2009b) Effect of 28-homobrassinolide on antioxidant capacity and photosynthesis in Brassica juncea plants exposed to different levels of copper. *Environ. Exp. Bot*. **66:**418-424

Fariduddin Q., Khalil R. R. A. E., Mir B. A., Yusuf M., Ahmad A. (2013) 24-Epibrassinolide regulates photosynthesis, antioxidant enzyme activities and proline content of *Cucumis sativus* under salt and/or copper stress. *Environ Monit Assess* **185**:7845-7856

Fariduddin Q. Yusuf M., I. Ahmad I., Ahmad A. (2014) Brassinosteroids and their role in response of plants to abiotic stresses. *Biol Plant* **58**: 9-17

Farooq M., Wahid A., Basra S.M.A., Din I. (2009) Improving water relations and gas exchange with brassinosteroids in rice under drought stress. *J Agron Crop Sci* **195**:262-269

Fedina E.O., Karimova F.G., Tarchevsky I.A., Toropygin I.Y., Khripach V.A. (2008) Effect of epibrassinolide on tyrosine phosphorylation of the Calvin cycle enzymes. *Russ J Plant Physiol 55*:193-20

Festucci-Buselli R.A., Contim L.A.S., Barbosa L.C.A., Stuart J., Otoni W.C. (2008) Biosynthesis and potential functions of the ecdysteroid 20-hydroxyecdysone - a review. *Botany* **86**:978-987

Fujioka S. (1999): Natural occurrence of brassinosteroids in the plant kingdom, In Brassinosteroids: Steroidal Plant Hormones, pp. 21-45. Eds Sakurai, A., Yokota T. and Clouse, S. D. Springer-Verlag, Tokyo.

Fung S., Siddall J.B. (1980) Stereoselective synthesis of brassinolide: a plant growth promoting steroidal lactone. *J American Chem Soc* **102**: 6580-6581

Golovatskaya I.F. (2004) Effect of ecdysterone on morphological and physiological processes in plants. *Russ J Plant Physiol* **51**:452-458

Griffiths P.G., Sasse J.M., Yokota T., Cameron D.W. (1995) 6-deoxotyphasterol and 3-dehydro-6-deoxoteasterone, possible precursors to brassinosteroids in pollen of *Cupressus arizonica*. *Biosci Biotechnol Biochem* **59**:956-959

Gruszka D. (2013) The Brassinosteroid Signaling Pathway - New Key Players and Interconnections with Other Signaling Networks Crucial for Plant Development and Stress Tolerance *Int J Mol Sci* **14:**8740-8774

Grove M.D., Spencer G.F., Rohwedder W.K., Mandava N., Worley J.F., Warthen J.D. Jr., Steffens G.L., Flippen-Anderson J.L., Cook J.C. Jr. (1979) Brassinolide, a plant growth promoting steroid isolated from *Brassica napus* pollen. *Nature* **281**:216-217

Hao J., Yin Y., Fei S.-Z. (2013) Brassinosteroid signaling network: implications on yield and stress tolerance. *Plant Cell Rep* **32**: 1017-1030

Haubrick L.L., Assmann S.M. (2006) Brassinosteroids and plant function: some clues, more puzzles. *Plant Cell Environ* **29**:446–457

Hayat S., Ahmad A. (2003) Soaking seeds of Lens culinaris with 28-homobrassinolide increased nitrate reductase activity and grain yield in India. *Ann Appl Biol* **143**:121–124.

Hayat S., Ahmad A., Mobin M., Hussain A., Fariduddin Q. (2000) Photosynthetic rate, growth and yield of mustard plants sprayed with 28-homobrassinolide. *Photosynthetica* **38**:469-471

Hayat S. Ahmad A. Hussain A. Mobin M. (2001a) Growth of wheat seedlings raised from the grains treated with 28-homobrassinolide. *Acta Physiol Plant* **23**:27-30

Hayat S., Ahmad A., Mobin M., Fariduddin Q., Azam Z.M. (2001b) Carbonic anhydrase, photosynthesis, and seed yield in mustard plants treated with phytohormones. *Photosynthetica* **39**:111-114

Hayat S., Ali B., Hasan S.A., Ahmad A. (2007) Brassinosteroid enhanced the level of antioxidants under cadmium stress in *Brassica juncea*. *Environ Exp Bot* **60**:33-41

Hayat S., Hasan S.A., Hayar Q., Ahmad A. (2010) Brassinosteroids protect *Lycopersicon esculentum* from cadmium toxicity applied as shotgun approach. *Protoplasma* **239**: 3-14

Hayat S., Maheshwari P., Wani A.S., Irfan M., Aleymeni M.N., Ahmad A. (2012) Comparative effect of 24 homobrassinolide and salicylic acid in the amelioration of NaCl stress in *Brassica juncea* L. *Plant Physiol Biochem* **53**:61-8

Hnilička F., Hniličková H., Martinková J., Bláha L. (2007) The influence of drought and the application of 24-epibrassinolide on the formation of dry matter and yield in wheat. *Cereal Res Commun* **35**:457-460

Holá D. (2011) Brassinosteroids and photosynthesis. In: S. Hayat, A. Ahmad (Eds.), Brassinosteroids: a class of plant hormone, Springer Science+Business Media B.V., Dordrecht-Heidelberg-London-New York, 143-192

Houimli S.M., Denden M., El Hadj S.B. (2008) Induction of salt tolerance in pepper (*Capsicum annuum*) by 24-epibrassinolide. *Eur Asia J Bio Sci* **2**:83-90

