Chapter 3

Brassinosteroids in Cereals – Presence, Physiological Activity and Practical Aspects

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Abstract Brassinosteroids (BRs) are plant steroid hormones that are characterised by a sterane skeleton of four rings with a number of functional groups attached (mainly hydroxyl). The first species from the *Poaceae* family in which BRs were found was rice (*Oryza sativa* L., cv. Arborio J1) – castasterone (13.6 pg g^{-1} F.W.) and dolichosterone (8.4 pg g^{-1} F.W.). BRs were also found in corn, wheat, rye, barley as well as *Phalaris canariensis* L. or ryegrass. There are significant differences between the different cereals in the types of BRs that are present and in their concentration. In agricultural and biological experiments whose aim was to clarify the role of these compounds in cereals, exogenous 28-homobrassinolide and 24-epibrassinolide and less often, brassinolide or other BRs were most commonly used. Recently, however, the number of articles in which BR-biosynthetic deficient mutants or BR-signalling mutants are being used in studies has increased. BR mutants of cereals include mutants of rice (i.e. *d61*), barley (i.e. *uzu*) and corn (*Brd1*). It is worth emphasising that in the case of cereal plants, studies on mutants have confirmed lot of the physiological functions of BRs that have previously been reported in works in which exogenous BR was applied. One can also mention the participation of BRs in regulating plant growth, CO_2 assimilation, proline and sugar production, their protective effects on the PSII (under stress conditions) or their participation in a complicated network of connections with other plant hormones. In addition to being a good model for studies of the role of BRs in cereals, mutants of cereal crops can be used in agricultural practice, i.e. to create new dwarf cultivars. This chapter will review the knowledge about brassinosteroids in cereals – their presence, physiological activity and practical applications.

Keywords Antioxidants · Brassinosteroid content · Photosynthesis · Plant stress response · Plant growth and development · *Poaceae*

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1 Introduction – Chemistry of Brassinosteroids

Brassinosteroids (BRs) are plant steroid hormones that are isolated from oilseed rape pollen (Grove et al. 1979). BRs have sterane as the main skeleton in the molecule; BRs are also defined as polyhydroxysteroids because they contain many hydroxyl groups per molecule. Low quantities (ng or pg g^{-1} fresh weight [F.W.]) of BRs are present in plants in a free form or in the form of conjugates (glycosides, conjugates with fatty acids). There are three main groups of BRs: C_{27}, C_{28} and C_{29}. They differ in the number of carbons in a molecule. An example of C_{27} is 28-norcastasterone, an example of C_{28} is 24-epibrassinolide or brassinolide, while 28-homobrassinolide represents C_{29} (Fig. 3.1). Synthetic analogues of BRs such as biobrass-6 (BB-6, Mazorra et al. 2004) are also known. Interestingly, some epoxybrassinosteroids (e.g. secasterone) have been discovered in the *Poaceae* family

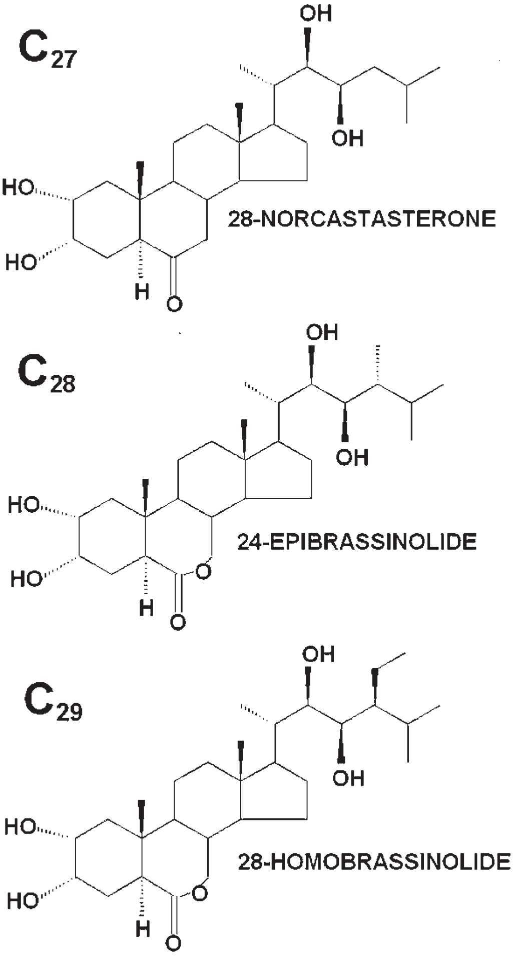

Fig. 3.1 Examples of the brassinosteroids that represent three structural groups: C_{27}, C_{28} and C_{29}, which are present in the *Poaceae* family. (Gamoh et al. 1990; Janeczko and Swaczynová 2010). 24-Epibrassinolide and 28-homobrassinolide are the BRs that are most often used in experiments using an exogenous application to plants from this family

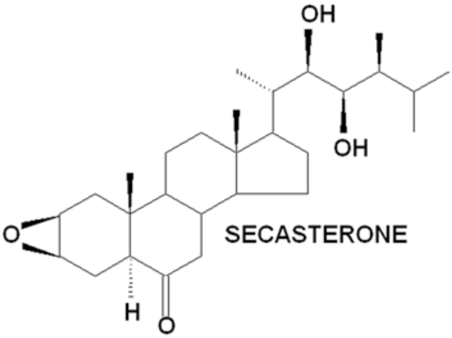

Fig. 3.2 Secasterone, the first naturally occurring 2,3-epoxybrassinosteroid was named after the species (*Secale cereale* L.) in which it was discovered. (Schmidt et al. 1995)

(*Secale cereale* L.) (Fig. 3.2). Sterols are the precursors of BR biosynthesis in plants. The first BR biosynthetic pathway that was discovered was described for brassinolide. The pathway starts with sterol – campesterol and goes through cathasterone, teasterone, typhasterol and castasterone in the early C6 oxidation pathway (Fujioka and Yokota 1997). BR receptors are present in cell membranes and are well described together with the signal transduction pathways (Clouse 2011). However, specific brassinosteroid binding has been reported in studies using the so-called radioligands not only in different cell membrane fractions, but also in the cytosol (Xu et al. 1994). This binding was weakened by trypsin, which indicates the protein nature of the binding structures. Hence, perhaps similar to animals and humans, plant steroid hormones have cytoplasmic or nuclear receptors. The primary function of BRs is the regulation of growth processes – plant mutants with a disturbed BR production show a dwarf phenotype (Morinaka et al. 2006; Makarevitch et al. 2012; Janeczko et al. 2016). BRs regulate the developmental processes (Yang et al. 2011) including fruit development (Symons et al. 2006). BRs also participate in the plant response to environmental stress (Krishna 2003).

Poaceae plants include many species that are very important from an agricultural point of view such as rice, wheat, maize or barley. These cereals, which are consumed in the form of groats, flakes, bread and other baked goods, are an important source of carbohydrates in the human diet. Research on the mechanisms that control the processes of the growth and development of these species as well as their resistance to stress factors is very important from a practical point of view (for farmers or plant breeders). In turn, brassinosteroids, which are hormones that have multidirectional physiological effects, are also of interest to many researchers. This chapter will review the knowledge about brassinosteroids in cereals – their presence, physiological activity and some possibilities for their practical application in agriculture.

2 Occurrence of Brassinosteroids and Their Changes in Plants of the *Poaceae* Family

BRs were discovered by Grove et al., in oil rapeseed pollen in 1979, while rice was the first species in the family *Poaceae* (5 years later) in which the occurrence of BR was confirmed (Abe et al. 1984). To date, the presence of BRs among *Poaceae* has been confirmed in wheat, maize, rye, barley, *Phalaris canariensis* L. and perennial ryegrass (Table 3.1). Some of the studies that have been carried out were qualitative analyses of BRs and several works have provided quantitative analyses. Brassinosteroids have been determined using gas chromatography coupled with mass spectrometry (GC-MS) (Abe et al. 1984, 1995; Suzuki et al. 1986; Yokota et al. 1994; Park et al. 1994; Schmidt et al. 1995; Antonchick et al. 2003; Kim et al. 2005), using liquid chromatography with fluorometric detection (Gamoh et al. 1990), using immunochemical methods (Taylor et al. 1993), using liquid chromatography coupled with mass spectrometry (Antonchick et al. 2005) and using high and ultra performance liquid chromatography coupled with tandem mass spectrometry with electrospray ionisation (HPLC or UHPLC-ESI-MS/MS) (Janeczko and Swaczynová 2010; Janeczko et al. 2010, 2011, 2013, 2015; Dockter et al. 2014; Pociecha et al. 2016; Gruszka et al. 2016a, b). Only small amounts of plant material are needed for an HPLC analysis usually (even less than 1 g of tissue), while for a GC analysis, more material (even kilograms) is required.

The level and profile of BRs in *Poaceae* varies and there are many factors that modify them. Differences have been found between individual families, plant species and cultivars (Table 3.1) as well as between plant organs (Asahina et al. 2014). Mutations are an important factor that causes changes in the BR content. Mutants with BR biosynthesis disorders are usually characterised by a reduced content of these compounds, whereas mutants with BR-perception disorders usually accumulate more of these compounds than the wild type (Dockter et al. 2014, Table 3.1).

