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A CASE STUDY

Significance of brassinosteroids in plant growth regulation

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SUMMARY

The brassinosteroids (BRs) are a group of plant originated steroidal lactones that exert pronounced growth promoting activities. BRs are engaged in a flurry of plant developmental aspects, including the stimulation of cell division and elongation, conferring stress tolerance, leaf development and vascular system differentiation. Though, rapid progress in BR biology is made, practical application of the BR-related knowledge to crop improvement has begun yet a little is accomplished towards the understanding of the mechanism involved. Moreover, the site of action of substances differs and need clear explanation. BR should also answer the questions concerning whether or not sterol hormones receptors predated the evolutionary divergence of the plants.

Key Words : Brassinosteroids (BRs), Structure, Biological activity, Vascular system, Steroidal lactones

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Recently, brassinosteroids (BRs) have gained fine reputation as the newest class of plant growth regulators. The use of such compounds has a great potential to increase plant productivity and yield. Since the discovery of brassinolide, steroidal lactones, from rape (*Brassica napus* L.) pollen, a large number of related compounds collectively called brassinosteroids (BRs) have been isolated from pollen, seeds, shoot and other parts of plant species. It is established now that they are all widely distributed in plants and are novel plant, promoting of growth steroidal lactones with structural similarities to animal steroids. Although, first isolation of BRs groups was obtained from rape pollen, later studies have shown that they may be found in wide range of plants including dicots, monocots, gymnosperms and algae. Over 60 kinds of brassinosteroids isolated so far, 31 have been fully characterized including 29 free and 2 conjugates. Extensive researches have shown that brassinosteroids are a new group of plant hormones that affect cell elongation, pollen tube growth, root growth and development, germination, senescence, photosynthesis, yield, regulation of gene expression in different plant species (Prakash *et al.,* 2003, Bao *et al.,* 2004 and Ozdemir F *et al.,* 2004). Plants that are deficient in BR biosynthesis or signal transduction pathways display characteristic growth-deficient phenotypes (Choe, 2004). The structure of brassinolide (the most active lactone steroid of BR family) was completely established by X- ray diffraction analysis of a single crystal which belong to the monoclinic space group $P2_1$ with a= 9.88 (2) A, b=7.63 (2), A, C= 17.98 (3) A and B=91.9 (1) (Grove *et al.,* 1979). There is one molecule of brassinolide and one molecule of water in the asymmetric unit. Generally, all brassinosteroids contain a steroid nucleus with a side chain at C-17 similar to the side chain in plant sterols. Other common features for all brassinosteroids, in addition to B oriented angular C-18 and C-19 methyl groups are (a) 2- orientation at C-5 (b) α-oriented hydroxyl group at C-22 and C-23 and (c) α - oriented hydroxyl group at C-2 and C-3 in ring A of the steroid nucleus (with exceptions).

Brassinosteroids greatly differ in functional group of C-24 (Sterol side chain) CH_3, brassinolide (BR_1) and brassinosteron (BR_2) = CH_2 dolicholide (BR3) and dolichosterene (BR4)= CH-CH3, homodolicholide (BR_{10}) and

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Brassinolide

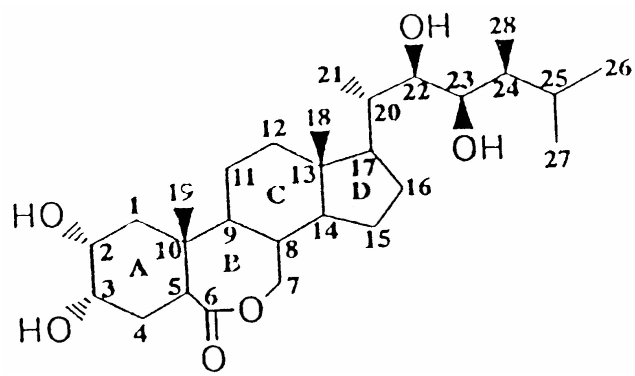

Fig. 1: The structure of brassinolide, a commonly occurring BR with high biological activity, showing numbered positions mentioned in the text. In natural BRs, hydroxylation can occur in ring A at positions 3-, and/ or 1-; also found are epoxidation at 2,3-, or a 3-oxo-group. In ring B, afternatives 6-oxo- and 6-deoxo-forms. In the side chain methyl-, ethyl-, methylene-, ethylidene, or non-alkyl groups can occur at 24-, and the 25-methyl-series is also represented.

homodolichosteron (BR_{11}) a C_2H_5 homobrassinosteren (BR12).

Biosynthesis :

Brassinosteroids occur in a wide variety of plant species and could be extracted from various plant parts like pollen, leaves, flowers, seeds, shoots, gall etc (Choe, 2006, Vert *et al.*2005). Now, substantial progress has been achieved how plants maintain their BRs levels in different parts and transport this endogenous substance to the place where it is required. Newer techniques like biochemical and molecular have shown that genes encoding various enzyme of certain steps in BRs synthesis may involve.