Cheng D.M., Yousef G.G., Lila M.A. (2010) Variation in phytoecdysteroid accumulation in seeds and shoots of *Spinacia oleracea* L. accessions. *Hortic Sci* **45**:1634-1638

Ishiguro M., Takatsuto S., Morisaki M., Ikekawa N. (1980) Synthesis of brassinolide, a steroidal lactone with plant growth promoting activity. *J Chem Soc Chem Commun*: 962-964

Ibn Maaouia-Houimli S., Dridi-Mouhandes B., ben Mansour-Gueddes S., Denden M. (2012) 24-Epibrassinolide ameliorates the adverse effect of salt stress (NaCl) on pepper (*Capsicum annuum* L.). *J Stress Physiol Biochem* 8:2332-40

Janeczko A., Filek W., Biesaga-Kościelniak J., Marcińska I., Janeczko Z. (2003) The influence of animal sex hormones on the induction of flowering in Arabidopsis thaliana: comparison with the effect of 24-epibrassinolide. *Plant Cell Tiss Organ Cult* **72**:147-151.

Janeczko A., Kościelniak J., Pilipowicz M., Szarek-Łukaszewska G., Skoczowski A. (2005) Protection of winter rape photosystem 2 by 24-epibrassinolide under cadmium stress. *Photosynthetica* **43**:293-298

Janeczko A., Oklešťková J., Pociecha E., Kościelniak J, Mirek M. (2011) Physiological effects and transport of 24-epibrassinolide in heat-stressed barley. *Acta Physiol Plant* **33**:1249-59

Jiang Y.P., Cheng F., Zhou Y.H., Xia X.J., Mao W.H., Shi K., Chen Z., Yu J.Q. (2012a) Cellular glutathione redox homeostasis plays an important role in the brassinosteroid induced increase in CO2 assimilation in *Cucumis sativus*. *New Phytol* **194**:932-943

Jiang Y.P., Cheng F., Zhou Y.H., Xia X.J., Shi K., Yu J.Q. (2012b) Interactive effects of CO2 enrichment and brassinosteroid on CO2 assimilation and photosynthetic electron transport in *Cucumis sativus. Environ Exp Bot* **75**:98-106

Kamlar M., Uhlík O., Kohout L., Šanda M., Macek T. (2010a) Affinity chromatography as the method for brassinosteroid-binding protein isolation. *J Biotechnol* **150**:S490

Kamlar M., Uhlík O., Chlubnová I., Kohout L., Harmatha J., Ježek R., Šanda M., Pišvejcová A., Macek T. (2010b) Affinity chromatography as a method of studying the mechanism of action of plant oxysterols. *Chem Listy* **104**:215-222

Kang Y., Guo S., Li J., Duan J. (2007) Effects of 24-epibrassinolide on antioxidant system in cucumber seedling roots under hypoxia stress. *Agric Sci China* **6**:281-289

Kartal G., Temel A., Arican E., Gozukirmizi N. (2009) Effects of brassinosteroids on barley root growth, antioxidant system and cell division. *Plant Growth Regul* **58**:261-267

Kęsy J., Trzaskalska A., Galoch E., Kopcewicz J. (2003) Inhibitory effect of brassinosteroids on the flowering of the short-day plant *Pharbitis Nil. Biol Plant* **47**:597-600.

Khripach V.A.; Zhabinskii V.N., de Groot A. (1999): Brassinosteroids A new Class of Plant Hormones, San Diego, CA: Academic Press, 263

Khripach V., Zhabinskii V., de Groot A. (2000) Twenty years of brassinosteroids: steroidal plant hormones warrant better crops for the XXI century. *Ann Bot* **86**:441-447

Khripach V.A., Zhabinskii V.N., Khripach N.B. (2003) New practical aspects of brassinosteroids and results of their ten-year agricultural use in Russia and Belarus. In: Hayat S, Ahmad A (eds) Brassinosteroids: bioactivity and crop productivity. Kluwer, Dordrecht-Boston-London, pp 189–230

Krishna P. (2003) Brassinosteroid-mediated stress responses. *J Plant Growth Regul* **22**:289-297

Kohout L. (1994): New method of preparation of brassinosteroids. *Collect Czech Chem Commun* **59:**457-460

Kohout L., Strnad M., Kamínek M. (1991) Types of brassinosteroids and their bioassays. In: Cutler, H.G., Yokota, T., Adam, G. (eds.): Brassinosteroids: Chemistry, bioactivity and applications (ACS Symposium series 474). American Chemical Society, Washington DC, 56-73

Krumova S., Zhiponova M., Dankov K., Velikova V., Balashev K., Andreeva T., Russinova E., Taneva S. (2014) Brassinosteroids regulate the thylakoid membrane

architecture and the photosystem II function. *J Photochem Photobiol B: Biology* 126:97-104

Kwon M., Choe S. (2005) Brassinosteroid biosynthesis and *dwarf* mutants. *J Plant Biol* **48**:1-15

Lafont R. (1998) Phytoecdysteroids in world flora: Diversity, distribution, biosynthesis and evolution. *Russ J Plant Physiol.* **45**:276-295

Lafont R., Harmatha J., Marion-Poll F., Dinan L., Wilson I.D.: (2013) The ecdysone handbook, 3rd edn, on-line, http://ecdybase.org. Accessed 25 March 2013

Lehmann M., Vorbrodt H.M., Adam G., Koolman J. (1988) Antiecdysteroid activity ofbrassinosteroids. *Experientia* **44**:355-356