The content and profile of BRs in cereals may be influenced by the exogenous application of BRs. In wheat, 24-epibrassinolide, when applied *via* plant spraying in the heading stage or *via* presowing seed soaking, did not accumulate in grains that were collected but did change the profiles of the BRs (Janeczko et al. 2010). 24-Epibrassinolide, when applied to the heading plants, decreased content of its precursor (24-epicastasterone) in the grains, which might be the result of negative feedback in the biosynthesis pathway of these BR. Interestingly, in this experiment it was also found that the solvent for the 24-epibrassinolide – ethanol, which was present in the working solutions, modified the composition of the BRs in the plants, for example, it increased the amount of brassinolide in the collected seeds (Janeczko et al. 2010). Changes in the content and profile of endogenous BRs *via* the exogenous application of BR were also noted in the study of Janeczko and Swaczynová (2010). The impact of exogenous BR on fluctuations in the endogenous BR content in a plant may be explained by their metabolising to other BRs (Joo et al. 2015) or the direct influence of the applied BRs on the biosynthetic pathways (e.g. on the basis of the aforementioned feedback).

Table 3.1 Brassinosteroids in plants from *Poaceae* family

Publication	Species	Organ	BRs
Abe et al. (1984)	**Rice** (*Oryza sativa* L.) cv. Arborio J1	Shoots	castasterone (13.6 pg $g^{-1}{}_{F.W.}$), dolichosterone (8.4 pg $g^{-1}{}_{F.W.}$)
Suzuki et al. (1986)	**Maize** (*Zea mays* L.)	Pollen	castasterone (120 ng $g^{-1}{}_{F.W.}$), typhasterol (6.6 ng $g^{-1}{}_{F.W.}$), teasterone (4.1 ng $g^{-1}{}_{F.W.}$)
Gamoh et al. (1990)	**Maize** (*Zea mays* L.)	Pollen	castasterone (27.2 ng $g^{-1}{}_{F.W.}$), 28-norcastasterone (18.3 ng $g^{-1}{}_{F.W.}$), dolichosterone (16.9 ng $g^{-1}{}_{F.W.}$)
Taylor et al. (1993)	**Perennial ryegrass** (*Lolium perenne* L.)	Pollen	25-methylcastasterone
Yokota et al. (1994)	**Wheat** (*Triticum aestivum* L.) cv. Chihoku	Bran	castasterone, 3-dehydroteasterone, teasterone, typhasterol, 6-deoxocastasterone
		Flour	kastasteron, teasterone, typhasterol, 6-deoxocastasterone
Park et al. (1994)	**Rice** (*Oryza sativa* L.) cv. Tongjinbyeo	Young seeds	castasterone, teasterone, 6-deoxocastasterone
Schmidt et al. 1995	**Rye** (*Secale cereale* L.)	Seeds	secasterone, castasterone, 28-homocastasterone, 28-norcastasterone, 6-deoxocastasterone, typhasterol, teasterone
Abe et al. (1995)	**Rice** (*Oryza sativa* L.) cv. Koshihikari	Bran	28-homotyphasterol, 28-homoteasterone, 6-deoxocastasterone
Shimada et al. (1996)	**Canary grass** (*Phalaris canariensis* L.)	Seeds	castasterone (5 ng g^{-1} seeds), teasterone (0.7 ng g^{-1} seeds)
Antonchick et al. (2003)	**Rye** (*Secale cereale* L.) cv. Sorom	Leaves of 18-d-old seedlings	secasterone (52 pg $g^{-1}{}_{F.W.}$), 2,3-diepisecasterone (20 pg $g^{-1}{}_{F.W.}$)
		Roots of 18-d-old seedlings	secasterone (107 pg $g^{-1}{}_{F.W.}$), 2,3-diepisecasterone (32 pg $g^{-1}{}_{F.W.}$)
Antonchick et al. (2003)	**Rye** (*Secale cereale* L.) cv. Petka	Leaves of 18-d-old seedlings	2,3-diepisecasterone (102 pg $g^{-1}{}_{F.W.}$)
		Roots of 18-d-old seedlings	2,3-diepisecasterone (22 pg $g^{-1}{}_{F.W.}$)

(continued)

Table 3.1 (continued)

Publication	Species	Organ	BRs
Antonchick et al. (2005)	**Rye** (*Secale cereale* L.) cv. Sorom	Seeds	castasterone (574 pg g^{-1} seeds), 2-epicastasterone 201 pg g^{-1} seeds), 3-epicastasterone (115 pg g^{-1} seeds)
		Leaves of 14-d-old seedlings	castasterone, 2-epicastasterone, 3-epicastasterone
Kim et al. (2005)	**Maize** (*Zea mays* L.) cv. Golden cross bantam	Primary roots	6-deoxocathasterone (0.1 ng g^{-1} $_{\text{F.W.}}$), 6-deoxoteasterone (1.0 ng g^{-1} $_{\text{F.W.}}$), 6-deoxotyphasterol (9.0 ng g^{-1} $_{\text{F.W.}}$)
Wu et al. (2008)	**Rice** (*Oryza sativa* L.) wild type	Flag leaves collected after beginning of flowering	6-deoxocathasterone (1.06 ng g^{-1} $_{\text{F.W.}}$), 3-epi-6-deoxocathasterone (2.23 ng g^{-1} $_{\text{F.W.}}$), 6-deoxoteasterone (0.18 ng g^{-1} $_{\text{F.W.}}$), 6-deoxo-3-dehydroteasterone (1.18 ng g^{-1} $_{\text{F.W.}}$), 6-deoxotyphasterol (8.96 ng g^{-1} $_{\text{F.W.}}$), 6-deoxocastasterone (1.84 ng g^{-1} $_{\text{F.W.}}$), teasterone (0.027 ng g^{-1} $_{\text{F.W.}}$), typhasterol (1.47 ng g^{-1} $_{\text{F.W.}}$), castasterone (0.68 ng g^{-1} $_{\text{F.W.}}$)
		Seeds collected 15 days after pollination stage	6-deoxocathasterone (0.48 ng g^{-1} $_{\text{F.W.}}$), 3-epi-6-deoxocathasterone (0.045 ng g^{-1} $_{\text{F.W.}}$), 6-deoxoteasterone (0.085 ng g^{-1} $_{\text{F.W.}}$), 6-deoxo-3-dehydroteasterone (0.075 ng g^{-1} $_{\text{F.W.}}$), 6-deoxotyphasterol (0.14 ng g^{-1} $_{\text{F.W.}}$), 6-deoxocastasterone (0.115 ng g^{-1} $_{\text{F.W.}}$), teasterone (0.040 ng g^{-1} $_{\text{F.W.}}$), typhasterol (0.08 ng g^{-1} $_{\text{F.W.}}$), castasterone (0.08 ng g^{-1} $_{\text{F.W.}}$)
Janeczko and Swaczynová (2010)	**Spring wheat** (*Triticum aestivum* L.) cv. Cytra	10-d-old seedlings (first + second leaf)	brassinolide (303 pg g^{-1} $_{\text{F.W.}}$), 24-epibrassinolide (258 pg g^{-1} $_{\text{F.W.}}$), castasterone (traces)
		Third leaf of 21-d-old seedlings	brassinolide (885 pg g^{-1} $_{\text{F.W.}}$), castasterone (785 pg g^{-1} $_{\text{F.W.}}$)
Janeczko et al. (2010)	**Spring wheat** (*Triticum aestivum* L.) cv. Torka	Mature seeds	brassinolide (127 pg g^{-1} $_{\text{F.W.}}$), castasterone (159 pg g^{-1} $_{\text{F.W.}}$), 24-epicastasterone (535 pg g^{-1} $_{\text{F.W.}}$)
Hartwig et al. (2011)	**Maize** (*Zea mays* L.) wild type	Shoots of 4-week old plants	6-deoxocathasterone (0.27 ng g^{-1} $_{\text{F.W.}}$), 6-deoxoteasterone (0.03 ng g^{-1} $_{\text{F.W.}}$), 3-dehydro-6-deoxoteasterone (0.28 ng g^{-1} $_{\text{F.W.}}$), 6-deoxotyphasterol (1.89 ng g^{-1} $_{\text{F.W.}}$), 6-deoxocastasterone (5.72 ng g^{-1} $_{\text{F.W.}}$), cathasterone (n.d.), teasterone (n.d.), typhasterol (0.045 ng g^{-1} $_{\text{F.W.}}$), castasterone (1.14 ng g^{-1} $_{\text{F.W.}}$), brassinolide (n.d.)

