Comprehensive Overview of the Brassinosteroid Biosynthesis Pathways: Substrates, Products, Inhibitors, and Connections

Andrzej Bajguz¹*, Magdalena Chmur¹ and Damian Gruszka²

¹ Faculty of Biology, University of Białystok, Białystok, Poland, ² Faculty of Natural Sciences, Institute of Biology, Biotechnology and Environmental Protection, University of Silesia, Katowice, Poland

Brassinosteroids (BRs) as a class of steroid plant hormones participate in the regulation of numerous developmental processes, including root and shoot growth, vascular differentiation, fertility, flowering, and seed germination, as well as in responding to environmental stresses. During four decades of research, the BR biosynthetic pathways have been well studied with forward- and reverse genetics approaches. The free BRs contain 27, 28, and 29 carbons within their skeletal structure: (1) 5α-cholestane or 26-nor-24α-methyl-5α-cholestane for C27-BRs; (2) 24α-methyl-5α-cholestane, 24β-methyl-5α-cholestanate or 24-methylene-5α-cholestanate for C28-BRs; (3) 24α-ethyl-5α-cholestanate, 24(Z)-ethyldene-5α-cholestanate, 25-methyl-5α-campestane or 24-methylene-25-methyl-5α-cholestanate for C29-BRs, as well as different kinds and orientations of oxygenated functions in A- and B-ring. These alkyl substituents are also common structural features of sterols. BRs are derived from sterols carrying the same side chain. The C27-BRs without substituent at C-24 are biosynthesized from cholesterol. The C28-BRs carrying either an α-methyl, β-methyl, or methylene group are derived from campesterol, 24-epicampesterol or 24-methylenecholesterol, respectively. The C29-BRs with an α-ethyl group are produced from sitosterol. Furthermore, the C29 BRs carrying methylene at C-24 and an additional methyl group at C-25 are derived from 24-methylene-25-methylcholesterol. Generally, BRs are biosynthesized via cycloartenol and cycloartanol dependent pathways. Till now, more than 17 compounds were characterized as inhibitors of the BR biosynthesis. For nine of the inhibitors (e.g., brassinazole and YCZ-18) a specific target reaction within the BR biosynthetic pathway has been identified. Therefore, the review highlights comprehensively recent advances in our understanding of the BR biosynthesis, sterol precursors, and dependencies between the C27-C28 and C28-C29 pathways.

Keywords: brassinazole, brassinolide, castasterone, inhibitors, mevalonate and nonmevalonate pathways, sterols
INTRODUCTION
Brassinosteroids (BRs) represent the sixth class of plant hormones. Since the discovery of brassinolide (BL) in 1979, about 70 naturally occurring compounds from this group have been reported as free molecules or conjugates with glucose and fatty acids. BRs are structurally very similar to androgens, estrogens, corticoids, and ecydosteroids. Their presence was reported both in lower and higher plants, especially in angiosperms; and also in all plant organs, including roots, stems, leaves, flowers, anthers, pollen, seeds, and grain (Bajguz and Tretyn, 2003; Yokota et al., 2017; Bajguz, 2019; Zullo and Bajguz, 2019). BRs play an essential role in the development and growth of plants. They elicit a broad spectrum of morphological and physiological responses as well as a tolerance against abiotic and biotic stress (Bajguz and Hayat, 2009; Bajguz and Piotrowska-Niczyporuk, 2014; Wei and Li, 2016; Wendeborn et al., 2017; Ahanger et al., 2018; Siddiqui et al., 2018; Nolan et al., 2020).

CHEMICAL STRUCTURE OF BRs
Based on the total number of carbons, BRs are divided into C27-, C28-, and C29-type. The basic structure of C27-BRs is a 5α-cholestane skeleton, C28-BRs: 5α-ergostane, and C29-BRs: 5α-stigmastane (Figure S1). Differences in the structure of these hormones are due to the type and orientation of oxygenated functions in the A- and B-ring, as well as the number and position of functional groups in the side chain of the molecule. These modifications arise during oxidation and reduction reactions. Based on the cholesterol (CR) side chain, BRs are divided by different substituents into C-23, C-24, C-25, 23-oxo, 24S-methyl, 24R-methyl, 24-methylene, 24S-ethyl, 24-ethylidene, 24-methylene-25-methyl, 24-methyl-25-methyl; without substituent at C-23, without substituent at C-24, and without substituents at C-23, C-24. BRs can also conjugate with glucose and fatty acids (Fujioka and Yokota, 2003; Bajguz, 2007; Piotrowska and Bajguz, 2011; Wendeborn et al., 2017; Ahanger et al., 2018). Two pathways for the synthesis of BRs are known: mevalonate (MVA) or non-MVA pathway (cycloartenol- and cycloartanol-dependent). So far, most of the reactions, enzymes, and genes were discovered and characterized by the C28- or C29-type of BR biosynthesis pathway (mostly in Arabidopsis thaliana, from which the majority of genes in this pathway were isolated). Their biosynthesis includes two major stages: biosynthesis of campesterol and 22α-hydroxycampesterol. The direct substrate of C27-BRs viz. cholesterol (CR) is finally converted to 28-norBL, whereas the biosynthesis of C29-BRs is initiated from β-sitosterol and leads to 28-homoBL. However, not all indirect compounds of this two pathways have been identified (Figure 1; Figure S1) (Fujioka et al., 2002; Kwon and Choe, 2005; Fujita et al., 2006; Ohnishi et al., 2006b; Chung and Choe, 2013; Roh et al., 2017; Kim et al., 2018; Rozhon et al., 2019).