Li L., van Staden J. (1998) Effects of plant growth regulators on the antioxydant system in callus of two maize cultivars subjected to water stress. *Plant Growth Regul* **24**:55-66

Li L., van Staden J., Jäger A.K. (1998) Effects of plant growth regulators on the antioxydant system in seedlings of two maize cultivars subjected to water stress. *Plant Growth Regul* **25**:81-87

Li K.R., Wang H.H., Wang Q.J., Fan J. (2008) Effects of brassonolide on survival, growth and drought resistance of *Robinia pseudoacacia* seedlings under water-stress. *New Forests* **35**:255-266

Li P, Chen L, Zhou Y, Xia X, Shi K, Chen Z, Ju J. (2013) Brassinosteroids-induced systemic stress tolerance was associated with increased transcripts of several defence-related genes in the phloem in *Cucumis sativus*. *PLoS ONE* **8**:e66582

Ma Y.H., Guo S.R. (2014) 24-epibrassinolide improves cucumber photosynthesis under hypoxia by increasing CO2 assimilation and photosystem II efficiency. *Photosyntetica* **52** (1): 96-104

Macháčková I., Vágner M., Sláma K. (1995) Comparison between the effects of 20-hydroxyecdysone and phytohormones on growth and development in plants. *Eur J Entomol* **92**:309-316

Mahesh K., Balaraju P, Ramakrishna B, Rao S.S.R. (2013) Effect of Brassinosteroids on germination and seedling growth of radish (*Raphanus sativus* L.) under PEG-6000 induced water stress. *Amer J Plant Sci,* **4**:2305-2313

Navrot N., Rouhier N., Gelhaye E., Jacquot J. (2007) Reactive oxygen species generation and antioxidant systems in plant mitochondria. *Physiol Plant* **129**: 185-195

Núñez M.V., Robaina C.R., Coll F.M. (2003) Synthesis and practical applications of brassinosteroid analogs. In: Hayat S, Ahmad A (eds) Brassinosteroids: bioactivity and crop productivity. Kluwer, Dordrecht-Boston-London, pp 87–118

Ogweno J.O., Song X.S., Shi K., Hu W.H., Mao W.H., Zhou Y.H., Yu J.Q., Nogués S. (2008) Brassinosteroids alleviate heat-induced inhibition of photosynthesis by increasing carboxylation efficiency and enhancing antioxidant systems in *Lycopersicon esculentum*. *J. Plant Growth Regul* **27**:49-57

Őzdemir F., Bor M., Demiral T., Tűrkan I. (2004) Effects of 24-epibrassinolide on seed germination, seedling growth, lipid peroxidation proline content and antioxidative system of rice (*Oryza sativa* L.) under salinity stress. *Plant Growth Regul* **42**:203–211

Piñol R., Simón E. (2009) Effect of 24-epibrassinolide on chlorophyll fluorescence and photosynthetic CO2 assimilation in *Vicia faba* plants treated with the photosynthesis-inhibiting herbicide terbutryn. *J Plant Growth Regul* **28**:97-105

Ramraj V.M., Vyas B.N., Godrej N.B., Mistry K.B., Swami B.N., Singh N. (1997) Effects of 28-homobrassinolide on yields of wheat, rice, groundnut, mustard, potato and cotton. *J Agric Sci* **128**:405-413

Rao A.A.R., Vardhini B.V., Sujatha E., Anuradha S. (2002) Brassinosteroids - a new class of phytohormones. *Curr Sci* **82**:1239-1245

Richter K., Koolman J. (1991) Antisteroid effects of brassinosteroids in insects. In: Cutler HG, Yokota T, Adam G, editors. Brassinosteroids: chemistry, bioactivity and applications. Washington DC: American Chemical Society p. 265-78.

Ritchie S.W., Hanway J.J., Benson G.O. (1993) How a corn plant develops. Spec. Rep. No 48. Iowa State University Extension, Ames

Ruley A.T., Sharma N.C., Sahi, S.V. (2004) Antioxidant defense in a lead accumulating plant, *Sesbania drummondii*. *Plant Physiol. Biochem.* **42**:899-906

Sairam R.K. (1994a) Effect of homobrassinolide application on metabolic activity and grain yield of wheat under normal and water-stress condition. *J Agron Crop Sci* **173**:11-16

Sairam R.K. (1994b) Effect of homobrassinolide application on plant metabolism and grain yield under irrigated and moisture stress conditions of two wheat varieties. *Plant Growth Regul* **14**:173-181

Sakurai A., Yokota T., Clouse S.D. (1999) Brassinosteroids: steroidal plant hormones. Springer-Verlag, Tokyo, Japan, 151-153

Sasaki H. (2002) Brassinolide promotes adventitious shoot regeneration from cauliflower hypocotyl segments. *Plant Cell Tissue Organ Culture* **71**: 111-116

Sasse J.M. (2003) Physiological actions of brassinosteroids: an update. *J Plant Growth Regul* **22**:276-288

Shahbaz M., Ashraf M., Athar H.R. (2008) Does exogenous application of 24-epibrassinolide ameliorate salt induced growth inhibition in wheat (*Triticum aestivum* L.)? *Plant Growth Regul* **55**:51-64

Sharma P., Bhardwaj R. (2007) Effect of 24-epibrassinolide on seed germination, seedling growth and heavy metal uptake in *Brassica juncea* L. *Gen Appl Plant Physiol* **33**:59-73

