บราสสิโนสเตอรอยด์ : บทบาททางสรีรวิทยาในพืช
Brassinosteroids : Physiological Roles in Plants

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ภาควิชาเกษตรและสิ่งแวดล้อม คณะวิทยาศาสตร์และเทคโนโลยี มหาวิทยาลัยราชภัฏสุรินทร์
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บทคัดย่อ

บทบาททางสรีรวิทยาของบราสสิโนสเตอรอยด์ในพืชเป็นไปในลักษณะที่คล้ายคลึงกับออกซินในบางกรณีและคล้ายกับไซโทไคนินหรือจิบเบอเรลลินในบางกรณี ดังนั้นจึงเห็นได้ว่าบทบาททางสรีรวิทยาที่แท้จริงของบราสสิโนสเตอรอยด์ในพืชจึงเป็นประเด็นที่มีความซับซ้อน ในกรณีของการใช้สารควบคุมการเจริญเติบโตของพืชชนิดนี้ เพื่อประโยชน์ทางการเกษตรนั้นพบว่าการใช้มุ่งเพื่อการเพิ่มผลผลิตและการปรับปรุงลักษณะต่างๆ ของพืชให้มีความเหมาะสมกับสภาพแวดล้อมในพืชหลายชนิด บราสสิโนสเตอรอยด์แสดงบทบาททางสรีรวิทยาหลายประการในการส่งเสริมการเจริญเติบโตและกระบวนการพัฒนาการของพืช เช่น การงอกของเมล็ด การออกดอก การชราภาพและความทนทานต่อความเครียด บทความนี้มีความมุ่งหมายที่จะกล่าวถึงบทบาททางสรีรวิทยาของบราสสิโนสเตอรอยด์ที่มีผลต่อการเจริญเติบโตและพัฒนาการของพืชอันจะนำไปสู่การประยุกต์ใช้สารควบคุมการเจริญเติบโตของพืชชนิดนี้ทางการเกษตรต่อไป

คำสำคัญ : บราสสิโนสเตอรอยด์ บทบาททางสรีรวิทยา

Abstract

Physiological roles of BRs in plant seem to be auxin-like at times and cytokinin - like or giberrellin - like at other time. Therefore, the original physiological roles in plant is complicated. In terms of agriculture uses, a considerable effort has been made in practical application of BRs for increasing yield and improving desirable characteristics in several crops. BRs play various physiological roles in growth-promoting activity and other developmental processes like seed germination, flowering, senescence, and stress tolerance. This paper attempts to enumerate BRs physiological roles in normal plant growth and development, leading to the improving of BRs application in agriculture.

Keywords : Brassinosteroids, Physiological roles

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Introduction

Plant hormones play a crucial roles in the endogenous regulation of plant growth and development. Recent studies on physiological effects of brassinosteroids (BRs) in plant have provided convincing evidence that BRs are a unique class of plant hormones that are essential for normal plant growth. BRs are a number of other naturally occurring organic compounds that exhibit strong biological activity when applied exogenously in low concentrations. Recently brassinolide, a steroidal compound, has been noted as the sixth plant hormone subsequent to auxin, gibberellin, cytokinin, ethylene and abscisic acid (Hamada, 1986). Currently, more than sixty BRs have been identified in many plants, including dicots, monocots, gymnosperms, green algae and ferns (Sakurai & Fujioka, 1994). The identity of the active compound known as “brassins” (so called because the first extracts were from the genus *Brassica*), was unclear. That they were not gibberellins (GAs) was indicated by some differences in growth pattern, such as swelling and curvature of stem, which do not occur in GA-induced growth (Srivastava, 2002).

