

# Brassinosteroids – A new class of phytohormones

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**Brassinosteroids are a new group of plant hormones with significant growth-promoting activity. Brassinosteroids were first isolated and characterized from the pollen of rape plant, *Brassica napus* L. Subsequently, they have so far been reported from 44 plants and are regarded probably ubiquitous in the plant kingdom. Brassinosteroids are considered as hormones with pleiotropic effects, as they influence varied developmental processes like growth, germination of seeds, rhizogenesis, flowering and senescence. Brassinosteroids also confer resistance to plants against various abiotic stresses. The article reviews the work relating to their discovery, distribution, physiological functions and economic importance. In addition, the case of brassinosteroids as the sixth group of phytohormones is discussed taking into account how they satisfy the basic features of plant hormones and evidences that they are essential for plant growth and development.**

PLANT growth is a complex, yet well organized and coordinated process. As early as 1880, Julius Sachs<sup>1</sup> suspected the existence of 'chemical messengers' which bring co-ordination of growth among different parts of the plant. However, the real impetus to this line of thinking came from the publication of a book, *The Power of Movements in Plants* by Charles Darwin<sup>2</sup>, in which he incorporated some of the observations made by him along with his son Francis Darwin on phototropic movements in the coleoptiles of canary grass. The book served as a springboard to an exciting line of research, which led to the identification of hormones in plants. While metabolism provides the power and building blocks for plant life, it is the hormones that regulate the pace of growth of individual parts and integrate these parts to produce the form that we recognize as a plant<sup>3</sup>. Until quite recently, plant growth and development was thought to be regulated only by five groups of hormones, namely auxins, gibberellins, cytokinins, abscisic acid and ethylene. However, there are compelling evidences for considering brassinosteroids, a group of steroidal substances first isolated from the pollen of rape plant (*Brassica napus* L.), as the sixth group of phytohormones.

## Discovery

The recognition that certain pollen extracts cause growth promotion paved the way for the discovery of brassinosteroids in plants. In 1970, Mitchell and co-workers<sup>4</sup> screened pollen from nearly sixty species and about half of them caused growth of bean seedlings. The growth-promoting substances from various pollen sources were named as 'brassins'. In 1974, a collective effort to identify 'the active factor(s)' of brassins was initiated by USDA scientists at the Northern Regional Research Center (NRRC), Peoria, the Eastern Regional Research Center (ERRC), Philadelphia and the Beltsville Agricultural Research Center (BARC), Maryland. About 227 kg (500 lb) of bee-collected rape (*B. napus*) pollen was processed through a pilot-plant-size solvent (2-propanol) extraction procedure at ERRC and partially purified at BARC. Four mg of crystals was obtained at NRRC and was subjected to X-ray crystallographic analysis to determine the structure. This biologically active plant growth promoter was named as 'brassinolide' and was found to be a steroidal lactone with an empirical formula of  $C_{28}H_{48}O_6$  (MW = 480).

In 1982, another steroidal substance with growth-promoting nature was isolated from the insect galls of chestnut (*Castanea crenata*)<sup>5</sup> and named as castasterone. The discovery of brassinolide and castasterone gave an impetus to the idea of the presence of steroidal hormones (till then considered as a monopoly of the animal system) with growth-promoting nature in the plant kingdom. Since then, extensive studies on isolation and identification of brassinolide and its related compounds from various sources have been undertaken. As the first steroidal hormone with growth-promoting nature was obtained from *B. napus*, the name 'brassinosteroids' was given to this new class of substances.

Brassinosteroids are a group of naturally occurring polyhydroxy steroids. Natural brassinosteroids so far identified have a common 5 $\alpha$ -cholestan skeleton, and their structural variations come from the kind and orientation of functionalities on the skeleton<sup>6</sup>. The compounds can be classified as  $C_{27}$ ,  $C_{28}$  or  $C_{29}$  brassinosteroids depending on the alkyl-substitution pattern of the side chain<sup>7</sup>. Till now, 42 brassinosteroids and four brassinosteroid conjugates have been characterized<sup>8</sup>. A sequential numerical suffix designates the brassinosteroids occurring in nature. BR1 denotes brassinolide and