Early Steps of BRs Biosynthesis
Biosynthesis of isopentenyl pyrophosphate (IPP), which is an indirect compound in the CR synthesis pathway, can occur via two pathways: non-MVA in lower plants and MVA in the most of higher plants. The initial compounds in non-MVA pathway are D-glyceraldehyde 3-phosphate and pyruvate, which are transformed into the 1-deoxy-D-xylulose 5-phosphate (DOXP) by the DOXP synthase. Then, DOXP is converted to 2-C-methyl-D-erythritol 4-phosphate (MEP) by the DOXP reductoisomerase. The next step leads to the formation of 4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol (CDP-ME) from MEP. The reaction is catalyzed by the CDP-ME synthase. Then, CDP-ME is converted to CDP-methyl-D-erythritol-2-phosphate by the CDP-ME kinase, and the obtained compound is transformed to 2-C-methyl-D-erythritol-2,4-cyclodiphosphate by the ME cyclodiphosphate synthase. Finally, with the action of reductases and dehydratases, the IPP is synthesized (Figure 1; Figure S1) (Lichtenhaler, 2000).

In the MVA pathway, three molecules of acetyl-CoA are combined to produce 3-hydroxy-3-methylglutaryl-CoA by the HMG-CoA synthase. The obtained compound is reduced to MVA by the HMG-CoA reductase. IPP is synthesized from MVA through the two indirect phosphorylation intermediates, such as MVA-phosphate and MVA-3-pyrophosphate (MVA-PP) (Mizierko, 2011). Enzymes involved in these reactions are the MVA kinase, phospho-MVA kinase, and MVA-3-phosphatase, respectively (Figure 1; Figure S1) (Wang et al., 2017).

Biochemical changes of IPP via geranyl pyrophosphate and farnesy1 pyrophosphate lead to the synthesis of squalene, which is oxidized to squalene-2,3-oxide via squalene epoxidase and the latter is converted to cycloartenol by the cycloartenol synthase. Cycloartenol is a key compound for the BR biosynthesis because it constitutes a substrate for multistep reactions in few pathways, which lead to the synthesis of C27-, C28-, or C29-BRs. Conversion of cycloartenol via cycloartenol and in a series of subsequent reactions to cholesterol/cholesterol and/or 6-oxocholestane leads to the synthesis of C27-BRs (Figure 1; Figure S1) (Wang et al., 2017).

Cycloartenol may also be a substrate of the C-24 methylation reaction, which is catalyzed by the sterol C-24 methyltransferase (SMT1), and leads to 24-methylcycloartenol. The next few reactions are catalyzed by C-4 sterol methyl oxidase (SMO1), cyclopropylsterol isomerase, obtusifoliol 14α-demethylase (CYP51), and sterol C-14 reductase, leading to the synthesis of 4α-methylgerostatrienol. Indirect products of these reactions are cycloecalenol and obtusifoliol. In next step, the sterol C-14 reductase which is encoded by the FACKEL/HYDRA2 gene catalyzes the reduction of 4α-methylgerostatrienol to 4α-methylgerostadienol, which is converted in the subsequent reaction to 24-methyleneophenol by the sterol 8,7 isomerase.
FIGURE 1 | Multistep reactions of brassinosteroids biosynthesis and their sterol biosynthetic precursors.
Biosynthesis of C_{27}-BRs

The C_{27}-BR biosynthesis pathway starts from the conversion of cycloartenol to cycloartenol (Figure 1; Figure S1) by the sterol side chain reductase 2 (SSR2) and proceeds through a synthesis of 31-norcycloartenol from cycloartenol by the C4-sterol methyl oxidase 3 (SMO3), and further biochemical changes of 31-norcycloartenol to cycloartenol (Sonawane et al., 2016). The reaction of 5α-cholesterol to cycloartenol (Roh et al., 2017).

Biosynthesis of C_{28}-BRs

24-methylenelophenol is a substrate of two independent pathways of sterol biosynthesis. The first leads to the biosynthesis of isocholesterol/β-sitosterol that are precursors of the C_{29}-BR biosynthesis (Xin et al., 2016); the second pathway, 24-converts methylenelophenol to campesterol, which is a substrate of the C_{28}-BR biosynthesis (Figure 1; Figure S1) (Choe, 2006; Sonawane et al., 2016).