(continued)

Table 3.1 (continued)

Publication	Species	Organ	BRs
	na1 mutant	Shoots of 4-week old plants	6-deoxocathasterone (0.025 ng g$^{-1}_{\text{F.W.}}$), 6-deoxoteasterone (0.01 ng g$^{-1}_{\text{F.W.}}$), 3-dehydro-6-deoxoteasterone (n.d.), 6-deoxotyphasterol (0.115 ng g$^{-1}_{\text{F.W.}}$), 6-deoxocastasterone (0.235 ng g$^{-1}_{\text{F.W.}}$), cathasterone (n.d.), teasterone (0.045 ng g$^{-1}_{\text{F.W.}}$), typhasterol (0.14 ng g$^{-1}_{\text{F.W.}}$), castasterone (0.065 ng g$^{-1}_{\text{F.W.}}$), brassinolide (n.d.)
Janeczko et al. (2011)	**Spring barley** (*Hordeum vulgare* L.) cv. Sezam	Seventh leaf	brassinolide (700 pg g$^{-1}_{\text{F.W.}}$), castasterone (930 pg g$^{-1}_{\text{F.W.}}$), 24-epibrassinolide (traces)
Dockter et al. (2014)	**Spring barley** (*Hordeum vulgare* L.)	Aerial part of 14-d-old seedlings	
	cv. Bowman		castasterone (1245 pg g$^{-1}_{\text{F.W.}}$)
	mutant BW084		castasterone (167 pg g$^{-1}_{\text{F.W.}}$)
	mutant BW091		castasterone (232 pg g$^{-1}_{\text{F.W.}}$)
	mutant BW333		castasterone (390 pg g$^{-1}_{\text{F.W.}}$)
	mutant BW033		castasterone (2097 pg g$^{-1}_{\text{F.W.}}$)
	mutant BW312		castasterone (4357 pg g$^{-1}_{\text{F.W.}}$)
	mutant BW885		castasterone (3448 pg g$^{-1}_{\text{F.W.}}$)
Asahina et al. (2014)	**Rice** (*Oryza sativa* L.) cv. Koshihikari	Aerial part of 7-d-old seedlings growing at white light	6-deoxocathasterone (605 pg g$^{-1}_{\text{F.W.}}$), 6-deoxoteasterone (177 pg g$^{-1}_{\text{F.W.}}$), teasterone (40 pg g$^{-1}_{\text{F.W.}}$), 6-deoxo-3-dehydroteasterone (549 pg g$^{-1}_{\text{F.W.}}$), 6-deoxotyphasterol (2897 pg g$^{-1}_{\text{F.W.}}$), typhasterol (463 pg g$^{-1}_{\text{F.W.}}$), 6-deoxocastasterone (900 pg g$^{-1}_{\text{F.W.}}$), castasterone (329 pg g$^{-1}_{\text{F.W.}}$)
		Roots of 7-d-old seedlings growing at white light	6-deoxocathasterone (723 pg g$^{-1}_{\text{F.W.}}$), 6-deoxoteasterone (288 pg g$^{-1}_{\text{F.W.}}$), teasterone (248 pg g$^{-1}_{\text{F.W.}}$), 6-deoxo-3-dehydroteasterone (546 pg g$^{-1}_{\text{F.W.}}$), 6-deoxotyphasterol (3904 pg g$^{-1}_{\text{F.W.}}$), typhasterol (780 pg g$^{-1}_{\text{F.W.}}$), 6-deoxocastasterone (142 pg g$^{-1}_{\text{F.W.}}$), castasterone (34 pg g$^{-1}_{\text{F.W.}}$)

(continued)

Table 3.1 (continued)

Publication	Species	Organ	BRs
Janeczko et al. (2015)	**Spring wheat** (*Triticum aestivum* L.) cv. Katoda	Aerial part of 7-d-old seedlings	brassinolide (4000 pg $g^{-1}{}_{\text{F.W.}}$), castasterone (80 pg $g^{-1}{}_{\text{F.W.}}$)
Janeczko (2016)	**Spring wheat** (*Triticum aestivum* L.) cv. Katoda	Flag leaf of well-watered plants	castasterone (21 ng $g^{-1}{}_{\text{F.W.}}$)
		Flag leaf of drought-stressed plants	castasterone (5.5 ng $g^{-1}{}_{\text{F.W.}}$)
	cv. Monsun	Flag leaf of well-watered plants	castasterone (19 ng $g^{-1}{}_{\text{F.W.}}$)
		Flag leaf of drought-stressed plants	castasterone (6.5 ng $g^{-1}{}_{\text{F.W.}}$)
Pociecha et al. (2016)	**Winter rye** (*Secale cereale* L.) cv. Dańkowskie Złote cv. Stach	Leaves of 3-week-old plants -before cold hardening	castasterone (2473 pg $g^{-1}{}_{\text{F.W.}}$) castasterone (2088 pg $g^{-1}{}_{\text{F.W.}}$)
	cv. Dańkowskie Złote cv. Stach	-after 3 weeks of cold hardening at +4 °C	castasterone (6389 pg $g^{-1}{}_{\text{F.W.}}$) castasterone (4872 pg $g^{-1}{}_{\text{F.W.}}$)
	cv. Dańkowskie Złote cv. Stach	-after 6 weeks of cold hardening at +4 °C	castasterone (6575 pg $g^{-1}{}_{\text{F.W.}}$) castasterone (7577 pg $g^{-1}{}_{\text{F.W.}}$)
Gruszka et al. (2016a)	**Barley** (*Hordeum vulgare* L.)	Leaves of 14-d-old seedlings	
	cv. Delisa		castasterone (3619 pg $g^{-1}{}_{\text{F.W.}}$)
	mutant *brd1-a*		castasterone (1485 pg $g^{-1}{}_{\text{F.W.}}$)
	mutant *brd1-b*		castasterone (1299 pg $g^{-1}{}_{\text{F.W.}}$)
	cv. Sebastian		castasterone (2413 pg $g^{-1}{}_{\text{F.W.}}$)
	mutant *brd1-c*		castasterone (1021 pg $g^{-1}{}_{\text{F.W.}}$)
	mutant *brd1-d*		castasterone (742 pg $g^{-1}{}_{\text{F.W.}}$)
Gruszka et al. (2016b)	**Barley** (*Hordeum vulgare* L.)	Third and fourth leaf of optimally watered plants in fifth-leaf stage of growth	
	cv. Bowman		castasterone (5800 pg $g^{-1}{}_{\text{F.W.}}$) 28-homocastasterone (52,690 pg $g^{-1}{}_{\text{F.W.}}$)

(continued)

Table 3.1 (continued)

Publication	Species	Organ	BRs
	mutant BW084		castasterone (1160 pg $g^{-1}{}_{\text{F.W.}}$) 28-homocastasterone (86,220 pg $g^{-1}{}_{\text{F.W.}}$)
	mutant BW091		castasterone (1296 pg $g^{-1}{}_{\text{F.W.}}$) 28-homocastasterone (93,405 pg $g^{-1}{}_{\text{F.W.}}$)
	mutant BW333		castasterone (5220 pg $g^{-1}{}_{\text{F.W.}}$) 28-homocastasterone (75,682 pg $g^{-1}{}_{\text{F.W.}}$)
	mutant BW312		castasterone (9600 pg $g^{-1}{}_{\text{F.W.}}$) 28-homocastasterone (43,110 pg $g^{-1}{}_{\text{F.W.}}$)
	mutant BW885		castasterone (7540 pg $g^{-1}{}_{\text{F.W.}}$), 28-homocastasterone (62,270 pg $g^{-1}{}_{\text{F.W.}}$) 24-epibrassinolide (1200 pg $g^{-1}{}_{\text{F.W.}}$)
	cv. Bowman	Third and fourth leaf of drought stressed plants in fifth-leaf stage of growth	castasterone (9280 pg $g^{-1}{}_{\text{F.W.}}$) 28-homocastasterone (47,900 pg $g^{-1}{}_{\text{F.W.}}$) 24-epibrassinolide (1186 pg $g^{-1}{}_{\text{F.W.}}$)
	mutant BW084		castasterone (2273 pg $g^{-1}{}_{\text{F.W.}}$) 28-homocastasterone (71,850 pg $g^{-1}{}_{\text{F.W.}}$) 24-epibrassinolide (1104 pg $g^{-1}{}_{\text{F.W.}}$)
	mutant BW091		castasterone (2270 pg $g^{-1}{}_{\text{F.W.}}$) 28-homocastasterone (72,808 pg $g^{-1}{}_{\text{F.W.}}$) 24-epibrassinolide (1296 pg $g^{-1}{}_{\text{F.W.}}$)
	mutant BW333		castasterone (7540 pg $g^{-1}{}_{\text{F.W.}}$) 28-homocastasterone (75,790 pg $g^{-1}{}_{\text{F.W.}}$) 24-epibrassinolide (928 pg $g^{-1}{}_{\text{F.W.}}$)
	mutant BW312		castasterone (19,428 pg $g^{-1}{}_{\text{F.W.}}$) 28-homocastasterone (28,740 pg $g^{-1}{}_{\text{F.W.}}$) 24-epibrassinolide (1200 pg $g^{-1}{}_{\text{F.W.}}$)
	mutant BW885		castasterone (14,208 pg $g^{-1}{}_{\text{F.W.}}$) 28-homocastasterone (57,480 pg $g^{-1}{}_{\text{F.W.}}$) 24-epibrassinolide (912 pg $g^{-1}{}_{\text{F.W.}}$)
Janeczko, Oklestkova, Novak, unpublished data 1	**Spring wheat** (*Triticum aestivum* L.) cv. Katoda	Aerial part of 21-d-old well-watered seedlings	28-homocastasterone (9 ng $g^{-1}{}_{\text{F.W.}}$)
		Aerial part of 21-d-old drought-stressed seedlings	28-homocastasterone (13 ng $g^{-1}{}_{\text{F.W.}}$)