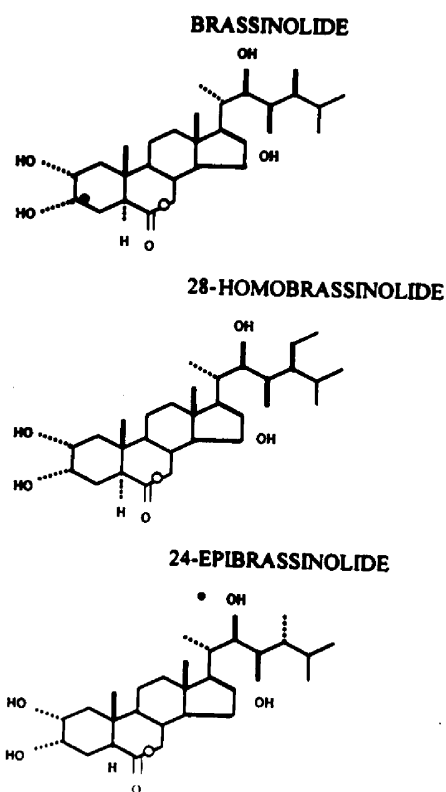
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others follow the sequence BR2, BR3, BR4 ... BR<sub>n</sub>. However all the brassinosteroids are not always biologically active<sup>9</sup>. Brassinolide, 24-epibrassinolide and 28-homobrassinolide (Figure 1) are the three biologically active brassinosteroids, being widely used in physiological studies.

## Distribution

As of now, brassinosteroids have been characterized from 44 plant species, which include 37 angiosperms (nine monocots and 28 dicots), five gymnosperms, one pteridophyte and one alga<sup>8</sup>. As brassinosteroids are observed in all the plants tested so far, Sasse<sup>10</sup> suggested that brassinosteroids are probably ubiquitous in the plant kingdom. Some of the plants and their parts in which brassinosteroids are reported are given in Table 1.

Brassinosteroids are present in plants at extremely low concentrations (nano-gram levels). Levels of endogenous brassinosteroids vary among plant tissues. Young growing tissues contain higher levels of brassinosteroids than mature tissues<sup>11</sup>. Pollen and immature seeds are the richest sources with a range of 1–100 ng per g fresh weight, while shoots and leaves usually have lower amounts, i.e. 0.01–0.1 ng per g fresh weight<sup>12</sup>.



**Figure 1.** Chemical structure of brassinosteroids.

**Table 1.** Distribution of brassinosteroids in the plant kingdom

Plant part	Plant species
Pollen	<i>Helianthus annuus</i> , <i>Alnus glutinosa</i> , <i>Brassica napus</i> , <i>Robinia pseudo-acacia</i> , <i>Vicia faba</i> , <i>Fagopyrum esculentum</i> , <i>Citrus unshiu</i> , <i>Citrus sinensis</i> , <i>Cupressus arizonica</i> , <i>Pinus thunbergii</i> , <i>Cryptomeria japonica</i>
Seed	<i>Gypsophila perfoliata</i> , <i>Beta vulgaris</i> , <i>Pharbitis purpurea</i> , <i>Brassica campestris</i> , <i>Raphanus sativus</i> , <i>Cassia tora</i> , <i>Lablab purpureus</i> , <i>Ornithopus sativus</i> , <i>Phaseolus vulgaris</i> , <i>Pisum sativum</i> , <i>Vicia faba</i> , <i>Cannabini sativa</i> , <i>Apium graveolens</i>
Shoot	<i>Arabidopsis thaliana</i> , <i>Ornithopus sativus</i> , <i>Pisum sativum</i> , <i>Lycopersicon esculentum</i>
Leaf	<i>Castanea crenata</i> , <i>Distylium recemosus</i> , <i>Thea sinensis</i>
Others	
Cultured cell	<i>Catharanthus roseus</i>
Panicle	<i>Rheum rhabarum</i>
Cambial region	<i>Cryptomeria japonica</i>
Gall	<i>Castanea crenata</i>
Strobilus	<i>Equisetum arvense</i>
Thallus	<i>Hydrodictyon reticulatum</i>

Based on Fujioka<sup>8</sup>.

## Are brassinosteroids phytohormones?

In addition to the five established groups of phytohormones, there are several other classes of compounds which are known to regulate plant growth and development. They include phenolics<sup>13,14</sup>, polyamines<sup>15</sup>, methyl jasmonates<sup>16,17</sup> and brassinosteroids<sup>18</sup>. Among these substances, however, there is consensus among scientists to consider brassinosteroids as the sixth class of phytohormones. It is appropriate to discuss to what extent brassinosteroids fulfil the conditions laid down to be considered as phytohormones.