The reaction is catalyzed by the C-4 sterol methyl oxidase 2 (SMO2) (Figure 1; Figure S1). Then, episterol is converted to 5-dehydroepisterol by the sterol C-5(6) desaturase encoded by the DWF7 gene (also known as SMO1), which is then converted to 24-methyleneCR (catalyzed by 7-dehydrocholesterol reductase encoded by the DWF5 gene) (Choe, 2006; Ohnishi, 2018). Further stages of the C_{28}-BR biosynthesis may proceed through two parallel pathways, called the late and early C-22 oxidation pathway. Reduction of 24-methyleneCR to campesterol initiates the late C-22 oxidation pathway and is catalyzed by the C-24(25)-sterol reductase in a two-step reaction in which 24-methyl-desmosterol is an intermediate (Dockter et al., 2014). The enzyme (also known as sterol side-chain reductase 1), which catalyzes the production of campesterol, is encoded by the DWFI gene. Campesterol is then transformed in the 5,4 isomerization reaction to (24R)-ergostan-4-en-3β-one. The latter is then converted through the DET2-mediated 5α-reduction to (24R)-5α-ergostan-3-one, which is transformed into campestanol (CN). In the parallel, early C-22 oxidation pathway, C-22α hydroxylation of 24-methyleneCR leads to the synthesis of 22-hydroxy-24-methyleneCR. The reaction of C-22α hydroxylation is catalyzed by the C-22α hydroxylase, which is encoded by the DWF4 gene. The enzyme belongs to the P450 cytochrome family (Fujiiyama et al., 2019). The next reactions are analogous to the late C-22 oxidation pathway and result in the synthesis of 22-hydroxy forms of the corresponding compounds.

However, an essential difference between the C-22 oxidation subpathways is the synthesis of 6-deoxocastasterone (6-deoxoCT) from (22S,24R)-22-hydroxy-5α-ergostan-3-one, without synthesis of campestanol (CN) (CN-independent pathway of BRs biosynthesis) as a result of the early C-22 oxidation.

On the other hand, in each stage of the late C-22 oxidation pathway, the compound can be hydroxylated by the C-22α hydroxylase into hydroxylated forms of early C-22 pathway (Choe, 2004; Ohnishi, 2018). Moreover, biochemical changes of 22-hydroxymethyleneCR can lead to the synthesis of 6-deoxodolichosterone, which may be further converted into dolichosterone (DS), and dolicholide (DL), as well as to castasterone (CS) and BL (Roh et al., 2017).

Campesterol may be a substrate of the BR biosynthesis in a parallel manner, both in late C-6 oxidation pathway (when hydroxylation of carbon atoms in the A-ring and both C-22 and C-23 positions of the side chain occurs before oxidation of C6) and early C-6 oxidation pathway (when hydroxylation takes place after oxidation of C6) (Figure 1; Figure S1) (Shimada et al., 2001). In A. thaliana both the early and late C6 oxidation pathways are functional (Fujiioka et al., 2000); however, the late C6 oxidation pathway plays a prominent role during morphogenesis, whereas the parallel early C6 oxidation dominates during skotomorphogenesis (Noguchi et al., 2000). Generally, the late C6 pathway is more prominent in plants (e.g., in potato, it is the only type of the C_{28}-BR biosynthesis). The late C6 pathway begins with hydroxylation of CN into the 6-deoxoCT by the 22α-hydroxylase. 6-deoxoCT may also be synthesized directly from 22-hydroxy5α-ergostan-3-one (the CN-independent pathway).

Then, 6-deoxoCT is hydroxylated through the C-23 hydroxylase
(encoded by the CPD gene) to the 6-deoxoteasterone, which is then C-3 oxidized into the 3-dehydro-6-deoxoteasterone (6-deoxo-3-DT) through the CYP90D3-C oxidase. In the next step, 6-deoxo-3-DT is converted to 6-deoxoTY. This reaction is catalyzed by the D11 CYP724B1 enzyme. Then, 6-deoxoTY is hyroxylated to 6-deoxoCS by the 2α-hydroxylase encoded by the DDWF1 gene. 6-deoxoCS is converted to castasterone (CS) (BR-6-oxidase1 and BR-6-oxidase2 catalyze the reaction). Then, CS is converted to BL via Baeyer-Villiger oxidation by the BR-6-oxidase2 (CYP85A2) (Choe, 2004; Choe, 2006; Vriet et al., 2013; Nakano and Asami, 2014; Ohnishi, 2018).