Sharma P., Dubey R.S. (2005) Modulation of nitrate reductase activity in rice seedlings under aluminium toxicity and water stress: role of osmolytes as enzyme protectant. *J Plant Physiol* **162**:854-864

Singh I., Shono M. (2005) Physiological and molecular effects of 24-epibrassinolide, a brassinosteroid on thermotolerance of tomato. *Plant Growth Regul* 47:111-119

Strasser B.J. (1997) Donor side capacity of photosystem II probed by chlorophyll a fluorescence analysis. *Photosynth Res* **52**:147-155

Swamy K.N., Rao S.S.R. (2008) Influence of 28-homobrassinolide on growth, photosynthesis metabolite and essential oil content on geranium (*Pelargonium graveolens* (L.) Herit. *Amer J Plant Physiol* **3**:173–179

Šimonovičová M., Tamás, L., Huttová, J., Mistrík. I. (2004) Effect of aluminum on oxidative stress related enzymes activities in barley roots. *Biol Plant* **48**:261-266

Takematsu T., Takeuchi Y. (1989) Effect of brassinosteroids on growth and yields of crops. *Proc Japan Acad* **65**:149-152

Thomson M.J., Mandava N.B., Flippen-Anderson J.L., Worley J.F., Dutky S.R., Robbins W.E., Lusby W.R. (1979) Synthesis of brassinosteroids: new plant growth-promoting regulators. *J Organ Chem* **44**:5002-5004

Torres-Ruiz B.L., Espinosa-Calderón A., Mendoza-Rodríguez M., Rodríguez de la O.J.L., Irizar-Garza M.B., Castellanos-Ruiz J.S. (2007) Effecto de brasinoesteroides en híbridos de maíz androestériles y fértiles. *Agronomía Mesoamericana* **18**:155-162

Uhlík O., Kamlar M., Kohout L., Ježek R., Harmatha J., Macek T. (2008) Affinity chromatography reveals RuBisCO as an ecdysteroid-binding protein. *Steroids* **73**:1433-1440

Vardhini B.V., Rao S.S.R. (1998) Effect of brassinosteroids on growth, metabolite content and yield of Arachis hypogaea. *Phytochemistry* **48**:927-930

Vardhini B.V., Rao S.S.R. (2003) Amelioration of osmotic stress by brassinosteroids on seed germination and seedling growth of three varieties of sorghum. *Plant Growth Regul* **41**:25-31

Viktorová J., Krásný L., Kamlar M., Nováková M., Macková M., Macek T. (2012) Osmotin, a pathogenesis-related protein. *Curr Prot Pept Sci* **13**:672-81.

Vlašánková E., Kohout L., Klemš M., Eder J., Reinöhl V., Hradilík J. (2009) Evaluation of biological activity of new synthetic brassinolide analogs. *Acta Physiol Plant* **31**:987-993

Vriet C., Russinova E., Reuzeau C. (2012) Boosting crop yields with plant steroids. *Plant Cell* **24**, 842–857

Voigt B., Whiting P., Dinan L. (2001) The ecdysteroid agonist/antagonist and brassinosteroid-like activities of synthetic brassinosteroid/ecdysteroid hybrid molecules. *Cell Mol Life Sci* **58**:1133-1140.

Xia X.J., Huang Y.Y., Wang L., Huang L.F., Yu Y.L., Zhou Y.H., Yu J.Q. (2006) Pesticides-induced depression of photosynthesis was alleviated by 24-epibrassinolide pretreatment in *Cucumis sativus* L. *Pest Biochem Physiol* **86**:42-48

Xia X.J., Huang L.F., Zhou Y.H., Mao W.H., Shi K., Wu J.X., Asami T., Chen Z., Yu J.-Q. (2009) Brassinosteroids promote photosynthesis and growth by enhancing activation of Rubisco and expression of photosynthetic genes in *Cucumis sativus. Planta* **230**:1185-96.

Yokota T. (1997) The structure, biosynthesis ans function of brassinosteroids. *Trends Plant Sci* **2**:137-143

Yang D.L., Yang Y., He Z., (2013) Roles of plant hormones and their interplay in rice immunity. *Mol Plant* **6**(3):675-685

Yu J.Q., Huang L.F., Hu W.H., Zhou Y.H., Mao W.H., Ye S.F., Nogués S. (2004) A role for brassinosteroids in the regulation of photosynthesis in Cucumis sativus. *J Exp Bot* **399**:1135-1143

Yuan G.-F., Jia Ch.-J., Zhen L., Sun B., Zhang L.-P., Liu N., Wang Q.-M. (2010) Effect of brassinosteroids on drought resistance and abscisic acid concentration in tomato under water stress. *Sci Hortic* **126**:103-108

Yusuf M., Fariduddin Q., Ahmad A. (2011) 28-homobrassinolide mitigates boron induced toxicity through enhanced antioxidant system in *Vigna radiata* plants. *Chemosphere* **85**:1574-84

Zhang S., Scheller H.V. (2004) Photoinhibition of Photosystem I at chilling temperature and subsequent recovery in *Arabidopsis thaliana*. *Plant Cell Physiol* **45**:1595-1602

Zhang M., Zhai Z., Tian X., Duan L., Li Z. (2008) Brassinolide alleviated the adverse effect of water deficits on photosynthesis and the antioxidant of soybean (*Glycine max* L.). *Plant Growth Regul* **56**:257-264