A textbook definition for a plant hormone is as follows: *A plant hormone is an organic compound synthesized in one part of a plant and translocated to another part, where in very low concentration it causes a physiological response*<sup>19</sup>. Salisbury<sup>20</sup> further elaborated this definition with an emphasis to distinguish hormones with the other biomolecules present in the plant system. Taking this elaboration into account, the following points are given to support the case of brassinosteroids for inclusion in the list of phytohormones:

As mentioned earlier brassinosteroids are reported in all plants tested so far (nine monocots, 28 dicots, five gymnosperms, one pteridophyte and one alga). Based on this, Sasse<sup>10</sup> suggested that brassinosteroids are probably ubiquitous in the plant kingdom. Ubiquity in the plant kingdom is considered as one of the prerequisites to consider a substance as a plant hormone.

Brassinosteroids are present in the plants in extremely low concentrations. The pollen and immature

seeds contain 1–100 ng per g fresh weight of brassinosteroids, while shoots and leaves possess still lower amounts in the range of 0.01–0.1 ng per g fresh weight<sup>12</sup>, which is a testimony to consider brassinosteroids as phytohormones.

Brassinosteroids were found physiologically active in very low concentrations. Brassinolide is active at 0.01 µg concentration in bean second-internode test and at 0.0005 µg concentration in rice lamina inclination assay.

Brassinosteroids are highly mobile in the plant system. Exogenously applied brassinolide to roots of intact young tomato and radish plants affected the hypocotyls and petioles<sup>21</sup>. The treatment of the bases of mung bean hypocotyls caused elongation of epicotyls<sup>22</sup>. These studies clearly demonstrated the mobility of brassinosteroids in the plant system. Though there are reservations among scientists to retain the concept of 'translocation' in the definition of plant hormones<sup>20</sup>, brassinosteroids still satisfy the 'translocation' property originally attributed to plant hormones.

The studies conducted with brassinosteroid-deficient and insensitive mutants provide convincing evidence that brassinosteroids are essential for plant growth and development. Brassinosteroid biosynthetic mutants such as *br1* (ref. 23), *cpd1* (ref. 24), *dwf1* (ref. 25) have been identified among de-etiolated dwarfs of *Arabidopsis thaliana*. The growth of mutants was found to be restored by exogenous application of brassinolide and not by gibberellin or IAA<sup>26</sup>. The dwarf mutant *lkb* of *Pisum sativum* was shown to be BR-deficient and application of brassinolide restored normal growth<sup>27</sup>. In another dwarf mutant of pea, *lk*, the endogenous level of castasterone was less than 25% that of the wild type. The dwarf tomato mutant, dumpy (*dpy*) is an intermediate dwarf, displaying a curled leaf phenotype with dark rugose leaves and suppressed axillary shoots. Exogenous application of brassinolide completely rescued the phenotype to wild type<sup>28</sup>. Another brassinosteroid-deficient tomato mutant is the *dwarf* (*d*) mutant. It has been shown that the DWARF protein, the product of the dwarf gene (*D*) is responsible for the conversion of 6-deoxacastasterone to castasterone<sup>29</sup>. Tomato mutant *curl-3* (*cu-3*) has shown to be brassinosteroid-insensitive<sup>30</sup>. The application of brassinazole, a specific inhibitor of brassinolide biosynthesis, to cress (*Lepidium sativum*) plants resulted in dwarfism and exogenous application of brassinolide reversed the dwarfism<sup>31</sup>, indicating the essentiality of brassinosteroids to plant growth and development.

## Bioassays

Two bioassays which are highly specific and sensitive to brassinosteroids were developed. They are (i) bean

second internode test, and (ii) rice lamina inclination test.

The bean second internode test was developed during the first isolation of brassinolide from the pollen of rape<sup>32</sup>. Cuttings of the second internode from seedlings of *Phaseolus vulgaris* L. cv Pinto, when treated with brassinolide in lanolin paste, showed elongation, curvature, swelling or splitting<sup>33</sup>. The effect depended on the amount of brassinolide. Brassinolide caused elongation, curvature and swelling at lower concentration of 0.01 µg and splitting (Figure 2) at a higher concentration of 0.1 µg.