(continued)

Table 3.1 (continued)

Publication	Species	Organ	BRs
	cv. Monsun	Aerial part of 21-d-old well-watered seedlings	28-homocastasterone (7 ng g^{-1} $_{F.W.}$)
		Aerial part of 21-d-old drought-stressed seedlings	28-homocastasterone (12 ng g^{-1} $_{F.W.}$)
Janeczko, Oklestkova, Novak, unpublished data 2	**Barley** (*Hordeum vulgare* L.) cv. Delisa	Aerial part of 7-d-old untreated plants	castasterone (0.42 ng g^{-1} $_{F.W.}$) 28-homobrassinolide 137 ng g^{-1} $_{F.W.}$) teasterone (1.12 ng g^{-1} $_{F.W.}$)
		Aerial part of 7-d-old plants treated with brassinazole (brassinosteroid biosynthesis inhibitor)	castasterone (0.36 ng g^{-1} $_{F.W.}$) 28-homobrassinolide (117 ng g^{-1} $_{F.W.}$) teasterone (0.60 ng g^{-1} $_{F.W.}$)

Original data expressed in pmol from part of articles have been recalculated to pg or ng (unification for the table purpose)

Stress is a very important factor that affects the BR content in *Poaceae* plants. For example, drought causes changes in the amount of individual BRs in wheat. The amount of 28-homocastasterone increased in aerial part of two cultivars of 21-day-old seedlings after a period of drought compared to plants that were optimally watered (Janeczko, Oklestkova, Novak, unpublished data 1, Table 3.1). The same phenomenon was observed in barley by Gruszka et al. (2016b, Table 3.1). On the other hand content of castasterone in flag leaf of drought stressed wheat plants was lower than in well-watered control (Janeczko 2016, Table 3.1). The BR content in tissues is also regulated by the plant growth temperature. Barley (genotype BW885) growing at 14 °C was characterised by a lower BR content (castasterone) than that growing at 26 °C (Dockter et al. 2014). The castasterone content increased in two rye cultivars (cv. Dańkowskie Złote and cv. Stach) during a few weeks of growth in the cold (cold-hardening process) (Pociecha et al. 2016). The presence or absence of light and its wave length also modifies the BR content in cereals (Asahina et al. 2014). For example, the castasterone content in the aerial parts of rice seedlings that were kept in the dark was on average 90 pg g^{-1} F.W. The authors considered this value to be 1 in order to make it easier to compared with the results that were obtained for plants growing in light. Plants cultured in far red light had 0.86 of the value that was observed in the dark, it was 1.68 for red light, 4.53 for blue light and plants growing in white light reached 4.30.

Finally, the content of BRs in *Poaceae* plants can be changed by using BR biosynthesis inhibitors such as brassinazole (BRZ). In about one-week-old barley seedlings, BRZ, which was applied *via* root watering on Petri dish, decreased the castasterone content by about 14% (Janeczko, Oklestkova, Novak, unpublished data 2, Table 3.1). The content of 28-homobrassinolide was decreased by about 15% and content of teasterone was lowered by about 46% (Janeczko, Oklestkova, Novak, unpublished data 2, Table 3.1).

3 Uptake and Transport of Brassinosteroids in Plants of the *Poaceae* Family

In agricultural and biological experiments on cereal plants, BRs are most often applied by spraying the aerial parts of the plant (Ramraj et al. 1997; Shahbaz and Ashraf 2007; Kroutil et al. 2010) and much less often through the root system (plant watering) (Janeczko and Swaczynová 2010) or through presowing seed soaking (Sairam 1994a). It has been found that the uptake and transport of BRs depend on method of their application. BRs that are applied by spraying are poorly transported or are immobile in all of the plant. The ^{14}C-labeled brassinosteroids (e.g. 24-epibrassinolide), when applied on the leaf of rice or wheat seedlings, were not transported to the other leaves, although they could penetrate inside the tissues at the application site or even slightly translocate within the leaf (Yokota et al. 1992; Nishikawa et al. 1994). In this case, BR transport seems to be partly dependent on the concentration in the working solution. Higher compound concentrations may promote greater uptake efficiency. After spraying wheat (two-leaf stage of growth) with 24-epibrassinolide (0.1 μM), this compound was not detected in the third leaf (Janeczko and Swaczynová 2010). When a higher concentration (2 μM) was used, 24-epibrassinolide was detected in trace amounts in the third leaf. We assume theoretically in this case that the 24-epibrassinolide that was detected in the leaves was the same as that applied exogenously to the plants. The protective barrier covering the leaf on which drops of the working solution containing the hormone flow down may be a factor that limits BR penetration into the leaf. This problem can be eliminated by using the so-called infiltration method. The BR solution is pumped directly into the apoplast under pressure (Janeczko et al. 2011). The introduction of 24-epibrassinolide at 0.005 and 0.25 mg dm^{-3} concentrations to the apoplast of 12-day-old barley seedlings with two leaves resulted in an increased concentration of this compound in the seventh leaf, which formed later, compared to the control. It is interesting, however, that a similar content of this BR was found in the leaves of older plants regardless of the concentration of 24-epibrassinolide in the solution that was applied to seedlings. Therefore, possible BR transport was under the control of internal homeostasis mechanisms, thus preventing the penetration of non-physiological BR concentrations into the developing leaves.

More efficient uptake and subsequently, BR transport can be obtained using the root application because roots are organs that are designed to uptake substances from the soil solution. After the root application of radiolabelled 24-epibrassinolide to wheat and brassinolide or castasterone to rice, radioactivity was detected in the aerial parts of plants (Nishikawa et al. 1994; Yokota et al. 1992). According to Yokota et al. (1992), radioactivity in the aerial parts of plants was detected 6 h after the root application and the majority of the determined there brassinosteroid pool was unmetabolised BRs. Wheat seedlings that were grown on Petri dishes and watered with a solution containing 24-epibrassinolide (0.1 and 2 μM) on the third day of vegetation accumulated an increased amount of this steroid in the leaves (Janeczko and Swaczynová 2010). The BR transport was also disproportionate to the applied concentration in this case. The root application of 24-epibrassinolide at a lower concentration caused amount of this compound in the leaves to increase 2-fold compared to the control. The quantity of 24-epibrassinolide in the leaves only increased 3-fold after the application of a 20-fold higher concentration (Janeczko and Swaczynová 2010). According to Nishikawa et al. (1994), BR transport probably occurs through the phloem. However, BRs induce physiological changes that involve the entire plant organism regardless of whether the BR transport takes place at a lower or higher efficiency (after root application) or whether it is applied locally (after spraying).

4 Selected Aspects of the Physiological Activity of Brassinosteroids in the *Poaceae* Family

Among the known brassinosteroids, exogenous 28-homobrassinolide and 24-epibrassinolide are the ones that are most commonly used in experiments on *Poaceae* plants, while brassinolide and other BRs are used less frequently. The experiments have mainly been carried out on popular species such as wheat, maize, rice and barley but also on less known finger millet. Currently, the availability of mutants, among others, rice and barley as well as maize mutants with BR biosynthesis or signalling disorders, allows for a more detailed explanation of some of the mechanisms of action of BR.

4.1 Plant Growth, Development and Yield

4.1.1 Plant Growth

Plant growth stimulation by brassinosteroids is the first known physiological activity of these compounds (Grove et al. 1979). Exogenous BRs stimulate the growth of plants in a concentration-dependent manner. For example, 24-epibrassinolide, when applied to two-week-old wheat seedling in concentration range from 0.005 to 2 mg dm^{-3}, most efficiently stimulated growth at concentrations of 0.25 and 0.5 mg (Janeczko et al. 2010) (Fig. 3.3). After root application of brassinolide solution (0.1–2 mg dm^{-3}) to germinated wheat seedlings, the most efficient growth stimulation of the aerial parts and roots was observed at a 1 mg dm^{-3} concentration (El-Feky and Abo-Hamad 2014).