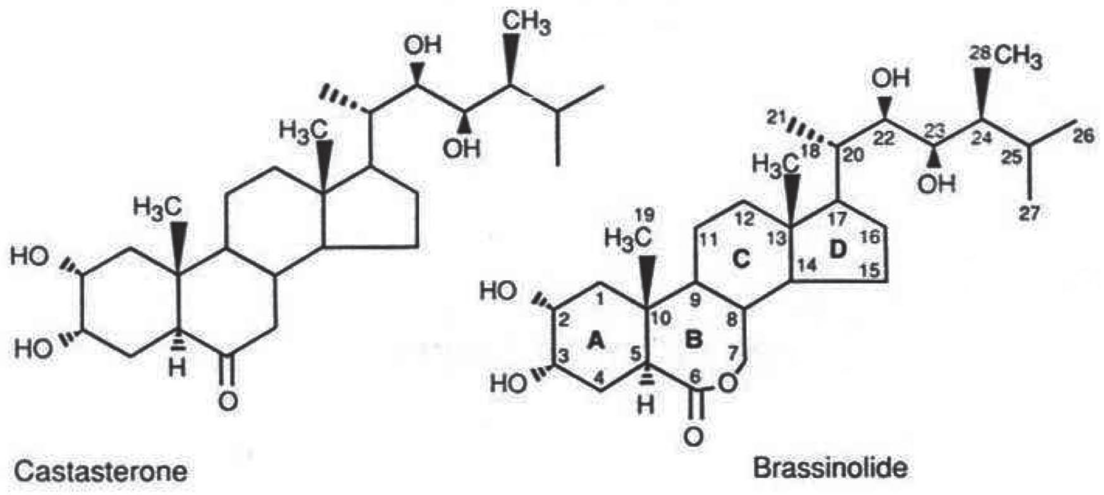
Physiologically, after the isolation and characterization of brassinolide (BL) as steroidal compounds in plant, they suddenly became very important phytophysiological compounds. A great number of steroidal compounds are already known as hormone in most animals and insects, but brassinolide is the first steroidal compound known to exhibit physiological role in plants. As a plant hormone, it must exist physiological role in every kind of plant, but the number of responses is still limited. In order to be recognized as a growth-promoting activities compounds, the application of BRs in various plants has been recently done.

Agriculturally, BRs have long been used for specific purpose in enhancing some desirable characteristics of agricultural crops. Physiological studies done in several laboratories have demonstrated that BRs can induce a broad spectrum of cellular responses such as stem elongation, pollen tube growth, leaf bending and epinasty, root inhibition, induction of ethylene biosynthesis, proton-pump activation, xylem differentiation, and regulation of gene expression (Mandava, 1988; Clouse & Sasse, 1998). To date, several studies in the field has been carried out on stress responses (Anuradha & Rao, 2001; Nakashita *et al*., 2003; Sharma & Bhardwaj, 2007; Alam *et al*., 2007). Although small number of other agricultural crops treats with BRs for increasing yield has been documented. It must, however, be mentioned that laboratory studies do not give a complete information of the physiological role of BRs when they are applied in the field.

Structure and distribution of BRs in plants

Brassinolide and castasterone (CS), the two most common BRs in higher plants, are C_{28} brassinosteroids (Figure 1). BRs are shown to have a steroidal structure and are the first naturally occurring steroid that has a seven-membered lactone ring as part of a fused ring system. They contain the typical steroid nucleus, with fused rings A, B, C and D and alkyl side at C-17 (Srivastava, 2002). Both the nucleus and the side chain contain various substituents in different isomeric configurations, which make the stereochemistry and nomenclature of BRs highly complicated (Mandava, 1988).

Detailed structure of both BL and CS have α-oriented hydroxyl groups (*cis* configuration) at C-2 and C-3 in ring A, an α orientation at C-5 (A/B ring junction), a ketone group at C-6 in ring B, β-oriented C-18 and C-19 methyl groups, α-oriented hydroxyl group at C-22 and C-23 in the side chain, and an α-methyl at C-24. BL, in addition, has a 7-oxa-6-ketone (or 7-oxalactone) (Srivastava, 2002). Other naturally occurring BRs perform differently in the presence or absence of a ketone or lactone in the B ring, number of



Castasterone

Brassinolide

Figure 1 Structures of brassinolide and castasterone

Source : (Srivastava, 2002; Grove *et al.*, 1979)

hydroxyl group in the A ring, and nature of substituents and their α vs β configuration in the side chain. The structure of BL, in addition, is similar to that of animal steroid hormones, including ecdysone, progesterone and testosterone (Friedrichsen & Chory, 2001). At the present time over sixty kinds of brassinosteroids have been found. Thirty-one are fully characterized, including 29 free compounds and 2 conjugates (Kim, 1991).