The rice lamina inclination test, originally developed as an auxin bioassay<sup>34</sup>, was found to be highly responsive to brassinosteroids. From etiolated seedlings of rice, segments consisting of the second leaf lamina, lamina joint and sheath were excised and floated on distilled water containing brassinosteroids. Bending of the lamina joint was observed, which was proportional to the concentration of the compounds applied. Brassinolide showed activity at a concentration of 0.005 µg/ml, but indoleacetic acid (IAA) showed only weak activity at 50 µg/ml. Because of such great difference in dose response in brassinosteroids and IAA, this test is considered more specific to brassinosteroids and is widely used to investigate the occurrence of brassinosteroids.

## Physiological functions

Brassinosteroids are plant hormones with significant growth-promoting activity. In addition to growth promotion, brassinosteroids also influence various other developmental processes like seed germination, rhizogenesis, flowering, senescence, abscission and maturation. Brassinosteroids also confer resistance to plants against various abiotic stresses. Due to multiple effects,



**Figure 2.** Brassinolide-induced splitting of second internode of bean. (From N. Bushan Mandava, with permission).

brassinosteroids are considered as plant hormones with pleiotropic effects<sup>10</sup>. Work on the influence of brassinosteroids on various growth and development processes is presented below.

### Growth

Stimulation of growth is considered as the important physiological role of brassinosteroids in plants. The initial studies with brassinolide were concentrated around its ability to induce cell elongation, swelling, curvature and splitting of the second internode and such activity is called 'brassin activity'. Elongation, curvature and splitting occurred when 0.01 mg of brassinolide was applied; even 0.01 µg induced splitting<sup>34</sup>.

Brassinosteroids are highly effective in stimulating growth in young vegetative tissues<sup>35</sup>. Brassinosteroids promoted elongation of soybean<sup>36</sup>, mung bean<sup>22</sup>, Azuki bean<sup>18</sup> and pea epicotyls<sup>37</sup>, bean<sup>38</sup>, sunflower and cucumber hypocotyls<sup>39</sup>, *Arabidopsis* peduncles<sup>40</sup> and wheat coleoptiles<sup>41</sup>. Hypocotyl elongation of Pakchoi (*Brassica chinensis*) by brassinosteroids was reported<sup>42</sup> and application to apical 3-mm region gave the best effect. Brassinosteroids also increase the root growth<sup>43</sup>. Enhanced root mass was observed in case of transplanted seedlings of *Pinus radiata*<sup>43</sup> due to root soak in 24-epibrassinolide. Increased root biomass of sugar beet by homobrassinolide treatment was reported<sup>44</sup>. By elegant experiments, Arteca and Arteca<sup>45</sup> proved that the exaggerated growth induced by brassinolide in *Arabidopsis thaliana* was independent of other known phytohormones.

Castasterone was found to stimulate the growth of *Catharanthus roseus* crown gall cells<sup>46</sup>. Brassinosteroids are also found to stimulate the growth of algae and fungi. A 2–3-fold increase in the growth of *Chlorella vulgaris* by brassinolide and 24-epibrassinolide was observed<sup>47</sup>. Strong growth promotion was observed in mycelial cultures of *Psilocybe cubensis* and *Gymnopilus purpuratus*<sup>48</sup>. However brassinosteroids specifically inhibited the growth of tobacco tumour cells caused by *Agrobacterium tumefaciens*<sup>49</sup>.

The promotion of growth by brassinosteroids is due to both cell division and cell elongation. 24-epibrassinolide increased cell division in cultured parenchymatous cells of *Helianthus tuberosus*<sup>50</sup>. Similar enhancement in the rate of cell division in the leaf protoplasts of *Petunia hybrida* by the use of brassinolide was observed<sup>51</sup>. The treatment of Chinese cabbage protoplasts by brassinosteroids resulted in the activation of cell division<sup>52</sup>.

Studies on soybean epicotyls revealed the ability of brassinosteroids to stimulate cell elongation; this elongation was found to be accompanied by proton extrusion and hyper polarization of cell membranes<sup>53</sup>. However, in cell suspension cultures of carrot, 24-

epibrassinolide induced cell enlargement without having any effect on cell multiplication<sup>54</sup>.