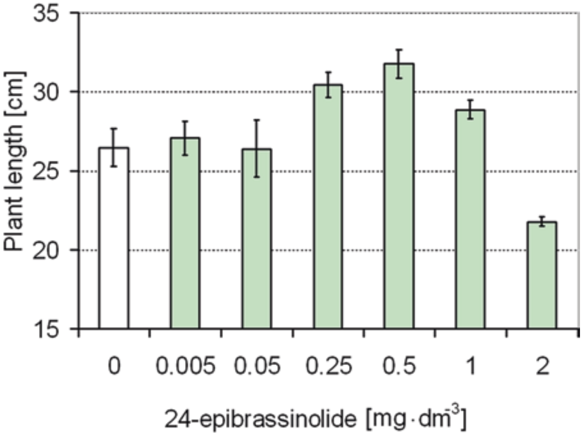

Fig. 3.3 Length of the aerial part of 4-week-old wheat seedlings 2 weeks after being sprayed with 24-epibrassinolide – a dose response curve. Mean values ± SE. (Based on Janeczko et al. 2010, modified)

The mutants of barley, rice and maize with brassinosteroid biosynthesis and signalling disorders were characterised by dwarfism, which confirms the significance of BRs for the growth processes of plants from the *Poaceae* family (Fig. 3.4a–d). Semi-dwarf mutants (*uzu*) were described in barley for the first time (Saisho et al. 2004). A monogenic, recessive mutation of the *HvBRI1* (*Uzu1*) gene encoding the transmembrane BR receptor was responsible for the mutant's phenotype. Recently, new mutations of the *uzu1* gene have been identified in barley. Mutations were induced *via* chemical and physical mutagenesis. Such mutants permitted a more detailed functional analyses of the gene and the encoded BR receptor. All of the mutations were 'missense type' mutations and resulted in substitutions of amino acids in different BR receptor domains, which is associated with BR-binding disorders (Gruszka et al. 2011a; Dockter et al. 2014). Mutants with BR biosynthesis disorders are also known in barley. Example are the semi-dwarf 522DK and 527DK mutants that were obtained by chemical mutagenesis (collection of the University of Silesia (Poland); Gruszka et al. 2011b, Fig. 3.4a, b). The mutants had missense mutations in the *HvDWARF* gene, which caused disturbances of the C6-oxidase activity in the BR biosynthetic pathway (Gruszka et al. 2011b). The mutants had a reduced content of endogenous castasterone compared to the wild type, i.e. 42% and 36% of the wild type values in the 522DK and 527DK, respectively (Janeczko et al. 2016). These mutants were more or less about 30% shorter than the wild type (Delisa) at every growth stage – from the coleoptile stage to the heading plants (Janeczko et al. 2016).

A dwarf maize mutant with a mutation in the *Brd1* gene encoding C-6 oxidase, which is the key enzyme responsible for BR conversions in the final steps of their biosynthesis, was described by Makarevitch et al. (2012). Plants with the mutation in this gene were five times shorter than the wild type and were also characterised by disturbances in their leaf and flower morphology.

A *d61* mutant phenotype, which is connected to the loss of function of the *OsBRI1* gene (BR receptor mutation), was described in rice (Morinaka et al. 2006). The identified alleles of this gene were numbered from 1 to 9 (mutants *d61-1*–*d61-9*). This

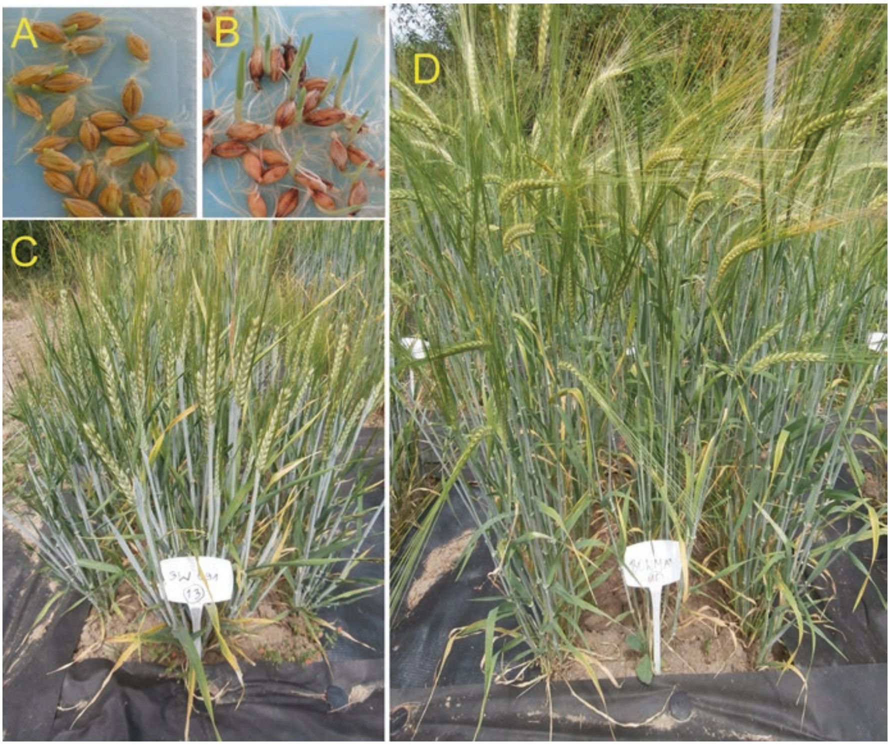

Fig. 3.4 Germination of the BR-deficient barley mutant 522DK (**a**) and the wild type cv. Delisa (**b**). Dwarf BR-deficient barley mutant BW091 (**c**) and wild type cv. Bowman (**d**) growing in a field. (Photo by A. Janeczko)

mutation is associated with the occurrence of dwarfism as well as with erect leaves. Earlier, Yamamuro et al. (2000) proved that OsBRI1 was responsible, among others, for internode elongation (inducing the formation of the intercalary meristem and the longitudinal elongation of internode cells) or for skotomorphogenesis in rice. Simultaneously, BRs also control leaf erectness in *Poaceae* (Sun et al. 2015). This mechanism is associated with the inhibition of the proliferation of specific abaxial cell groups in the lamina joint parts by BRs in rice. A U-type cyclin (CYC U4;1), whose activity and expression is controlled by BR signalling, has also been identified (Sun et al. 2015). Cyclins are engaged in the cell cycle as well as the activity of cyclins and cyclin-dependent kinases determine the progression of the cell cycle. Crosstalk with typical growth hormones is another mechanism by which BR influence *Poaceae* growth. In rice, BRs regulate cell elongation by modulating the metabolism of gibberellins (GAs) (Tong et al. 2014). BRs regulate the expression of the GA metabolic genes (i.e. by inducing *D18*/*GA3ox-2* expression), thereby promoting GA1 accumulation and cell elongation in rice. Excess BRs inactivate GAs through the upregulation of the GA inactivation gene (*GA2ox-3i*) and additionally repress BR biosynthesis, which results in growth inhibition. GAs may also inhibit

BR biosynthesis and BR response. On the other hand, barley BR mutants produced lower levels of cytokinins, thus the crosstalk between these hormones and BRs may also be important for growth regulation in *Poaceae* (Janeczko et al. 2016). Finally, cell elongation in *Poaceae* (rice) may also be simultaneously induced by BR and IAA (Yang et al. 2006). The authors characterised a brassinolide upregulated gene in rice, *OsBLE3*, which was expressed in the roots and leaf sheaths and this expression was BR dose-dependent. The reduced *OsBLE3* expression (in *OsBLE3* antisense transgenic rice) was accompanied by growth retardation. The presence of auxin response elements in the 5′-flanking region of the *OsBLE3* gene indicated that the expression of this gene was under the control of auxin. Simultaneously, the *OsBLE3* transcript level was reduced in the BR-deficient mutant compared to the wild type. According to Yang et al. (2006), OsBLE3 is engaged in cell elongation in rice through a dual regulation by brassinosteroid (brassinolide) and auxin (IAA).

4.1.2 Plant Development and Yield

The action of brassinosteroids on the development of plants of the *Poaceae* family is multidirectional. The application of 24-epibrassinolide in winter wheat during suboptimal vernalisation (low-temperature treatment required for the induction of development) slowed down the generative development by delaying plant entry into the heading stage compared to the control (Janeczko et al. 2015). Plants that had been treated with a BR biosynthesis inhibitor (brassinazole) headed faster than control. Plants that had been treated with the inhibitor, the effect of which was simultaneously compensated for the addition of exogenous 24-epibrassinolide, headed at a similar time as the controls. This suggests that the BRs in wheat may be a negative regulator in the generative development induction process (Janeczko et al. 2015). On the other hand, plant heading time was delayed 20 days in spring barley that had BR biosynthesis disorders and a decreased castasterone content (mutants 522DK and 527DK) (Janeczko et al. 2016). The role of BRs in the induction of generative development in *Poaceae* requires further research and differences between species must be taken into account. The action of BR, however, is also observed at later developmental stages – e.g. during pollen development. Holá et al. (2010) sprayed three maize lines with 24-epibrassinolide and one synthetic analogue of castasterone (2α,3α,17β-trihydroxy-5α-androstan-6-one) in field. The BRs were used in development stages V3/4 and V6/7 (i.e. 41 and 55 days from sowing) at concentrations of 10^{-8}–10^{-14} M. The spraying in stage V3/4 delayed male anthesis and silking, whereas spraying in stage V6/7 accelerated these processes regardless of the BR concentration and genotype. In turn, the final number of ears that were developed by each plant at the end of the flowering was dependent on the BR concentration and the time of application. The most effective were BRs in the 10^{-14} M concentration that were applied in V3/V4, while the application of BRs in V6/V7 decreased the ear number/plant. The work carried out by Czech researchers drew attention to the fact that the use of BR in field maize cultivation not only requires the precise determination of the BR concentrations, but also the selection of the most suitable plant developmental stage for BR application and even taking into account the specificity of the line/cultivar.

The importance of natural BRs in the later stages of maize development was also confirmed in mutant studies of this species (Hartwig et al. 2011). Authors studied maize dwarf mutant *nana plant1* (*na1*), which has feminised male flowers. The mutant carried a loss-of-function mutation in the *DET2* homologue, which is a gene in the BR biosynthetic pathway and accumulated (24R)-24-methylcholest-4-en-3-one. It was accompanied by a decrease of the downstream BR metabolites. The expression of *na1* throughout their development, especially in the anthers, allowed a hypothesis that BRs promoted the masculinity of the male inflorescence and participated in the sex determination process in maize to be formulated.