BRs are growth-promoting natural products found at low levels in pollen, seeds, and young vegetative tissues throughout the plant kingdom (Clouse & Sasse, 1998). They have been found in a wide range of plants, including dicots, monocots, gymnosperms and algae (Kim, 1991). Although plant roots have not yet been investigated, it is likely that they will also contain BRs. Among the naturally occurring BRs, BL and CS are considered to be the most important, because of their wide distribution, as well as their potent biological activity (Arteca, 1995). Apparently, BL was the first steroid hormone reported for the plant kingdom (Grove *et al.*, 1979). It also carried out the bean second-internode bioassay and found that BL produced a nearly 200% increase in elongation of the internode compared to that of untreated controls (Howell *et al.*, 2007). The benefit of knowing BRs structures and their distribution in plant can support plant researchers and agriculturists in finding more biological activity of BRs in order to use them for agricultural purposes as much as possible. Bajguz & Tretyn (2003), moreover, reported that the BL, 24-epibrassinolide, is believed to be the most powerful of the BRs. BRs are now classified as steroidal plant hormones. They have been identified in 27 higher plant families and 3 lower plant families.

Presently, BRs have been characterized from 44 plant species, which include 37 angiosperms (nine monocots and 28 dicots), five gymnosperms, one pteridophyte and one alga. They are present in many plant parts at extremely low concentrations (nanogram levels). Levels of endogenous BRs vary among plant tissues. Young growing tissues contain higher levels of BRs than mature tissues. Pollen and immature seeds are the richest sources with a range of 1-100 ng per g fresh weight, while shoots leaves usually have lower amounts (Rao *et al.*, 2002; Srivastava, 2002). Some of the plant species and their parts in which BRs are reported are given in Table 1.

Table 1 Distribution of BRs in the plant kingdom

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

Source : Rao *et al*., (2000)

Table 2 Some physiological effects of brassinosteroids in plants

Cell level	Whole plant level
Stimulation of elongation and fission	Growth promotion
Effect on hormonal balance	Increase in the success of fertilization
Effect on enzyme activity; H^+-pump activation	Shortening the period of vegetative growth
Activation of protein and nucleic acid synthesis	Size and quantity of fruits increase
Effect on the protein spectrum and on the amino acid composition of proteins	Effect on the content of nutritive components and fruit quality improvement
Effect on the fatty acid composition and on the properties of membranes	Increased resistance to unfavourable environmental factors, stress and diseases
Enhancement of the photosynthetic capacity and of translocation of products	Crop yield increase

Source : Khripach *et al*., (2000)

Recent works on physiological roles and agricultural uses

BRs significantly influence various physiological processes like seed germination, rhizogenesis, flowering, senescence, abscission and maturation. BRs also remarkably confer resistance to plants against various stresses as shown in Table 2.

As they play an important role in plant development, their physiological action must be taken into account. Recent works on the influence of BRs on growth and developmental processes is presented below.

Growth

The initial studies with BRs in plants were concentrated around its ability to induce growth such as cell elongation, swelling, curvature and splitting of the second internode. Due to stimulation of growth is considered as the most well-documented physiological role of BRs in various plants. Most of recent studies with BRs have been carried out to ascertain their role both in molecular and physiological level.

Stimulatory effects of BRs on elongation are among the most documented physiological effects. It has been observed in many assay systems such as radish, tomato, sunflower and cucumber hypocotyls, normal and dwarf pea, mung bean and azuki bean epicotyls, *Arabidopsis* peduncle, and wheat coleoptiles (Brosa, 1999). However, BRs have been shown to promote elongation of vegetative tissue in a wide variety of plants at very low concentration. Recent work of Wimolphan (2004) revealed that wheat plants applied with the BR at only 0.005 to 0.02 ppm were significantly taller than untreated wheat plants. Obviously, cell elongation is associated with an increase of acid secretion. Thus, the stimulation of elongation in vegetative tissue is confirmed by BRs action.