### Germination

As early as 1949, Evenari<sup>55</sup> suggested that several plant growth regulators also act as germination regulators. Now it is well established that brassinosteroids also promote seed germination. The application of brassinolide caused enhancement in seed germination of *Lepidium sativus*<sup>56</sup>. Similarly, seed treatment of *Eucalyptus camaldulensis* with 24-epibrassinolide resulted in substantial improvement in the percentage of seed germination<sup>57</sup>. Brassinolide, 24-epibrassinolide and 28-homobrassinolide promoted the germination of groundnut seeds<sup>58</sup>. Brassinosteroids not only promoted seed germination, but also reversed the inhibitory effect of abscisic acid<sup>59</sup>. The ability of brassinosteroids to promote seed germination was also observed in the case of *Brassica napus*<sup>60</sup>, rice<sup>61,62</sup>, wheat<sup>63</sup>, *Orabanchae minor*<sup>64</sup>, tomato<sup>65</sup> and tobacco<sup>66</sup>.

### Flowering

Foliar application of brassinosteroids resulted in increase in the number of flowers in strawberry<sup>67</sup>. In grape fruits, spraying of brassinosteroids in autumn increased the number of flowers, while such application in late winter reduced flower production<sup>68</sup>.

### Senescence

Senescence, the aging phenomenon in plants, is regulated by phytohormones. Brassinosteroids also play a crucial role in regulating the processes of senescence. Brassinolide accelerated senescence in *Xanthium* and *Rumex* explants<sup>38</sup>. The ability of brassinosteroids to promote senescence in detached cotyledons of cucumber seedlings<sup>69</sup> and leaves of mung bean seedlings<sup>70</sup> was reported. Brassinosteroid-deficient *Arabidopsis* mutants exhibited delay in chloroplast senescence<sup>23</sup>.

### Stress tolerance

Brassinosteroids increase the resistance of plants against various abiotic stresses. Brassinosteroid-treated tomato and rice plants grew better than control plants under low-temperature conditions<sup>71</sup>. Brassinosteroids improved the tolerance of maize<sup>72</sup> and cucumber<sup>73</sup> seedlings against low-temperature stress. Brassinosteroids were found to be involved in increasing resistance to chilling in brome grass<sup>74</sup> and rice<sup>75</sup>. In rice, 24-epibrassinolide increased the resistance against chilling stress (1–5°C) and the tolerance was associated with increased ATP, proline levels and SOD activity, thus

indicating brassinosteroid involvement in membrane stability and osmoregulation<sup>75</sup>.

Brassinosteroids increased tolerance to high temperature in wheat leaves<sup>76</sup> and brome grass<sup>74</sup>. The tolerance in plants to high temperature due to application of brassinosteroids is associated with induction of *de novo* polypeptide (heat shock protein) synthesis<sup>76</sup>. Application of brassinosteroids resulted in increased tolerance to drought stress in sugar beet<sup>44</sup>. The ability of 28-homobrassinolide to confer resistance to moisture stress in wheat was also established<sup>77</sup>.

The ability of brassinosteroids to counteract the inhibitory effects of salinity on seedling growth of groundnut was reported<sup>59</sup>. In *Eucalyptus camaldulensis*, treatment of seeds with 24-epibrassinolide resulted in increase in seed germination under saline conditions<sup>57</sup>. Similarly, 24-epibrassinolide and 28-homobrassinolide were found to alleviate the salinity-induced inhibition of germination and seedling growth in rice<sup>78</sup>. Seed treatment with very dilute solutions of brassinosteroids considerably improved the growth of rice plants in saline media (Figure 3). Kumaro and Takatsuto<sup>79</sup>, who were impressed by the ability of brassinosteroids to confer resistance of plants against a wide variety of environmental stresses stated, 'The role of brassinosteroids in protecting the plants against environmental stresses will be an important research theme and may contribute greatly to the usage of brassinosteroids in agricultural production'.

### Other effects

Brassinosteroids were found to induce roots on stem cuttings of Norway spruce (*Picea elbies*)<sup>80</sup>. Brassinolide retarded abscission of leaves of *Citrus*<sup>81</sup>. The ability of brassinolide to induce gravitropic curvature in maize-primary roots has been recently recognized and brassi-

nolide influence is independent of IAA<sup>82</sup>. Application of brassinosteroids increased nodulation and nitrogen fixation in groundnut<sup>83</sup>.