The early C6 oxidation pathway begins from hydroxylation of CN to 6α-hydroxyCN and its subsequent oxidation to 6-oxo-CN. The latter is transformed to CT by the 22α-hydroxylase. Cathasterone (CT) is converted in the consecutive reactions to teasterone (TE), 3-dehydroteasterone (3-DT), typhasterol (TY), cathasterone (CT) is converted in the consecutive reactions to deoxoCS by 6α-hydroxylase encoded by the DDWF1 gene. 6-deoxoCS is converted to castasterone (CS) (BR-6-oxidase1 and BR-6-oxidase2 catalyze the reaction). Then, CS is converted to BL via Baeyer-Villiger oxidation by the BR-6-oxidase2 (CYP85A2) (Choe, 2004; Choe, 2006; Vriet et al., 2013; Nakano and Asami, 2014; Ohnishi, 2018).

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Thus, it is most conceivable that all the biosynthetic pathways of BRs in plants are funneled into CS to carry out the relevant biological activities. It is known that the early C-6 oxidation pathways of C29-, C28-, and C29-BRs are commonly interrupted in plant tissues (Fujita et al., 2006; Joo et al., 2012; Joo et al., 2015; Kim et al., 2018). A recent study of barley (Hordeum vulgare) BR mutants indicated that the accumulation of 28-homoCS is inversely correlated with the accumulation of CS: mutants deficient in the biosynthesis of CS accumulate the highest concentrations of 28-homoCS, on the other hand, the BR-insensitive line, in which the highest concentration of CS was observed, accumulates the lowest concentration of 28-homoCS (Gruszka et al., 2016).

**Biosynthesis of C29-BRs**

The C29-BR biosynthesis is the least known and described route of BR biosynthesis (Figure 1; Figure S1). In this pathway 24-methylenelophenol is converted by sterol methyltransferase 2 (SMT2) into 24-ethylidenelophenol that is transformed into avenasterol by the 22α-hydroxylase. Cathasterone (CT) is converted in the consecutive reactions to teasterone (TE), 3-dehydroteasterone (3-DT), typhasterol (TY), castasterone (CS), and BL, respectively (Shimada et al., 2001; Fujioka et al., 2002; Kwon and Choe, 2005; Ohnishi et al., 2006a; Ohnishi et al., 2006b; Lee et al., 2010; Lee et al., 2011; Zhao and Li, 2012; Chung and Choe, 2013; Joo et al., 2015; Kim et al., 2018; Ohnishi, 2018; Roh et al., 2020).

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**Links Between C27-C28 and C28-C29 Pathways**

The C27-BR biosynthetic pathway links with the C28 pathway through the following reactions: 28-norTE → TE, 28-nor-3-DT → 3-DT, 28-norTY → TY, and 28-norCS → CS. On the other hand, C29-BRs conversion to C28-BRs occurs through the following reactions: 28-homoTE → TE, 28-homoTY → TY, 28-homoCS → CS, 28-homoDS → DS, and 28-homoDS → DS → CS. Therefore, it is suggested that the biosynthetic connection of C27- and C29-BRs with C28-BRs occurs mainly between the end products of the pathways. Five pathways are biosynthetically connected to produce CS, an active BR, in plants (Figure 1; Figure S1). Direct substrates to synthesize CS are: 28-norCS, DS, 6-deoxoCS by 6α-hydroxyCS, 28-homoDS, and 28-homoCS. Thus, it is most conceivable that all the biosynthetic pathways of BRs in plants are funneled into CS to carry out the relevant biological activities. It is known that the early C-6 oxidation pathways of C27-, C28-, and C29-BRs are commonly interrupted in plant tissues (Fujita et al., 2006; Joo et al., 2012; Joo et al., 2015; Kim et al., 2018). A recent study of barley (Hordeum vulgare) BR mutants indicated that the accumulation of 28-homoCS is inversely correlated with the accumulation of CS: mutants deficient in the biosynthesis of CS accumulate the highest concentrations of 28-homoCS, on the other hand, the BR-insensitive line, in which the highest concentration of CS was observed, accumulates the lowest concentration of 28-homoCS (Gruszka et al., 2016).

**INHIBITORS OF BR BIOSYNTHESIS**

Inhibitors are tools useful not only for investigating biosynthetic pathways, but also for manipulating the BR level in crop plants. Till now, 17 inhibitors (KM-01, brassinozole (Brz), Brz2001, Brz220, propiconazole, YCZ-18, yucaizol, fenarimol, spironolactone, triadimefon, imazalil, 4-MA, VG106, DSMEM21, fnastrade, AFA76, and brassinopride) have been discovered (Figure 2), however, the site of action of only nine compounds is known. The sites of action of inhibitors are as follows:

- campestanol → 6-deoxoCT for brassinazole, Brz2001, Brz220, triadimefon, and spironolactone;
- 6-deoxoCT → 6-deoxoTE for brassinazole, Brz2001, Brz220, propiconazole, and fenarimol;
- 6-deoxoTE → 6-deoxo-3DT for YCZ-18, yucaizol, propiconazole, and fenarimol;
- 6-oxocampestanol → CT for brassinazole, Brz2001, Brz220, and triadimefon;
- CT → TE for brassinazole, Brz2001, Brz220, propiconazole, and fenarimol;
- TE → 3DT for YCZ-18, yucaizol, propiconazole, and fenarimol (Rozhon et al., 2019) (Figure 1; Figure S1).