BRs also invole in the gravitropic response of primary root of maize (Kim *et al*., 2000). Based on their study, exogenously applied of BL and CS enhanced gravitropic curvature of maize roots in an indole-3-acetic acid dependent manner. Similarly, the low concentrations of BRs such as 24-epicastasterone and 24-epibrassinolide promote root elongation in *Arabidopsis* wild-type plants up to 50% and in BR-deficient mutants such as dwf 1-6 (cbb1) and cbb3 up to 150% (Müssig *et al*., 2003). Also BRs are required for lateral root development in *Arabidopsis* and that BRs act synergistically with auxin to promote lateral root formation (Bao *et al*., 2004). According to their observations, BRs significantly perform the regulation of auxin transport, providing a mechanism for two hormonal interactions in plants and supporting the hypothesis that BRs promote lateral root development through increasing acropetal auxin transport. Similar enhancement was observed in onion root tips. Low concentration of BL between 0.005 to 0.05 ppm nearly doubled the mean root length that were significantly greater than of the controls. In contrast, the highest length that were less than controls (Howell *et al*., 2007).

Germination

The application of BRs also promote seed germination in several crops such as *Lepidium sativus*, *Eucalyptus camaldulensis*, ground nut, *Brassica napus*, rice, wheat, tomato and tobacco (Rao *et al*., 2002). As well, BRs was found to stimulate the germination in *Arabidopsis*, they are needed for normal germination (Steber & McCourt, 2001).

Flowering

There is a small number of report in using BRs to induce flowering in plants. Also, the gap of knowledge with respect to effects of BRs on flowering in economic crops must be realized. Since the promotive effects of BRs have also shown at very low concentration. Thus, the possibility of BRs contamination in environment is less. Nevertheless, foliar application of BRs resulted in increase in the number of flowers in strawberry was observed (Rao *et al*., 2002).

Senescence

BRs have various physiological and morphological effects on plants, including ethylene production which is the cause of senescence. BL retarded abscission of leaves of *Citrus* was reported (Iwahori *et al*., 1990). BRs increase chlorophyll breakdown (Vardhini & Rao, 2002) but inhibit anthocyanin biosynthesis (Brosa, 1999). On the contrary, BRs accelerate senescence (He *et al*., 2001; Rao *et al*., 2002) but the studies about this topic are not enough to clarify their effect in regulating senescence process.

It is known that phytohormones play an important role in the regulation of senescence of plants. In order to support the aforementioned detailed studies, recent study of Vardhini & Rao (2002) allows us to know that BRs lowered chlorophyll levels in tomato pericarp disc and performed the ability in accelerating fruit-senescence which was associated with increase in ethylene production. Recently, promoting of senescence of leaf has been shown in leaf segments taken from wheat leaves specially at high concentration (Sağlam-Çağ, 2007). This finding has supported the works of many researchers who claimed that exogenously applied BRs accelerated the loss of photosynthetic pigments of leaves and cotyledons during the senescence.

Stress tolerance

Is it possible to grow crops under unfavourable conditions, such as high salinity, drought, insufficient nutrients or manifestation of plant diseases. Can BRs overcome those unfavourable conditions, and be used as a substitute for some anti-phytopathogen substances. The answer of those questions are still not clear enough. However, in the case of increasing plant resistance to stresses, BRs were found to reverse the inhibitory effect on germination and seedling growth of rice induced by salinity stress (Anuradha & Rao, 2001; Kagale *et al*., 2007). As well, BRs enhanced seedling tolerance to drought and cold stresses in both *Arabidopsis thaliana* and *Brassica napus* (Kagale *et al*., 2007). Cold resistance of rape seedling treated with BR_{27} was also reported (Janeczko *et al*., 2007). BRs were also found to improve growth of *Brassica napus* under Cu metal stress. Since BRs, 24-epibrassinolide, blocked copper metal uptake and accumulation in the plants (Sharma & Bhardwaj, 2007). In *Brassica juncea*, treatment of plants with 28-homobrassinolide resulted in partially neutralized the toxic effect of nickle (Alam *et al*., 2007)