### Mode of action

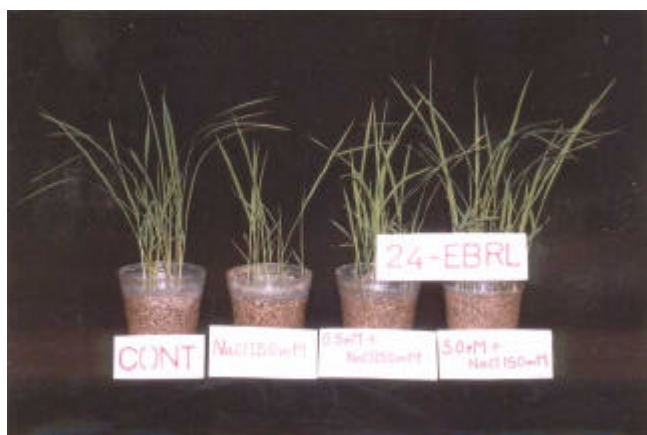
Our understanding of the molecular mechanism of action of brassinosteroids is still in its infancy stage. Recently, a leucine-rich protein (BRL 1) has been identified from *A. thaliana*, which is considered as a brassinosteroid receptor. Unlike in animal systems, where receptors for steroid hormones are intracellular, the brassinosteroid receptor (BRL 1) is located in the plasma membrane, functions at the cell surface and transduces extra-cellular signals<sup>84</sup>. A hypothetical scheme for further signal transduction is proposed. The binding of brassinosteroid molecule to the receptor causes activation of the kinase domain and subsequent phosphorylation of additional kinases and/or phosphatases<sup>85</sup>. However, there are vital gaps and several loose ends in the model proposed. Further research may be able to abridge the gaps and tie up the loose ends, and provide a comprehensive model on the molecular mechanism of action of brassinosteroids.

### Agricultural uses

Immediately after the discovery of brassinosteroids in plant systems, studies were initiated to explore the possibilities of using these new substances for improving the yield of economically useful plants. Meudt and his associates<sup>86,87</sup> with the use of brassinolide demonstrated improvement in the yield of lettuce, radish, bush bean and pepper. Foliar spray of brassinolide substantially improved the yields of wheat<sup>88</sup> and mustard<sup>89</sup>, rice<sup>11</sup>, corn<sup>11</sup> and tobacco<sup>11</sup>. Brassinosteroids were also found to increase the growth and yield of sugar beet<sup>44</sup>, legumes<sup>90</sup> and rape seed<sup>91</sup>. Application of 28-homobrassinolide significantly increased tuber yields in potato<sup>92</sup>. Application of 24-epibrassinolide increased yields of corn, tobacco, watermelon, cucumber and grape<sup>93</sup>. Foliar application of brassinolide, 24-epibrassinolide<sup>94</sup> and 28-homobrassinolide<sup>95</sup> was found highly effective in enhancing the yields of groundnut and tomato<sup>96</sup>. Exogenous application of brassinosteroids considerably improved the growth and economic yield of radish<sup>97</sup>. In China, 28-homobrassinolide has been registered as a plant growth regulator in case of tobacco, sugarcane, rape seed and tea. In Russia, 24-epibrassinolide has been registered as a regulator for potato, tomato, cucumber, pepper and barley<sup>98</sup>.

### Future prospects

Twenty years of brassinosteroid research brought into light several vital functions to this new group of phyto-



**Figure 3.** Alleviating influence of 24-epibrassinolide on salinity stress-induced growth inhibition of rice (20-day plants grown in Growth Chamber Model RD 2000, Nippon, Japan).

hormones in plant growth and development. As Clouse and Fieldman<sup>28</sup> aptly pointed out, after a long period of neglect by plant scientists, brassinosteroids are now receiving a great deal of international attention. Future researches may bring into light many more significant roles to this group of steroid hormones. New discoveries of the physiological properties of brassinosteroids allow us to consider them as highly promising, environment-friendly natural substances suitable for wide application in plant protection and yield promotion in agriculture<sup>99</sup>. One of the major constraints to employ brassinosteroids in large scale in fields is their higher costs. Progress in the chemical synthesis of brassinosteroids and their analogues has led to economically feasible approaches that have brought their practical application in agriculture within reach. Pesticidal companies in China and Japan started synthesizing brassinosteroids on a commercial scale. In India, a private agro-chemical industry introduced homobrassinolide in the market. A greater role of brassinosteroids in realizing better crop yields during the 21st century is predicted. As the cost of brassinosteroids is brought down to affordable levels, the chemical promise held by brassinosteroids to improve crop yields can be soon accomplished.

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