Zhang Y.P., Zhu X.H., Ding H.D., Yang S.J., Chen Y.Y. (2013) Foliar application of 24-epibrassinolide alleviates high-temperature-induced inhibition of photosynthesis in seedlings of two melon cultivars. *Photosynthetica* **51**:341-9

Zhao Y.J., Chen J. (2003) Studies on physiological action and application of 24-epibrassinolide in agriculture. In: Hayat S, Ahmad A (eds) Brassinosteroids: bioactivity and crop productivity. Kluwer, Dordrecht-Boston-London, pp 159–170

Zhao B., Li J. (2012) Regulation of brassinosteroid biosynthesis and inactivation. *J Integr Plant Biol* **54**:746-759

9. Přílohy

9.1. Další publikace související s tématem práce

9.1.1. Články ve sbornících

Fridrichová L., Jelénková I., Holá D., Kočová M., Rothová O., Trubanová N., Panchártek D., Seňková J., Palovská M. (2012) Odezva bobu obecného (*Vicia faba* L.) na nedostatek vody a ošetření 24-epibrassinolidem. *In:* Hnilička, F. (ed.): Vliv abiotických a biotických stresorů na vlastnosti rostlin 2012, Praha, Česká republika, 1.-2.2.2012. Sborník příspěvků, 115-119

Rothová O., Kočová M., Holá D., Trubanová N., Kohout L., Šíša M. (2007) Má ošetření obilek kukuřice 24-epibrassinolidem pozitivní vliv na odolnost rostlin vůči suchu? *In*: Bláha, L. (ed.): Vliv abiotických a biotických stresorů na vlastnosti rostlin 2007, Praha, Česká republika, 21.-22.3.2007. Sborník příspěvků, 318-323

Rothová O., Holá D., Kočová M., Bartáková J., Benešová M., Kohout L. (2006) Vliv brassinosteroidů na vybrané fotosyntetické a morfologické charakteristiky u různých genotypů kukuřice vystavených chladu. *In*: Bláha, L., Hnilička, F. (eds.): Vliv abiotických a biotických stresorů na vlastnosti rostlin 2006, Praha, Česká republika, 17.5.2006. Sborník příspěvků, 67-71

9.1.2. Přednášky, plakátová sdělení a abstrakty z konferencí

Kamlar M., Rothová O., Uhlík O., Holá D., Rezek J., Kočová M., Harmantha J., Kohout L., Spiwok V., Macek T. (2013) Oxysterols increase CO_2 fixation by RuBisCo and the activity of the oxygen evolving complex. Phytotechnologies, Syracuse, New York, USA, 1.-4.10.2013, Book of Abstracts, p. 135

Rothová O., Holá D., Kočová M., Tůmová L., Hnilička F., Hniličková H., Kamlar M., Macek T. (2013) 20-hydroxyecdysone regulates photosynthesis differently in maize and spinach: competition or cooperation with 24-epibrassinolide? 11. dny doktorandů experimentální biologie rostlin a 13. konference experimentální biologie rostlin, Košice, Slovenská republika, 9.-13.9.2013, Sborník abstraktů, p. 126

Tůmová L., Kočová M., Holá D., Rothová O., Jelénková I., Trubanová N., Panchártek D, Palovská M., Řehořová K. (2013) Brassinosteroids as possible regulators of the response of young plants of *Vicia faba* L. and *Zea mays* L. to water deficit. 11. dny doktorandů experimentální biologie rostlin a 13. konference experimentální biologie rostlin, Košice, Slovenská republika, 9.-13.9.2013, Sborník abstraktů, p. 73

Fridrichová L., Trubanová N., Holá D., Kočová M., Rothová O., Jelénková I., Panchártek D. (2012) Brassinosteroids as regulators of changes in response two genotypes of *Zea mays* L. to water deficit. 10th International Conference of Ph.D. Students of Experimental Plant Biology, Brno, Česká republika, 3.-5.9.2012, Book of Abstracts, p. 93

Rothová O., Kuklíková R., Fridrichová L., Holá D., Kočová M. (2012) Application of synthetic brassinosteroid differentially affects various maize and faba been cultivars exposed to drought and re-watering conditions. Plant Biology Congress, Freiburg, Německo, 30.7.-3.8.2012, Book of Abstracts, p. 788

Holá D., Rothová O., Kočová M., Fridrichová L., Macek T. (2012) Phytoecdysteroids together with brassinosteroids stimulate oxygen-evolving activity of photosystem II. Plant Biology Congress, Freiburg, Německo, 30.7.-3.8.2012, Book of Abstracts, p. 771

Fridrichová L., Trubanová N., Kočová M., Holá D., Rothová O., Jelénková I., Panchártek D. (2012) 24-epibrassinolide improves the response of young maize and faba been plants during drought stress: joint analysis of plant biochemistry, physiology, morphology and development. Plant Biology Congress, Freiburg, Německo, 30.7.-3.8.2012, Book of Abstracts, p. 766

Rothová O., Trubanová N., Holá D., Kočová M., Fridrichová L., Kohout L. (2011) Má ošetření 24-epibrassinolidem pozitivní vliv na odolnost různých genotypů kukuřice? Genetická konference GSGM 2011, Lednice, Česká republika, 14.-16.9.2011, Book of Abstracts, p. 62

Fridrichová L., Seňková J., Holá D., Kočová M., Rothová O., Jelénková I., Trubanová N. (2011) Brassinosteroids as regulator of changes in response of *Zea mays* L. to water deficite. 9th International Conference of Ph.D. Students of Experimental Plant Biology, Praha, Česká republika, 5.-6.9.2011, Book of Abstracts, p. 16