Finally, BRs in *Poaceae* may participate in final steps of development – grain production and filling. Wu et al. (2008) created a transgenic rice expressing the gene encoding sterol C-22 hydroxylases. The enzyme controlled the BR levels and the obtained plants were characterised by an increased BR content downstream of 6-deoxocathasterone. For example, the content of 3-*epi*-6-deoxocathasterone was doubled, as was the content of 6-deoxotyphasterol. Transgenic plants with an increased BR level produced more tillers and seeds than the wild type. Seed yield increased by 15–44% depending on the growth conditions. The glucose pool was higher in the flag leaves and the plants had an increased glucose accumulation compared to the starch in the seeds. The significance of BRs for the production of grains and more broadly biomass by a plant was also demonstrated in the work of Morinaka et al. (2006). The authors compared biomass and grain production in the wild-type and the *d61-7* dwarf rice mutant (BR receptor mutation). The wild-type biomass was 38% higher than *d61-7* at the standard planting density, but the *d61-7* biomass was 35% higher than the wild type at a high planting density. Erect leaves of this mutant allowed for better light penetration in the field in this case. The small size of the *d61-7* grains, however, did not allow a higher yield to be obtained than for the wild type.

Research on mutants and transgenic plants (Morinaka et al. 2006; Wu et al. 2008), which provide information about the role of BRs in the processes of biomass accumulation and yielding, confirmed the results that had been obtained earlier in the experiments using exogenous BRs in rice by Ramraj et al. (1997) or Fujii and Saka (2001). The study of Fujii and Saka (2001) showed that exogenous brassinolide influenced the transport/accumulation of assimilates in the grains, thereby increasing the concentration of starch and sucrose in the forming seeds. On the other hand, an increase in the rice yield was found in a field experiment (India) after the application of 28-homobrassinolide (Ramraj et al. 1997). A double BR spraying at a concentration of 1 mg dm^{-3} was the most effective. The control yield was 4.90 t/ha, while 6.27 t/ha was obtained from the plants that had been treated with 28-homobrassinolide. The BR-sprayed fields were characterised by an increased number of panicles per square metre. Brassinosteroids also stimulate yield of wheat. 28-Homobrassinolide stimulated the wheat yield in field and pot experiments (Sairam 1994a, b). The field experiment (India) compared the wheat culture in a season with frequent droughts to artificially irrigated plants and found an increased number of seeds in the ears, the number of ears per m^2 and 1000 seed weight in both groups of plants under the influence of 28-homobrassinolide. The compound was applied by seed soaking (6 h before sowing) and by spraying 25-day-old seedlings with 28-homobrassinolide (0.01 and 0.05 ppm) (Sairam 1994a). The cultivar C306 responded better to 28-homobrassinolide than HD2329 in that experiment. For example, the number of seeds that were collected from m^2 was 328 in the C306 under artificial irrigation, while 456 seeds were obtained in the plants after 0.05 ppm steroid spraying (Sairam 1994a). In a 3-year field experiment (India), 28-homobrassinolide stimulated the yield of wheat cv. Lok-1 (Ramraj et al. 1997). The average control yield from three seasons was 5.70 t/ha and an average of 6.70 t/ha was obtained for the best 28-homobrassinolide combination (spraying in two developmental stages, a concentration of 0.5 mg dm^{-3}) (Ramraj et al. 1997). Another BR – 24-epibrassinolide – when applied to wheat (plant spraying or seed priming) also increased the yield of this species (Ali et al. 2008; Hniličká et al. 2007; Janeczko et al. 2010). However, the effect of 24-epibrassinolide on the chemical composition of the grain was low and additionally depended on the cultivar and growth conditions (Hniličká et al. 2007, Janeczko et al. 2010). An increase in the content of soluble sugars in seeds (by 25% after hormonal seed priming), but not the starch content, was found in a pot experiment (Janeczko et al. 2010). A decrease in the fat content was observed (34% after 24-epibrassinolide spraying), but no significant changes in the soluble protein content were found. The influence of the hormone on the content of carbohydrates, proteins and lipids was very slight in a field cultivation (Janeczko et al. 2010). Hniličká et al. (2009) observed a weak, although in most cases positive effect, of 24-epibrassinolide spraying (10^{-9} M at the beginning of the flowering stage) on the protein, lipid and starch content in six wheat cultivars, which had been subjected to drought and a temperature increase to 33 °C (in the late stage of stem growth) in a pot experiment. Calorimetric analysis of the amount of energy that was accumulated in the grains (determined based on the combustion of a grain sample in an oxygen atmosphere in a calorimetric vessel) showed that its greater resources were stored by the plants that had been treated with 24-epibrassinolide. The action of BR in other *Poaceae* plants was also tested in maize and *Eleusine coracana* L. (finger millet). 24-Epibrassinolide and a castasterone analogue influenced the yield of field-grown maize that had a strong dependence on the cultivar, concentration and yield parameter (Holá et al. 2010). For example, the application of the castasterone analogue (10^{-14} M) increased the dry weight of the whole ear and cob in line 2023 when the plants were treated with the hormone in stage V3. The effect in line CE704 was the opposite. An increased yield from 1636 kg/ha (control) to 1990 kg/ha was found in the *Eleusine coracana* L. plants, to which BR had been applied by 8-h seed soaking (0.1 ppm) before sowing (Nithila et al. 2007).

It seems that the effect of BRs on the yield in *Poaceae* is mainly based on the regulation of the processes that are related to photosynthesis efficiency and, as was mentioned above, the transport of assimilates. An increase in the chlorophyll content that was caused by BR was found in wheat (Sairam 1994a, b), which is important in terms of the efficiency of solar energy absorption and the performance of the photosynthetic light reactions. Barley mutants with a reduced BR level also had a lower chlorophyll content in the leaves (Janeczko et al. 2016). The effect of BR on accumulation of photosynthetic pigments and the photosynthetic light reactions is different in maize. According to Rothová et al. (2014), the application of 24-epibrassinolide and a castasterone analogue increased photosynthetic pigment accumulation and selected parameters that characterise PSII efficiency. For example, a positive effect on the oxygen-evolving complex (OEC) was observed. On the other hand, PSI efficiency in maize was not affected by these two steroids (Honnerová et al. 2010). An increased maximum quantum yield of primary photochemistry of PS II (Fv/Fm) was shown in transgenic rice with an increased BR accumulation (Wu et al. 2008) compared to the wild type. This rice was also characterised by an increased CO_2 assimilation in the photosynthetic dark reactions. The increased activity of the CO_2-binding enzyme Rubisco (carboxylase-ribulose-1,5-bisphosphate carboxylase) and net photosynthesis were previously recorded in wheat after exogenous BR application (Braun and Wild 1984; Sairam 1994a, b; Hniličkaet al. 2008). Simultaneously, BR-deficient barley mutants were characterised by a lower Rubisco activity (Janeczko et al. 2016). These mutants also had a reduced sucrose accumulation along with increased glucose and fructose levels, thereby suggesting that BR could also affect the enzymatic system that is involved in sugar (sucrose) biosynthesis. This is consistent with studies in which exogenous BRs increased the production of sugars and their transport (Fujii and Saka 2001; Wu et al. 2008). These phenomena are an important element of the mechanism by which BRs stimulate the biomass accumulation, including yield. As presented, BRs act during the entire plant life cycle in the *Poaceae* family and are responsible for the direct or indirect regulation of many growth, developmental and yield processes.

4.2 Plant Stress Response

During the vegetation period, plants are naturally exposed to different environmental factors – biotic (pathogens) and abiotic (drought or excess of water, too low or too intense light, cold, frost, too high temperatures etc.) The occurrence of these stress factors during the growth of the crop plants of the *Poaceae* family (such as rice, maize, wheat) and especially their higher severity can cause significant damage to crops that result in yield losses. Brassinosteroids are one of the plant hormones that stimulate the processes that counteract the negative effects of stress.

There are many publications that show that BRs counteract the effects of many types of stresses in the species of the *Poaceae* family. In this review, only a few examples will be given, together with an explanation of some of the mechanisms of action of BR.