The first reported BR inhibitor, i.e., KM-01 was isolated from a microbial culture medium. It inhibited BR activity in a rice lamina inclination test. Despite the unclear site of action, KM-01 exhibits highly potent activity (Kim et al., 1994; Kim et al., 1995; Kim et al., 1998). However, brassinazole (Brz) represents the first specific BR biosynthesis inhibitor, which blocks the conversion of campestanol to 6-deoxoCT, 6-deoxoCT to 6-deoxoTE, 6-oxocampestanol to CT, and CT to TE in the BR biosynthetic pathways. Brz2001 is a modified form of Brz containing an allyl moiety instead of the methyl group. Both inhibitors block the
FIGURE 2 | Inhibitors of sterol and brassinosteroid biosynthesis. Numbered inhibitors have a known site of action presented in Figure 1.
same reactions (Asami and Yoshida, 1999; Asami et al., 2000; Asami et al., 2001; Asami et al., 2003b). Brz and Brz2001 can induce morphological changes, including dwarfism, altered leaf color, and curling in de-etiolated barley (Sekimata et al., 2001). Brz decreased the level of BRs in the barley leaves, but not in roots. The inhibition effect of Brz on plant growth is reversed by exogenous BR (Bajguz and Asami, 2004; Bajguz and Asami, 2005; Bajguz et al., 2019).

Propiconazole, a triazole compound, also affects similar to Brz (Hartwig et al., 2012). Another triazole-type BR biosynthesis inhibitors, YCZ-18, and yucaizol, bind to the CYP90D1 enzyme and inhibit the BR-induced cell elongation. However, only BL negates the inhibitory effect of YCZ-18 or yucaizol. Therefore, it was suggested that they function differently from Brz (Oh et al., 2015a; Oh et al., 2015b). Fenarimol is known for inhibiting cytochrome P450 monoxides involved in 14α-demethylation during the biosynthesis of ergosteryl. Simultaneously, it inhibits the conversion of CT to TE, and evokes the phenotype of BR-deficient mutants with short hypocotyls, de-etiolate dark-grown seedlings, and dark green downward curled leaves of light-grown A. thaliana (Wang et al., 2001; Oh et al., 2015a). Plants treated with triadimeton show reduced elongation of stems and petioles, dark green and thicker leaves, delayed senescence, and increased expression levels of the CPD gene. The phenotypes could be recovered with CT, TE, TY, CS, and BL (Asami et al., 2003a). On the other hand, imazalil causes severe hypocotyl shortening in A. thaliana, which could be reversed by the application of 24-epibrassinolide (Werbrouck et al., 2003). Seedlings of A. thaliana treated with spironolactone showed dark, downward curled leaves, and shortened hypocotyls, which could be reversed by BL application (Asami et al., 2004). Although A. thaliana mutants viz. cd; det-2-1, or cbb1 treated with brassinopride enhanced apical hook formation, the normal phenotype was recovered by BL (Gendron et al., 2008). Voriconazole, fluconazole, and fenpropimorph (Figure 2) inhibit cycloeucalenol-obtusifoliol isomerase and have a reductive impact on BRs level (Rozhon et al., 2013; Rozhon et al., 2019).

REFERENCES
Ahanger, M. A., Ashraf, M., Bajguz, A., and Ahmad, P. (2018). Brassinosteroids regulate growth in plants under stressful environments and crosstalk with other potential phytohormones. J. Plant Growth Regul. 37, 1007–1024. doi: 10.1007/s4334-018-9855-2
Asami, T., and Yoshida, S. (1999). Brassinosteroid biosynthesis inhibitors. Trends Plant Sci. 4, 348–353. doi: 10.1016/S1360-1385(99)01456-9
Asami, T., Min, Y. K., Nagata, N., Yamagishi, K., Takatsuto, S., Fujioka, S., et al. (2000). Characterization of brassinazole, a triazole-type brassinosteroid biosynthesis inhibitor. Plant Physiol. 123, 93–99. doi: 10.1104/pp.123.1.93
Asami, T., Mizutani, M., Fujioka, S., Goda, H., Min, Y. K., Shimada, Y., et al. (2001). Selective interaction of triazole derivatives with DWF4, a cytochrome P450 monooxygenase of the brassinosteroid biosynthetic pathway, correlates with brassinosteroid deficiency in planta. J. Biol. Chem. 276, 25687–25691. doi: 10.1074/jbc.M103524200
Asami, T., Mizutani, M., Shimada, Y., Goda, H., Kitahata, N., Sekimata, K., et al. (2003a). Triadimeton, a fungicidal triazole-type P450 inhibitor, induces brassinosteroid deficiency-like phenotypes in plants and binds to DWF4 protein in the brassinosteroid biosynthesis pathway. Biochem. J. 369, 71–76. doi: 10.1042/BJ20028835
Asami, T., Nakano, T., Nakashita, H., Sekimata, K., Shimada, Y., and Yoshida, S. (2003b). The influence of chemical genetics on plant science: Shedding light on functions and mechanism of action of brassinosteroids using biosynthesis inhibitors. J. Plant Growth Regul. 22, 336–349. doi: 10.1007/s4334-003-0065-0
Asami, T., Oh, K., Jikumaru, Y., Shimada, Y., Kaneko, I., Nakano, T., et al. (2004). A mammalian steroid action inhibitor spironolactone retards plant growth by inhibition of brassinosteroid action and induces light-induced gene expression in the dark. J. Steroid Biochem. 91, 41–47. doi: 10.1016/j.jsbmb.2004.01.011
Bajguz, A., and Asami, T. (2004). Effects of brassinazole, an inhibitor of brassinosteroid biosynthesis, on light- and dark-grown Chlorella vulgaris. Planta 218, 869–877. doi: 10.1007/s00425-003-1170-9