Rothová O., Holá D., Kočová M., Fridrichová L. (2010) Exogenně aplikované brassinosteroidy ovlivňují morfologii, vývoj a výnosové parametry kukuřice v závislosti na jejich typu, koncentraci, genotypu rostlin a ontogenetické fázi, při níž byly aplikovány. 12. konference experimentální biologie rostlin, Praha, Česká republika, 14.-17.9.2010. Bulletin České společnosti experimentální biologie rostlin a Fyziologické sekce Slovenské botanické společnosti (sborník abstraktů), p. 168

Rothová O., Holá D., Kočová M., Kohout L., Šíša M. (2008): The effect of brassinosteroids on selected growth, reproductive and yield parameters of three genotypes of maize grown in field conditions. The XVI. Congress of the Federation of European Societies of Plant Biology, Tampere, Finsko, 17.-22.8.2008.Abstract in: Physiol. Plant. 133, P10-041

Rothová O., Kočová M., Bartáková J., Holá D., Benešová M., Šíša M., Kohout L. (2006) Inbred and hybrid genotypes of maize differ in their response to brassinosteroids under conditions of cold stress. The XV. Congress of the Federation of European Societies of Plant Biology, Lyon, Francie, 17.-21.7.2006. Book of Abstracts, p. 166

Holá D., Bartáková J., Kočová M., Rothová O., Benešová M., Kohout L., Šíša M. (2006) Brassinosteroids improve the response of photosynthetic apparatus in maize leaves to low-temperature conditions. The XV. Congress of the Federation of European Societies of Plant Biology, Lyon, Francie, 17.-21.7.2006. Book of Abstracts, p. 153

9.2. Publikace nesouvisející s tématem práce

9.2.1. Články v časopisech s IF

Holá D., Kočová M., Rothová O., Hlízová E., Fridrichová L., Lhotáková Z., Albrechtová J. (2012) A universal method for the isolation of photochemically active broken chloroplasts from conifer needles and its possible application in photosynthetic studies. *Photosynthetica* 50: 291-304

Benešová M., Holá D., Fischer L., Jedelský P.L., Hnilička F., Wilhelmová N., Rothová O., Kočová M., Procházková D., Honnerová J., Fridrichová l., Hniličková H. (2012) The Physiology and Proteomics of Drought Tolerance in Maize: Early Stomatal Closure as a Cause of Lower Tolerance to Short-Term Dehydration? *PLoS ONE* 7(6): e38017

Holá D., Benešová M., Honnerová J., Hnilička F., Rothová O., Kočová M., Hniličková H. (2010) The evaluation of photosynthetic parameters in maize inbred lines subjected to water deficiency: Can these parameters be used for the prediction of performance of hybrid progeny? *Photosynthetica* 48: 445-558

Kočová M., Holá D., Wilhelmová N., Rothová O. (2009) The influence of low-temperature stress on the genetic determination of the photochemical activity of mesophyll chloroplasts and activity of antioxidant enzymes in maize (Zea mays L.) leaves. *Biol. Plant.* 53: 475-483

Holá D., Kutík J., Kočová M., Rothová O. (2008) Low-temperature induced changes in the ultrastructure of maize mesophyll chloroplasts strongly depend on the chilling pattern/intensity and considerably differ among inbred and hybrid genotypes. *Photosynthetica* 46: 329-338

Holá D., Kočová M., Rothová O., Wilhelmová N., Benešová M. (2007) Recovery of maize (Zea mays L.) inbreds and hybrids from chilling stress of various duration: Photosynthesis and antioxidant enzymes. *J. Plant Physiol*. 164: 868-877

9.2.2. Články v recenzovaných časopisech bez IF

Hnilička F., Hniličková H., Holá D., Rothová O., Kočová M. (2010) Short-term effect of drought on the photosynthetic accumulation of energy in maize. *Növénytermelés.* Suppl. 4: 489-492

Hnilička F., Hniličková H., Holá D., Kočová M., Rothová O. (2008) Vliv sucha na rychlost fotosyntézy a transpirace juvenilních rostlin kukuřice. *Úroda* 8: 48-49

Holá D., Suda J., Kočová M., Rothová O., Salava J., Chodová D. (2007) Endopolyploidy pattern and efficiency of photosynthetic electron transport in *Kochia scoparia* (L.) Schrad. biotypes resistant/sensitive to atrazine and/or ALS inhibitors. *Herbologia* 8: 71-83

9.2.3. Články ve sbornících

Holá D., Kočová M., Rothová O., Tůmová L., Lhotáková Z., Albrechtová J. (2013) Primární fotosyntetické procesy u dvou druhů lesních stromů dlouhodobě vystavených zvýšené koncentraci CO2. *In*: Hnilička F. (ed.): Vliv abiotických a biotických stresorů na vlastnosti rostlin 2013, Praha, Česká republika, 13.-14.2.2013. Sborník příspěvků, 8-11

Hnilička F., Hniličková H., Holá D., Rothová O., Kočová M. (2010) Vliv vodního deficitu na obsah sušiny nadzemní biomasy juvenilních rostlin kukuřice. *In*: Fuksa P. (ed.): Aktuální témata v pícninářství a trávníkářství 2010, Praha, Česká republika, 8.12.2010. Sborník příspěvků z odborného semináře, 36-41