4.2.1 Salt Stress

Salinity is a problem of agricultural soils in many countries, hence much work has been devoted to research that is aimed at improving the conditions of plant growth under this stress. Brassinosteroids alleviate the negative effects of salt stress on

Poaceae plants. An example is 28-homobrassinolide that was applied by presowing seed soaking (12 h, 10^{-4}–10^{-8} M concentrations) in maize (Arora et al. 2008). The hormone increased the activity of the antioxidant enzymes (superoxide dismutase (SOD), guaiacol peroxidase, catalase (CAT), glutathione reductase (GR) and ascorbate peroxidase (ASP) in the leaves of 30-day-old maize that had been exposed to salt stress (NaCl – 25, 50 and 75 mM). The hormone reduced the peroxidation of cell lipids (measured by the accumulation of malondialdehyde (MDA) and increased the protein content (Arora et al. 2008). According to the authors, 28-homobrassinolide alleviated the oxidative stress in the salt-treated maize plants. The ameliorative effects of another BR – 24-epibrassinolide – in mitigating the phytotoxicity of NaCl stress in the seedlings of maize were also reported by Agami (2013). The application of the hormone improved growth, increased photosynthetic pigment and proline content as well as the antioxidant activity of CAT and peroxidases. In addition to changes in the efficiency of the antioxidant system, BRs regulated maize's hormone metabolism under salt stress. Brassinolide used for seed soaking and plant spraying (0.25 ppm) abolished the adverse effect of salinity on plant hormone production (IAA, GA3 and zeatin) (El-Khallal et al. 2009). In wheat, spraying plants with 24-epibrassinolide stimulated biomass production and increased the leaf surface area under saline conditions in two cultivars – S-24 – saline-resistant and MH-97 – susceptible to this stress factor (Shahbaz et al. 2008). The application of this hormone to the roots in wheat growing in a hydroponic culture under saline conditions also resulted in an increase in the total yield (among others, through an increase of the 100-seed weight) in the two tested wheat cultivars (Ali et al. 2008). The best effects were reported for 0.104 and 0.052 μM concentrations (Ali et al. 2008). The results of Tofighi et al. (2017) were also interesting, as these authors claimed that BR increased wheat salinity tolerance by cooperating with arbuscular mycorrhizal fungi (*Glomus mosseae*). BR prevented a decrease in chlorophyll and increased the nitrate reductase activity in rice growing under saline conditions (Anuradha and Rao 2003). This compound also increased the content of the proline osmoprotectant, proteins and the activity of antioxidant enzymes as well as reduced the damage to cell membranes (Sharma et al. 2013). An increased activity of antioxidant enzymes in rice growing in salt stress was noted also under the influence of one of the BR analogues (BB-16) (Núñez et al. 2003).

4.2.2 Drought Stress

Water deficiency is one of the most important factors that limits crop yield. Drought excludes agricultural cultivation in many areas. Regions with sufficient water resources may also endure years with periodic droughts due to changes in climate. BRs are one of the regulators that can minimise the effects of drought on plant growth and yield. The effect of 24-epibrassinolide on the yield of spring wheat cv. Torka was evaluated in a field experiment conducted in the climatic conditions of central-eastern Europe (Poland) (Janeczko et al. 2010). 24-Epibrassinolide was administered *via* 48-h presowing seed soaking (1 mg dm^{-3}) and spraying the plants in the heading stage (0.25 mg dm^{-3}). Although the average rainfall for July in Polish climatic conditions usually reaches 90 mm/month and is sufficient for plants, drought unexpectedly occurred in that month during an experiment in 2006 (rainfall 14 mm/month). This allowed the effect of the hormone to be evaluated under natural drought conditions. BR raised the crop yield in the field cultivation by about 20% compared to the untreated control. The basis for the increase in the yield was the formation of a higher number of seeds by the plant (Janeczko et al. 2010). Hnilička et al. (2007) also observed a slight increase in the seed and straw yield in six wheat cultivars that had been sprayed with 24-epibrassinolide (10^{-9} M concentration, greenhouse conditions) under drought stress and that were then subjected to an increased temperature of 33 °C (in the late stage of stem growth). Sairam (1994a, b) conducted research on selected mechanisms of BR action in wheat in drought conditions. Author found, among others, that in wheat BR (28-homobrassinolide) stimulated the activity of the enzymes that are associated with nitrogen metabolism: nitrate reductase and glutamate synthetase. This compound also decreased stress-induced cell membrane damage. Farooq et al. (2009, 2010) described a beneficial effect of BR on rice plants in drought conditions. BRs possibly enhanced plant growth because of the improved assimilation of carbon. The BR-treated plants were also characterised by an ability to maintain a better tissue water status. While drought increased H_2O_2 and MDA production, BRs counteracted this effect, among others, by enhancing the capacity of the antioxidant system. Of the two BRs that were used, 24-epibrassinolide and 28-homobrassinolide, the former was more active. Moreover, the application *via* spraying was more effective than seed priming. The study of Janeczko et al. (2016) characterised physiologically and biochemically BR-deficient barley mutants (522DK and 527DK) and the wild type Delisa. The aim of the study was to answer the question of whether/how disturbances in the production of brassinosteroids in barley affect the plant's metabolism under drought. In drought conditions, BR synthesis disorders were accompanied by a decrease in the production of other plant hormones (ABA and cytokinins), although this effect was not observed for auxins. The mutants produced less osmoprotectant proline compared to the wild type during drought. They also accumulated less sucrose, although the Rubisco activity was at a similar level in both the mutants and the wild type. The accumulation of the transcript of the gene encoding the protective protein – hsp90 – from the heat shock protein group was statistically significantly reduced in the 527DK mutant. A reduced kestose accumulation (one of the fructans considered to be cell membrane stabilising factor) was revealed in 527DK under drought. Finally, PSII efficiency in conditions of drought was lower in the mutants – especially in 527DK. The findings of Gruszka et al. (2016b) for drought-stressed barley mutants with disturbances in BR biosynthesis and signalling can serve as an interesting conclusion to this chapter. The authors proved that all of the mutants and the wild type plants increased the production/accumulation of BRs in drought conditions, which may support the presented data and show that BRs play an important role in protecting plants against drought.

4.2.3 Heavy Metal Stress

Many heavy metals such as copper, manganese, iron or cobalt are naturally present in living organisms and are often components of the enzyme and protein molecules that are required for cell function. However, an excess of these elements is toxic to cells. Many works have shown that the negative effects of heavy-metal poisoning were alleviated or limited by BRs in *Poaceae* plants. 24-Epibrassinolide (0.1 mg dm^{-3}), which was sprayed on maize plants that were then subjected to the stress of a high manganese concentration in soil (150–750 mg kg^{-1}, a phenomenon that is particularly dangerous in acidic soils), reduced the unfavourable physiological changes that are caused by excess of this element (Wang et al. 2009). This was manifested by an increase in the chlorophyll content, net photosynthesis intensity and dry matter accumulation. A decreased accumulation of H_2O_2 was observed in plants together with an increased activity of antioxidant enzymes (including SOD, CAT, GR, ASP). Bhardwaj et al. (2007) studied the effects of 28-homobrassinolide on maize seedling growth, lipid peroxidation and antioxidative enzyme activities under nickel stress. The hormone reduced the toxicity of the heavy metal on seedling growth and also influenced the protein content. Lipid peroxidation was increased under the heavy metal stress, but decreased in the BR-treated plants. The hormone also increased the activity of the antioxidant enzymes (except SOD). The application of 24-epibrassinolide was effective in ameliorating the stress that was caused by chromium in rice (Sharma et al. 2016). The application of the hormone as a pre-soaking treatment resulted in better plant growth, a lower accumulation of chromium by the tissues and a strengthened defense system by upregulating the gene-encoding antioxidant enzymes such as Mn-SOD, Cu/Zn-SOD, CAT or GR.

4.2.4 Temperature Stress

Among abiotic stresses, temperature stress is a particularly serious problem in agriculture and horticulture. Some species such as maize are very sensitive to cold, while frost, especially when there is insufficient snow cover on fields, can cause significant yield losses of winter cereals. High-temperature stress is dangerous when combined with drought during the vegetation season. Many hormones control the plant response to high or low temperatures and brassinosteroids appear to be among them. An increased concentration of abscisic acid (ABA) – a stress hormone – occurred in maize as a defensive response to cold stress (Janowiak et al. 2003). Moreover, the cold-tolerant cultivars of maize accumulated more of this hormone (Janowiak et al. 2003). Studies related to changes in the level of brassinosteroids in plants under temperature fluctuations are scarce. The content of one of the BRs, castasterone, which was measured in the barley line BW885 at 14 °C, was 7.43 pmol g^{-1} F.W., but increased to 10.31 pmol g^{-1} F.W. after the plants were moved to 26 °C (Dockter et al. 2014). Simultaneously, Pociecha et al. (2016)

observed an increased castasterone content from 4–5 pmol g^{-1} F.W. (control) to 14–16 pmol g^{-1} F.W. in winter rye (*Secale cereale* L.) plants after 6 weeks of plant cold hardening. The described changes may suggest that BRs play a role in the processes of acclimation to changing temperature conditions.

4.2.4.1 Frost

Sudden drops in temperature during winter followed by the periods of higher, dehardening temperature in countries that cultivate winter cereals is a factor that causes frost damage (especially in the absence of snow cover) that later affects yielding. Research conducted by Pociecha et al. (2016) showed that 24-epibrassinolide (0.25 mg dm^{-3}), when applied before the cold hardening of winter rye, significantly increased frost tolerance. Plants had less frost damage and a higher survival rate. 24-Epibrassinolide also improved frost tolerance in winter wheat (Janeczko 2016, Fig. 3.5). Wheat seedlings that had been sprayed with the hormone, cold-acclimated at +5 °C and then exposed to −12 °C had a better survival rate than untreated plants (Fig. 3.5). According to Pociecha et al. (2016), 24-epibrassinolide increased the Rubisco activity in both of the cultivars that were tested as well as the sucrose content (but in a cultivar-dependent manner). An increased sucrose concentration is a well-known phenomenon in the process of cold hardening and its function is to reduce the freezing point of the cell aqueous solution, which improves survival in frost conditions. In one of the cultivars that was tested, BR also stimulated the accumulation of protective fructooligosaccharide (nystose) by 55% compared to the cold-hardened plants that had not been sprayed with BR.