SUPPLEMENTARY MATERIAL
The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2020.01034/full#supplementary-material

SUPPLEMENTARY FIGURE 1 | Multistep reactions of brassinosteroids biosynthesis and their sterol biosynthetic precursors.

AUTHOR CONTRIBUTIONS
AB and MC prepared a draft of figures and text. AB and DG corrected and finalized the review.

Bajguz, A., and Asami, T. (2005). Suppression of Wolfia arrhiza growth by brassinazole, an inhibitor of brassinosteroid biosynthesis and its restoration by endogenous 24-epibrassinolide. Phytochemistry 66, 1787–1796. doi: 10.1016/j.phytochem.2005.06.005
Bajguz, A., and Hayat, S. (2009). Effects of brassinosteroids on the plant responses to environmental stresses. Plant Physiol. Bioch. 47, 1–8. doi: 10.1016/j.plaphy.2008.10.002
Bajguz, A., and Piotrowska-Niczyporuk, A. (2014). “Brassinosteroids implicated in growth and stress responses,” in Phytohormones: A Window to Metabolism, Signaling and Biotechnological Applications. Eds. L.-S. P. Tran and S. Pal (New York, NY: Springer), 163–190.
Bajguz, A., and Tretyn, A. (2003). The chemical characteristic and distribution of brassinosteroids in plants. Phytochemistry 62, 1027–1046. doi: 10.1016/S0031-1872(02)00656-8
Bajguz, A., Orczyk, W., Golebiowska, A., Chmur, M., and Piotrowska-Niczyporuk, A. (2019). Occurrence of brassinosteroids and influence of 24-epibrassinolide with brassinazole on their content in the leaves and roots of Hordeum vulgare L. cv. Golden Promise. Plant. Sci. 249, 123–137. doi: 10.1016/j.plaphy.2018.03.081-3
Bajguz, A. (2007). Metabolism of brassinosteroids in plants. Plant Physiol. Bioch. 45, 95–107. doi: 10.1016/j.plaphy.2007.01.002
Bajguz, A. (2019). “Brassinosteroids in microalgae: Application for growth improvement and protection against abiotic stresses,” in Brassinosteroids: Plant Growth and Development. Eds. S. Hayat, M. Yusuf, R. Bhardwaj and A. Bajguz (Singapore: Springer Singapore), 45–58.
Choe, S. (2004). “Brassinosteroid biosynthesis and metabolism,” in Plant Hormones: Biosynthesis, Signal Transduction, Action! Ed. P. J. Davies (Dordrecht: Kluwer Academic Publishers), 156–178.
Choe, S. (2006). Brassinosteroid biosynthesis and inactivation. Physiol. Plant. 126, 539–548. doi: 10.1111/j.1399-3054.2006.00681.x
Chung, Y., and Choe, S. (2013). The regulation of brassinosteroid biosynthesis in Arabidopsis. Crit. Rev. Plant Sci. 32, 396–410. doi: 10.1080/07352689.2013.797856
Dockter, C., Gruszka, D., Braumann, I., Druka, A., Druka, I., Franckowiak, J., et al. (2014). Induced variations in brassinosteroid genes define barley height and
sturdiness, and expand the green revolution genetic toolkit. *Plant Physiol.* 166, 1912–1927. doi: 10.1104/pp.114.250738

Fujisaki, S., and Yokota, T. (2003). Biosynthesis and metabolism of brassinosteroids. *Annu. Rev. Plant Biol.* 54, 137–164. doi: 10.1146/annurev.arplant.54.031902.134921

Fujisaki, S., Noguchi, T., Watanabe, T., Takatsuto, S., and Yoshida, S. (2000). Biosynthesis of brassinosteroids in cultured cells of *Catharanthus roseus*. *Phytochemistry* 53, 549–553. doi: 10.1016/S0031-1872(99)00582-8