Hnilička F., Hniličková H., Holá D., Rothová O., Kočová M. (2010) Změny rychlosti fotosyntézy a transpirace u kukuřice v závislosti na působení nízkou teplotou. *In*: Hnilička, F. Bláha, L., Martinková J. (eds.): Vliv abiotických a biotických stresorů na vlastnosti rostlin 2010, Praha, Česká republika, 10.-11.2.2010. Sborník příspěvků, 127-130

Hnilička, F., Holá, D., Hniličková, H., Rothová, O., Kočová, M. (2009) Vliv postupného vodního deficitu na obsah energie v biomase juvenilních rostlin kukuřice. *In*: Černošková, E. (ed.): 31. Mezinárodní slovenský a český kalorimetrický seminář (sborník příspěvků), Univerzita Pardubice, 51-54

Hnilička, F., Holá, D., Rothová, O., Kočová, M., Hniličková, H. (2009) Vliv vodního deficitu na tvorbu sušiny kukuřice. *In*: Hejnák, V. (ed.): Proceedings of the II. Prague Plant Scientific Workshop, ČZU v Praze, Praha, 22-25

Hnilička F., Hniličková H., Holá D., Kočová M., Rothová O. (2009) Stomatální inhibice výměny plynů kukuřice pod vlivem vodního deficitu. In: Bláha, L. (ed.): Vliv abiotických a biotických stresorů na vlastnosti rostlin 2009, Praha, Česká republika, 4.-5.3.2009. Sborník příspěvků, 199-202

Holá D., Benešová M., Haisel D., Hnilička F., Hniličková H., Honnerová J., Kočová M., Kučera T., Rothová O., Procházková D., Šonská A., Wilhelmová N. (2009) Fyziologické a biochemické parametry inbredních a hybridních genotypů kukuřice vystavených vodnímu deficitu. *In*: Bláha, L. (ed.): Vliv abiotických a biotických stresorů na vlastnosti rostlin 2009, Praha, Česká republika, 4.-5.3.2009. Sborník příspěvků, 130-134

Hnilička F., Hniličková H., Holá D., Kočová M., Rothová O. (2008) Vliv půdního sucha na obsah energie juvenilních rostlin kukuřice. *In*: Černošková, E. (ed.): 30. Mezinárodní český a slovenský kalorimetrický seminář, Rožnov pod Radhoštěm, Česká republika, 26.-30. 5. 2008, Sborník příspěvků, 133-136

Hnilička F., Hniličková H., Holá D., Rothová O., Kočová M. (2008) Vliv vodního deficitu na výměnu plynů juvenilních rostlin kukuřice (Zea mays L.). *In*: Hejnák, V., Skalický M., (ed.): I. Prague Plant Scientific Workshop, Praha, Česká republika, Sborník příspěvků, 38-41

Hnilička F., Hniličková H., Holá D., Kočová M., Rothová O. (2008) Vliv sucha na rychlost fotosyntézy a transpirace kukuřice. *In*: Bláha, L., Hnilička, F. (eds.): Vliv

abiotických a biotických stresorů na vlastnosti rostlin 2008, Praha, Česká republika, 12.-13.2.2008. Sborník příspěvků, 220-224

Kočová M., Holá D., Rothová O., Kholová J., Johnová E., Bartáková J. (2007) Vliv hypoxických a posthypoxických podmínek na fyziologické a morfologické znaky kukuřice. *In*: Bláha, L. (ed.): Vliv abiotických a biotických stresorů na vlastnosti rostlin 2007, Praha, Česká republika, 21.-22.3.2007. Sborník příspěvků, 332-339

Holá D., Kočová M., Rothová O., Kholová J., Benešová M., Červinková H. (2007) Změny fyziologických a morfologických znaků u tří generací kukuřice (*Zea mays* L.) v důsledku vodního deficitu. *In*: Bláha, L. (ed.): Vliv abiotických a biotických stresorů na vlastnosti rostlin 2007, Praha, Česká republika, 21.-22.3.2007. Sborník příspěvků, 324-331

Červinková H., Holá D., Kočová M., Rothová O., Kutík J. (2007) Ultrastruktura chloroplastů kukuřice (Zea mays L.) pod vlivem zaplavení. *In*: Bláha, L. (ed.): Vliv abiotických a biotických stresorů na vlastnosti rostlin 2007, Praha, Česká republika, 21.-22.3.2007. Sborník příspěvků, 312-317

9.2.4. Přednášky, plakátová sdělení a abstrakty z konferencí

Holá D., Lhotáková Z., Kočová M., Rothová O., Radochová B., Mašková P., Tůmová L., Albrechtová J. (2013) Primary photosynthetic processes in leaves of european beech and norway spruce exposed to elevated CO2. 11. dny doktorandů experimentální biologie rostlin a 13. konference experimentální biologie rostlin, Košice, Slovenská republika, 9.-13.9.2013, Sborník abstraktů, p. 171

Kočová M., Holá D., Rothová O., Lhotáková Z., Fridrichová L. (2012) Response of forest tree species to elevated carbon dioxide concentration: I. Differencesin primary photosynthetic reactions in European beech and Norway spruce. Plant Biology Congress, Freiburg, Německo, 30.7.-3.8.2012, Book of Abstracts, p. 360

Albrechtová J., Lhotáková Z., Lipavská H., Mašková P., Kubínová Z., Kočová M., Holá D., Rothová O., Radochová B., Janáček J., Čapek M., Kubínová L., Šprtová M., Urban

O., Kubásek J., Marek M. (2012) Response of forest tree species to elevated carbon dioxide concentration: IV. Interrelation between metabolic and structural characteristics in European beech and Norway spruce. Plant Biology Congress, Freiburg, Německo, 30.7.-3.8.2012, Book of Abstracts, p. 291