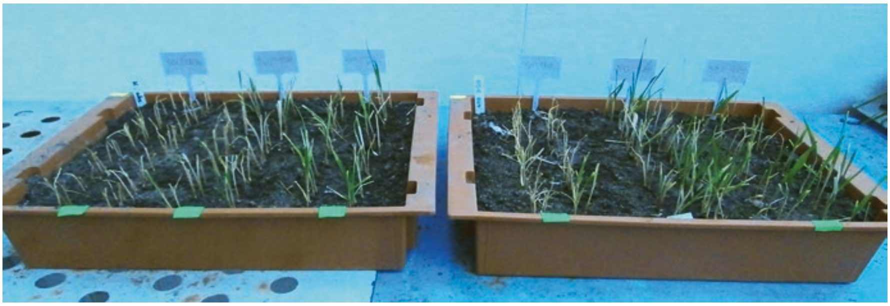

Fig. 3.5 Regrowth of winter wheat after exposure to −12 °C. Dying plants of cv. Bystra (low frost tolerance) and cv. Nutka (moderate frost tolerance), visible in control (*left pot*); only plants of the highly tolerant cv. Smuga survived. BR application (*right pot*) before the low temperature treatment increased the survival of the cv. Nutka plants and even some plants of cv. Bystra also regrew. (Janeczko 2016, data from project 2013/09/B/NZ9/01653). Order of cultivars in pot: cv. Bystra - first two rows, cv. Nutka - rows 3 and 4, cv. Smuga - rows 5 and 6.

4.2.4.2 Cold

Among the plants of the *Poaceae* family, maize is particularly cold sensitive. Temperatures below 10 °C can seriously damage young maize seedlings. An experiment of Singh et al. (2012) exposed maize seedlings to cold stress (net house with a maximal temperature of 17.6–24.5 °C and a minimal temperature of 2.8–7.4 °C; 21 days). The authors showed a decrease in plant height by about 35% and F.W. by about 24%; the data were compared to the controls that were growing in a green house (25/18 °C (d/n)). The application of 24-epibrassinolide (1 μM) to the plants that were growing in the net house increased plant height, fresh and dry weight (15, 36 and 2%, respectively) compared with the plants without the application of BR. Seedlings that were exposed to the cold in the net house had a slightly increased glucose, starch and sucrose content compared to the control plants that were cultured in the controlled conditions of the greenhouse. Additionally, 24-epibrassinolide elevated the content of these sugars (15–45%) compared to the stressed plants without the BR treatment. Cold also decreased the chlorophyll content in the maize in the net house, but this effect was neutralised by 24-epibrassinolide.

4.2.4.3 High Temperatures

High temperature in natural conditions, when associated with drought stress, is a very important cause of the limitation of photosynthesis and inhibition of growth. Thussagunpanit et al. (2015a, b) studied the effect of high temperature on rice plants. The decrease in the chlorophyll content was milder in rice that had been treated with 24-epibrassinolide prior to exposure to high temperature (40/30 °C; 7 days). After hormone application, the heat-stressed rice had a better PSII performance and significantly improved electron transport rate (Thussagunpanit et al. 2015a). The protective effect of 24-epibrassinolide (0.25 mg dm^{-3}, leaf infiltration before heat stress) on PSII performance was also found in barley seedlings (Janeczko et al. 2011). Energy absorption by the antennas, energy transferred to the reaction centre and energy transferred to the electron transport chain were higher in the first leaves of the seedlings by 23, 49 and 69%, respectively, when compared to the values that were recorded in the stressed plants without the application of BR. In rice, high temperature also decreased the leaf net CO_2 assimilation and transpiration parameters by 17 and 31%, respectively, and increased the leaf internal CO_2 concentration by 8% compared to non-stressed plants (Thussagunpanit et al. 2015a, b). The application of 24-epibrassinolide counteracted this effect.

4.2.5 General Comments

In opinion of author of this chapter, of all of the mechanisms of the action of BRs, two appear to be the most important and especially help *Poaceae* plants to achieve a better stress tolerance: (1) a modulation of the antioxidant cell system (Xia et al. 2015) and

(2) physicochemical modifications of the properties of the cell membrane (Filek et al. 2017). Of course, we should not forget that BRs regulate the expression of many genes as well as cooperate with other hormones, but it is the stabilisation of the cell membrane and the ability to maintain the proper redox balance in a cell that provide a favourable environment for the functioning of all of the other biochemical processes.

The cell membranes are involved in thermal sensing (Horváth et al. 2012) and are generally responsible for the cell-environment contacts. The proper functioning of the membranes affects all of the processes that are localised in the membranes such as the light phase of photosynthesis (the proper structure of the photosynthetic antennas, the efficiency of the photosystems) and also some parts of the dark phase (e.g. the aquaporin channels that enable CO_2 transport are located in the membranes). As was mentioned earlier, although the action of BR on the membranes in stress conditions is manifested by a reduction in membrane permeability and lipid peroxidation, BRs also modulate the physicochemical properties of the cell membranes (Filek et al. 2017). Two brassinosteroids with different chemical structures, 24-epibrassinolide and 24-epicastasterone, when introduced into lipid the monolayers, changed their physicochemical properties. Studies were performed using a Langmuir bath to analyse the monolayer formation of lipids that had been isolated from wheat leaves growing at 20 °C and in the cold (5 °C). 24-epibrassinolide increased the area per lipid molecule in the monolayers, which resulted in the formation of more flexible surface structures. This effect is very similar to the effect of sterols on membranes and is associated with a higher fluidity of membranes, which guarantees (especially in low temperatures) a better stress tolerance for the entire plant. Interestingly, the second BR that was studied, 24-epicastasterone, induced the different effects, which showed the importance of the BR chemical structure for their interaction with cell membranes and further physiological effects. Xia et al. (2015) described the maize model of interaction between BRs and the antioxidant system and the effects of these interactions in plants – growth or stress response (Fig. 3.6). According to Xia et al. (2015), activation of the BR receptor led to the production of reactive oxygen species (ROS) (e.g. H_2O_2). However, the temporal and spatial changes in their levels depended on the BR concentrations (the stress factors increase the accumulation of BRs (Gruszka et al. 2016b; Pociecha et al. 2016). High BRs levels cause the long-term accumulation of reactive oxygen species, which in turn triggers the miogen-activated protein kinase phosphorylation cascade. In this case, ROS and kinase stimulate the ABA biosynthesis – the main hormone that is associated with the induction of stress tolerance. Low BRs levels, on the other hand, cause a transient increase in the ROS concentration, which stimulates a cell's antioxidant system, which ultimately leads to shifting the redox balance of the cell towards the reducing processes. This acts as a signal, e.g. for the stimulation of the photosynthesis and growth processes.

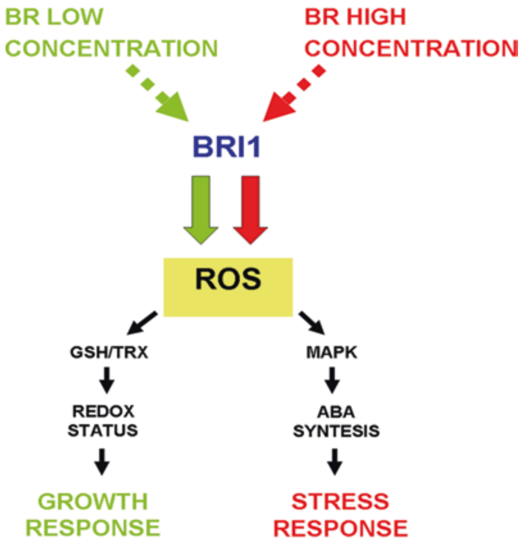

Fig. 3.6 BR concentration-dependent model of growth and stress response in maize (Xia et al. 2015, modified). *BRI1* brassinosteroid receptor domain, *MAPK* specific mitogen activated protein kinase, *GSH/TRX* glutathione/thioredoxin systems

5 Future Perspectives

The discussed experiments that tested the effects of exogenously applied BR treatments provide an overview of the physiological functions of these compounds in *Poaceae*. Recently, the number of studies that use research models that involve biosynthesis and perception BR mutants of *Poaceae* plants has also significantly increased. Importantly, studies on mutants confirmed lot of BR physiological functions previously reported in works that used exogenous BRs. For instance, the role of BRs in the regulation of CO_2 assimilation, proline and sugar production or their protective effect on the PSII complex under stress conditions can be mentioned. Simultaneously, the results from studies in which BRs were exogenously applied on plants growing in stress conditions were a good starting point for the production of agrochemicals that contain BRs – natural and biodegradable substances (Khripach 2010). Such agrochemicals could be useful for protecting cereal crops in changing climatic conditions. On the other hand, manipulating the endogenous BR levels or elements of its signalling pathways (classical breeding methods or genetic engineering) may help to obtain new cereal cultivars – dwarfs or those with a higher resistance to stress (Morinaka et al. 2006; Dockter et al. 2014).

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