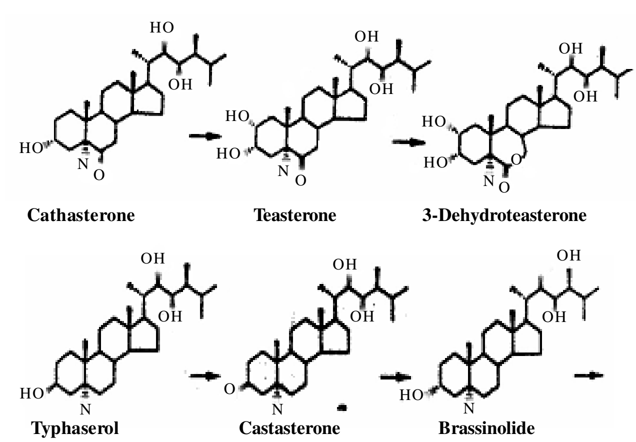

Fig. 2: Biosynthesis of brassinolide via early C6-oxidation path way

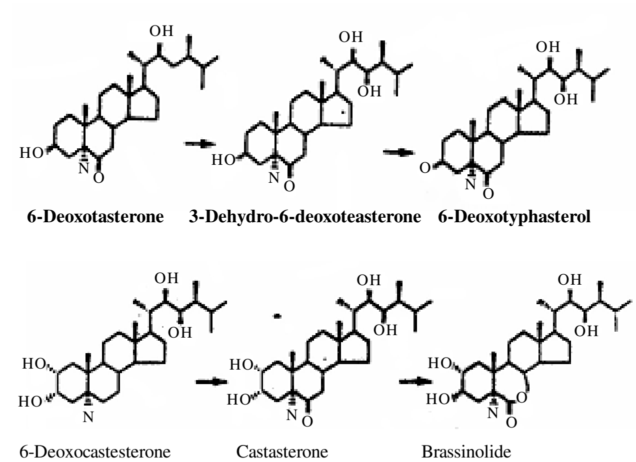

Fig.3: Biosynthesis of brassinolide via late C6-oxidation path way

Starting from the initial precursor camp esterol (CR), the BR intermediates undergo a series of hydroxylation, reduction and epimerization and a Baeyer villigen type oxidation leading to the most oxidized form brassinolide (BL). Castasterene (CS) oxidation, the last step in BR biosynthesis is not found in some species *e.g.* Mungbean. In that case CS plays a role as the major BR rather than BL (Yokota *et al.,* 1991). There might be two pathway *i.e.* early oxidation pathway and late oxidation pathway. The BL biosynthetic pathway is divided into the sterol specific pathway, squalene to campesterol and the BR-specific pathway, campesterol to brassinolide. The complete pathway, intermediate, enzymes involved are depicted in diagram. Currently, it has been shown that once 24-methylenecholesterol is made, it seems to serve as a precursor for BR biosynthesis (Hong, 2005) .

Transport :

It is not yet known whether long distance transport is important for the endogenous effects of BRs, but short distance effects can probably be assumed from results in pollen, seeds and model system. Labelled BRs supplied exogenously are translocated probably via the xylem conjugates of plant hormones are considered to be transport, storage or inactivated form. Two examples of 23 glucosyl conjugates of BRs were known and reversible acyl conjugation of the 3- position has now been reported (Abe, 1996).

Interaction with hormones :

Brassinosteroids have been evaluated in several bioassays for plant hormones and are considered to be involved in action of other plant hormones. It is an imperative need to work on more versatile test system to characterize brassinolide and its related compounds that would permit the study of their interaction with other hormones and related growth substances. In many assay systems, they act synergistically with auxins. However, antiauxins nullify the effects of brassinosteroids. On the other hand, BRs only have additive effects on some crop species when applied with auxins, for instance, the similarity between the effects of BR and auxin on elongation of apical hook segments of dwarf pea, maize mesocotyl etc were observed (Yopp *et al.,* 1981). They also demonstrated strong synergistic interaction between auxin and BR. This synergism however, occurs only when the tissue is pretreated with BR and exposed to auxin. Further, evidence for BR-auxin synergism comes from the data on auxin induced ethylene production in etiolated mungbean hypocotyls segments. BR stimulates this response in a synergistic manner. However, in light grown mungbean section, BR does not increase ethylene production. Moreover promotion of growth of pea plants by exogenous IAA has been demonstrated and a synergistic interaction between IAA and BRs in the promotion of cucumber hypocotyls section has been observed. BR serves as modulator in enhancing the auxin response.

The interaction between GA_3 and BR was investigated to determine the synergism between the two growth promoters and the dependency of BRs on endogenous GAs reports to reject synergism between the two. BR works in additive manner at 10^{-8}nm to 10^{-9}nm. Similarly, there are reports, which reveal that BRs act in additive manner when used in combination with cytokinins. When BR was tested with cytokinin assays, it was only weakly active and BRs and Kinetin showed opposite effects in bioassays involving the retardation of senescence of dark grown *Xanthium* leaf discs. BR promoted the senescence. On the other hand, in dark grown Amaranthus seedlings, kinetin promoted betacyanin formation but BR was inactive in this case. Cytokinin caused defoliation in *A. indiane*. So, the same reports with BRs are collected. ABA interacts strongly with BR and prevent the effects of BRs. BR alone and in combination with auxin induces the synthesis of ethylene perhaps between SAR and ACC. Abscissic acid interacts very strongly with the BR induced elongation in beans (*Phaseolus vulgaris*) and mungbean (*P. aureus*) that are reversible.