Along with growth acceleration, BRs perform their potential in enhancing plant resistance to phytopathogens. The ability of BRs to reduce the infection of phytophthora up to a maximal 40% was reported (Savel'eva *et al*., 1999). Application of 24-epibrassinolide prolonged dormancy of potato tubers and increased their resistance to sprouting and diseases (Korableva *et al*., 1999). Furthermore, field application of 24-epibrassinolide to barley plants in doses of about 5-15 $mgha^{-1}$ significantly decreased the extent of leaf diseases induced by mixed fungal infection (Pshenichnaya *et al*., 1997). However, a protective effect of 24-epibrassinolide against fungi which established in field trails with cucumber was reported (Churikova *et al*., 1999). A newly discovered aspect of the protective action of BRs on other crops such as tobacco and rice has been put forward. Wild-type tobacco treated with BL exhibited enhanced resistance to the viral pathogen tobacco masaic virus (TMV), the bacterial pathogen *Pseudomonas syringae* pv. tabaci (*Pst*), and the fungal pathogen *Oidium* sp. Similarly, BL induced resistance in rice to rice blast and bacterial blight diseases caused by *Magnaporthe grisea* and *Xanthomonas oryzae* pv. oryzae (Nakashita *et al*., 2003).

These data indicate that exogenously apply of BRs can act efficiently in plants as plant- resistance enhancers and immuno-modulators when applied at the appropriate concentration and stage of plant development. Since BRs confer their various ability on shifting a complex sequence of biochemical processes and the production of various chemical defence compound in plants, they are considered as promising phytohormone from a practical point of view. Based on the employment of very small amounts, they friendly exhibit their effects on environment in general. In this regard, the protective effect of BRs against some phytopathogens has helped to open up new approaches for plant diseases management in agriculture.

Increasing yield

Application of BL obviously increased ear weights of both tillers and main stem, in comparison with untreated controls by the spray of BL at concentrations of 10^{-4} to 10^{0} ppm from the beginning of anthesis. Similarly, BL treatment at the early silking stage had significant influence on the length of the unfertile tip portion of the ear and the number of vacant kernels in corn (Hamada, 1986). Certainly, foliar spray of different concentrations of 28-homobrassinolide significantly increased grain yield in wheat, rice and mustard, pod yield in groundnut, tuber yield in potato and seed cotton yields, over control (Ramraj *et al*., 1997). Exogenous application of 24-epibrassinolide was found to be most effective in enhancing the number of nodules and weight of nodulated roots in groundnut (Vardhini & Rao, 1999).

In other crops, BRs were also found to increase the growth and yield of sugar beet, legumes, rape seed, tobacco, watermelon, cucumber, grape, groundnut, tomato and sugarcane (Rao *et al*., 2002). Wheat applied with BR at the tillering + jointing + heading stage tended to produce yield and yield components significant greater than the wheat applied with BR at the tillering or jointing or heading stage only (Wimolphan, 2004). Recently, an increase of number of fruits per plant has been shown in commercial yellow passion fruit orchards in the first year of production (Gomes *et al*., 2006). In addition, BL increased the seed size and the length of pods containing three seeds in brassinosteroid-deficient mutant faba bean was reported (Fukuta *et al*., 2006).

Conclusion and perspectives

Brassinosteroids are a new class of plant hormones with the A-D ring structure of steroids to which an alkyl side chain is attached. A great number of BRs occur in plants, with the most potent substances being brassinolide and castasterone. BRs are widely distributed throughout the plant kingdom. They are present in nearly all parts and organs in flowering plants, with the highest concentrations is found in pollen grains and in immature seeds. To date, 42 brassinosteroids and four brassinosteroid conjugates have been characterized (Rao *et al*., 2002). BRs have served multi-functions in plant growth and development as well as other plant hormones. They perform their physiological roles in various plants like cell elongation, swelling, curvature, germination of seed, floral stimulation, senescence, stress tolerance and increasing yield. BRs have been considered as promising compounds for application both in molecular level and physiological level. Since BRs have a broad spectrum of stimulative and protective activities that have a positive effects on the quantity and quality of crops, the possibility of using them as a plant growth regulator to enhance yield in other crops is likely. New discoveries of the physiological properties of BRs pave us to recognize them as highly promising natural substances suitable for broad application in agriculture. Even though, one of the major constraints to employ BRs in large scale in fields was reported by Rao *et al* (2002) is their higher costs.

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