Kočová M., Holá D., Rothová O., Lhotáková Z., Radochová B. (2010) Rozdíly ve vybraných fotosyntetických parametrech *Fagus sylvatica (L.)* v závislosti na koncentraci CO_2, ozářenosti a poloze listů. 12. konference experimentální biologie rostlin, Praha, Česká republika, 14.-17.9.2010. Bulletin České společnosti experimentální biologie rostlin a Fyziologické sekce Slovenské botanické společnosti (sborník abstraktů), p. 131

Hnilička F., Holá D., Hniličková H., Rothová O., Kočová M., Martinková J. (2010) Změny obsahu energie biomasy juvenilních rostlin kukuřice (*Zea mays* L.) v závislosti na působení vodního deficitu. 12. konference experimentální biologie rostlin, Praha, Česká republika, 14.-17.9.2010. Bulletin České společnosti experimentální biologie rostlin a Fyziologické sekce Slovenské botanické společnosti (sborník abstraktů), p. 160

Holá D., Hnilička F., Benešová M., Rothová O., Kočová M., Haisel D., Wilhelmová N., Kholová J., Hniličková H., Procházková D., Fridrichová L., Seňková J. (2010) Odlišná reakce inbredních a hybridních genotypů kukuřice na stres suchem: srovnávací analýza fyziologie, morfologie a růstu rostlin. 12. konference experimentální biologie rostlin, Praha, Česká republika, 14.-17.9.2010. Bulletin České společnosti experimentální biologie rostlin a Fyziologické sekce Slovenské botanické společnosti (sborník abstraktů), p. 143

Rothová O., Kočová M., Benešová M ., Holá D., Kučera T., Wilhelmová N., Procházková D., Haisel D., Hnilička F. (2009) Drought-induced modulation of heterosis in maize: do hybrids behave differently from their parents? Eco-physiological aspects of plant responses to stress factors (8th International conference), Krakow, Polsko, 16.-19.9.2009. Abstract in: *Acta Physiol. Plant.* 31 (Suppl. 1), S-71

Holá D., Benešová M., Kočová M., Rothová O., Kučera T., Hnilička F., Haisel D., Wilhelmová N., Procházková D. (2009) Relationship between various morphological, physiological, biochemical and proteome parameters of leaves in drought-stressed maize. Eco-physiological aspects of plant responses to stress factors (8th International conference), Krakow, Polsko, 16.-19.9.2009. Abstract in: *Acta Physiol. Plant.* 31 (Suppl. 1), S-61

Fridrichová L., Kuklíková R., Holá D., Kočová M., Rothová O., Benešová M., Honnerová J. (2009) Inter- and intraspecific variability in *Vicia faba* L. and *Zea mays* L. response to water deficit. Eco-physiological aspects of plant responses to stress factors (8th International conference), Krakow, Polsko, 16.-19.9.2009. Abstract in: *Acta Physiol. Plant.* 31 (Suppl. 1), S-57

Kočová M., Holá D., Rothová O., Kholová J., Červinková H., Fridrichová L. (2008): The inheritance of photosynthetic and growth parameters in drought-stressed plants of two inbred lines of maize and their F1 and F2 hybrids. The XVI. Congress of the Federation of European Societies of Plant Biology, Tampere, Finsko, 17.-22.8.2008. Abstract in: *Physiol. Plant* 133, P09-088

Holá D., Rothová O., Kočová M., Hnilička F., Benešová M., Bartáková J. (2008) The use of photosynthetic parameters as secondary selection traits for the assessment of maize tolerance to drought: parent-progeny analysis. XVI. Congress of the Federation of European Societies of Plant Biology, Tampere, Finsko, 17.-22.8.2008 Abstract in: *Physiol. Plant* 133, P09-064

Kočová M., Holá D., Rothová O., Kholová J., Benešová M., Bartáková J., Červinková H. (2007) Reakce inbredních a hybridních genotypů kukuřice (*Zea mays* L.) na změny vodního režimu. Konference experimentální biologie rostlin (11. dny fyziologie rostlin), Olomouc, Česká republika, 9.-12.7.2007. Bulletin České společnosti experimentální biologie rostlin a Fyziologické sekce Slovenské botanické společnosti (sborník abstraktů), p. 113

Červinková H., Holá D., Kočová M., Rothová O., Kutík J. (2007) Působí stres zaplavení kořenového systému na fotosyntetický aparát kukuřice (*Zea mays* L.)*?* Konference

experimentální biologie rostlin (11. dny fyziologie rostlin), Olomouc, Česká republika, 9.-12.7.2007. Bulletin České společnosti experimentální biologie rostlin a Fyziologické sekce Slovenské botanické společnosti (sborník abstraktů), p. 58

9.2.5. Knihy určené pro výuku

Rothová O., Kolková J. (2012) DNA - molekula života. Výukový a metodický text. Přírodní vědy a matematika na středních školách, aktuálně s aplikacemi - projekt OPPA. Nakladatelství P3K s. r. o., Praha. ISBN 978-80-87186-91-6

Kolková J., Morávková A., Peštová I., Rothová O., Vilímová V. (2012) Biologie. Aktivně, aktuálně a s aplikacemi Přírodní vědy a matematika na středních školách, aktuálně s aplikacemi - projekt OPPA. Nakladatelství P3K s. r. o., Praha. ISBN 978-80-87186-97-8