Fujisaki, S., Takatsuto, S., and Yoshida, S. (2002). An early C-22 oxidation branch in the brassinosteroid biosynthetic pathway. *Plant Physiol.* 130, 930–939. doi: 10.1104/pp.008722

Fujita, S., Ohnishi, T., Watanabe, B., Yokota, T., Takatsuto, S., Fujisaki, S., et al. (2006). Arabidopsis CYP90B1 catalyses the early C-22 hydroxylation of C27, C28 and C29 sterols. *Plant J.* 45, 765–774. doi: 10.1111/j.1365-3137.2005.02639.x

Fujiyama, K., Hino, T., Kanadani, M., Watanabe, B., Lee, H. J., Mizutani, M., et al. (2019). Structural insights into a key step of brassinosteroid biosynthesis and its inhibition. *Nat. Plants* 5, 589–594. doi: 10.1038/s41477-019-0436-6

Gendron, J. M., Haque, A., Gendron, N., Chang, T., Asami, T., and Wang, Z. Y. (2008). Chemical genetic dissection of brassinosteroid-ethylene interaction. *Mol. Plant* 1, 368–379. doi: 10.1093/mp/san005

Gruzda, D., Janeczko, A., Dzurak, M., Pociucha, E., Oldekostka, J., and Szerzejko, I. (2016). Barley brassinosteroid mutants provide an insight into phytohormonal homeostasis in plant reaction to drought stress. *Front. Plant Sci.* 7, 1824. doi: 10.3389/fpls.2016.01824

Hartwig, T., Corvalan, C., Best, N. B., Budka, J. S., Zhu, J. Y., Choe, S., et al. (2012). Propionocoenzyme is a specific and accessible brassinosteroid (BR) biosynthesis inhibitor for *Arabidopsis* and maize. *PloS One* 7, e36625. doi: 10.1371/journal.pone.0036625

Joo, S. H., Kim, T. W., Son, S. H., Lee, W. S., Yokota, T., and Kim, S. K. (2012). Biosynthesis of a cholesterol-derived brassinosteroid, 28-norcastasterone, in *Drechslera avenae*. *Tetrahedron Lett.* 53, 1731–1734. doi: 10.1016/j.tetlet.2012.04.039

Joo, S. H., Jang, M. S., Kim, M. K., Lee, J.-E., and Kim, S.-K. (2015). Biosynthesis and metabolism of dolichosterone in *Catharanthus roseus*. Establishment of biosynthetic pathways to generate castasterone as the first committed intermediate. *Plant Cell* 37, 138–2047. doi: 10.1104/pp.124.1.201

Kushiro, M., Nakano, T., Sato, K., Yamagishi, K., Asami, T., Nakano, A., et al. (2019). Recent advances in brassinosteroid biosynthetic pathway: insight into novel brassinosteroid shortcut pathway. *Plant Cell* 31, 335-348. doi: 10.1105/tpc.19.00335

Ohnishi, T., Schaller, H., Goh, C. H., Kwon, M., Choe, S., An, C. S., et al. (2005). Arabidopsis CYP90B1 catalyses the early C-22 hydroxylation of C27, C28 and C29 sterols. *Plant J.* 45, 765–774. doi: 10.1111/j.1365-3137.2005.02639.x

Oh, K., Matsumoto, T., Yamagami, A., Hoshi, T., Nakano, T., and Yoshizawa, Y. (2015a). Fanenilom, a Pyridimine-Type Fungicide, Inhibits Brassinosteroid Biosynthesis. *Int. J. Mol. Sci.* 16, 17273–17288. doi: 10.3390/ijms16081727

Oh, K., Matsumoto, T., Yamagami, A., Ogawa, A., Yamada, K., Suzuki, R., et al. (2015b). YCZ-18 Is a New Brassinosteroid Biosynthesis Inhibitor. *PloS One* 10, e0120812. doi: 10.1371/journal.pone.0120812

Ohnishi, T., Satsunami, A. M., Watanabe, B., Fujita, S., Bancos, S., Koncz, C., et al. (2006a). C-23 hydroxylation by Arabidopsis CYP90C1 and CYP90D1 reveals a novel shortcut in brassinosteroid biosynthesis. *Plant Cell* 18, 3275–3288. doi: 10.1105/tpc.106.045443

Ohnishi, T., Watanabe, B., Sakata, K., and Mizutani, M. (2006b). CYP724B2 and CYP724B3 function in the early C-22 hydroxylation steps of brassinosteroid biosynthetic pathway in tomato. *Biocsi. Biotech. Bioch.* 70, 2071–2080. doi: 10.1217/bb.60.03

Ohnishi, T. (2018). Recent advances in brassinosteroid biosynthetic pathway: insight into novel brassinosteroid shortcut pathway. *J. Pestic. Sci.* 43, 159–167. doi: 10.1584/psticides.D18-040

Piotrowska, A., and Bajguz, A. (2011). Conjugates of abscisic acid, brassinosteroids, ethylene, gibberellins, and jasmonates. *Phytochemistry* 72, 2097–2112. doi: 10.1101/phytochemistry.2011.08.012

Roh, J., Yeom, H. S., Jang, H., Kim, S., Youn, J. H., and Kim, S. K. (2017). Identification and biosynthesis of C-24 ethylidene brassinosteroids in *Arabidopsis thaliana*. *Plant Biol.* 60, 533–538. doi: 10.1111/j.1365-3102.2016.05360

Rohzon, W., Akter, S., Fernandez, A., and Poppenberger, B. (2019). Inhibitors of brassinosteroid biosynthesis and signal transduction. *Molecules* 24, 4372. doi: 10.3390/molecules24234372

Schneider, B. (2002). “Pathways and enzymes of brassinosteroid biosynthesis,” in *Prog Bot*. Eds. K. Esser, U. Littge, W. Breysslagh and F. Hellwig (Berlin, Heidelberg: Springer-Verlag), 286–306.

Sekimoto, K., Kimura, T., Kaneko, I., Nakano, T., Yoneyama, K., Takeuchi, Y., et al. (2001). A specific brassinosteroid biosynthesis inhibition inhibitor, Brz2001: evaluation of its effects on *Arabidopsis*, cress, tobacco, and rice. *Planta* 213, 716–721. doi: 10.1007/s00428-000-05546

Shimada, Y., Fujioka, S., Miziorko, H. M. (2011). Enzymes of the mevalonate pathway of isoprenoid biosynthesis. *Int. J. Mol. Sci.* 12, 4534-4559. doi: 10.3390/ijms12045344

Shimada, Y., Fujioka, S., Miyauchi, N., Kushiro, M., Takatsuto, S., Nomura, T., et al. (2000). Engineering a key step of brassinosteroid biosynthesis and signal transduction. *Mol. Plant–Mol. Biol.* 33, 16205. doi: 10.1038/nplants.2016.205
Vriet, C., Russinova, E., and Reuzeau, C. (2013). From Squalene to Brassinolide: The Steroid Metabolic and Signaling Pathways across the Plant Kingdom. *Mol. Plant* 6, 1738–1757. doi: 10.1093/mp/stt096

Wang, J. M., Asami, T., Yoshida, S., and Murofushi, N. (2001). Biological evaluation of 5-substituted pyrimidine derivatives as inhibitors of brassinosteroid biosynthesis. *Biosci. Biotech. Bioch.* 65, 817–822. doi: 10.1271/bbb.65.817

Wang, H., Wei, Z., Li, J., and Wang, X. (2017). “Brassinosteroids,” in *Hormone Metabolism and Signaling in Plants*. Eds. J. Li, C. Li and S. M. Smith (London: Academic Press), 291–326.

Wei, Z. Y., and Li, J. (2016). Brassinosteroids regulate root growth, development, and symbiosis. *Mol. Plant* 9, 86–100. doi: 10.1016/j.molp.2015.12.003

Wendeborn, S., Lachia, M., Jung, P. M. J., Leipner, J., Brocklehurst, D., De Mesmaeker, A., et al. (2017). Biological activity of brassinosteroids - direct comparison of known and new analogs in planta. *Helv. Chim. Acta* 100, e1600305. doi: 10.1002/hlca.201600305

Werbrouck, S. P. O., Saibo, N. J. M., Dhuyvetter, H., De Schepper, S., Van Der Straeten, D., and Debergh, P. C. (2003). Physiological and morphological evidence of brassinosteroid-biosynthesis inhibition by the fungicide imazalil. *Physiol. Plant* 119, 69–77. doi: 10.1034/j.1399-3054.2003.00155.x

Xin, P. Y., Yan, J. J., Li, B. B., Fang, S., Fang, J. S., Tian, H. L., et al. (2016). A comprehensive and effective mass spectrometry-based screening strategy for discovery and identification of new brassinosteroids from rice tissues. *Front. Plant Sci.* 7, 1786. doi: 10.3389/Fpls.2016.01786

Yokota, T., Okuishi, T., Shibata, K., Asahina, M., Nomura, T., Fujita, T., et al. (2017). Occurrence of brassinosteroids in non-flowering land plants, liverwort, moss, lycophyte and fern. *Phytochemistry* 136, 46–55. doi: 10.1016/j.phytochem.2016.12.020

Zhao, B. L., and Li, J. (2012). Regulation of brassinosteroid biosynthesis and inactivation. *J. Integr. Plant Biol.* 54, 746–759. doi: 10.1111/j.1744-7909.2012.01168.x

Zullo, M. A. T., and Bajguz, A. (2019). *Brassinosteroids: Plant Growth and Development*. Eds. S. Hayat, M. Yusuf, R. Bhardwaj and A. Bajguz (Singapore: Springer Singapore), 1–44.

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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