Biological activities :

Like auxins and gibberellins, the promotive effect of BRs on cell elongation is now well established. However, the precise biochemical mechanisms responsible for cell wall loosening remain elusive (Cosgrove, 1997). Brassinosteroids characteristically evoke both cell elongation and cell division resulting in elongation, swelling, curvature and splitting of the second internodes. Such activity is called brassin activity. BRs produce activity at a lower concentration (nm to pm range) as compared to gibberellin or auxin. Dwarfism in dwarf mutants of several species could be rescued by the brassinolide, a member of BRs family (Choe *et al*., 1998). Experiments have shown that BRs stimulate longitudinal growth of young tissues via cell elongation and cell division. Sterol synthesis has been shown as cause of dwarfism in certain mutants and signaling is necessary for normal plant growth and development (Aspiroz *et al.,* 1998). Severe dwarfism in dim mutant (DIMINUTO/ DWARF) (dim/dwfi) is recovered by addition of exogenous brassinolide (Klahre *et al.,* 1998). BR also induces elongation of normal and dwarf pea epicotyl, mungbean epicotyl, cucumber hypocotyl, sunflower hypocotyl. It has also been reported that application of BRs to intact plants such as lettuce, cucumber, mustard and wheat grown under hydroponics conditions, contributes to the growth of whole plants. It was also reported that brassinosteroids could stimulate hypocotyl elongation in Pakchoai by increasing wall relaxation without a concomitant change in wall mechanical properties and a passive dilution of the osmotic pressure of the cell sap. The lower endogenous BR levels and the ability of brassinolide to enhance internode elongation imply that the dwarf phenotype of Ikb mutant is likely to be consequence of BR deficiency. Deficiency of BL causes dwarfism in the IKD mutant *Pisum sativum* L (Nomura *et al.,* 1997). Early experiments with BRs described promotive effects on both expansion and division but promotion of division proved hard to confirm in model systems and inhibition was often seen. However, a study of the effects of 24 epi BL on potato plants from Chinese cabbage showed an enhancement in the rate of cell division, as was cluster and colony formation. But differentiation of the protoplasts and regeneration of the cell wall were promoted (Nakajima *et al.,* 1996).

Generally, exogenous application of BRs to root tissue inhibits extension and lateral root formation with only sporadic promotion of elongation or adventitious rooting when extremely low concentrations are used. BRs may be involved in the center of lateral root initiation as uniconazole inhibits it to a substantial extent. This is further supported by the recent findings (Yokota *et al.,* 1991). Alkamides isolated from plants promote growth and alter root development (Ramirez *et al.,* 2004). Brassinolide regulate root nodule formation in Soybean (Terakado *et al.,* 2004) . Application of BRs to grape berries significantly promoted ripening (Symons *et al.,* 2006).

Stress response :

BRs have been found to have prominent effects in plants facing external stresses like temperature, salinity, and water. An appreciable work on chilling stress was carried out and it was demonstrated that both cold and thermo tolerance were enhanced in cell culture of broom grass. However, germination in solutions of 24 epiBL did not center heat shock tolerance in seeding of moth bean. In rice, 24 epi BL treatments reduced electrolyte leakage during chilling at 1-5^{0}C, reduced malonodialdehyde content and showed the decrease in activity of super oxide dismutase. While levels of ATP and initially proline was enhanced and this resistance was due to BR induced effects on membrane stability and hormone regulations. Like other hormones, in wheat variety sensitive to drought stress, treatment of 28-homo, BL increased soluble protein and relative water content and reduced ion leakage. Similarly treatment with 24-pi BL enhanced growth of gram plants and increased relative water extent and decreased stomatal transpiration rate in sorghum in water stressed conditions. Earlier work conducted with BRs has revealed that induction of BR in saline condition overcome the salinity and caused reduction in groundnut (Vidyavardhini and Ram Rao, 1997). Similarly, enhanced salt tolerance by BRs in rice has been confirmed and a protective effect on ultra structure of barley leaf is reported. In many cases, BR treatment brought about a protection against various pathogens. However, it is not a general case, because BR induced susceptibility of potato to *Phytophthora infestans*. Further, promotive effect of BRs on percent and rate of germination of *Eucalyptus cameldulensis* was observed in 150 mM NaCl stress.

Conclusion :

Last two decades has seen unprecedented progress in the research of brassinosteroids, which emerged as a potent growth promoting substances contributing normal plant growth and development. However, the main constraint at this moment is the availability of BRs and/or its analogues from commercial viewpoint. Plant physiologists, biochemists are actively engaged in isolating these substances (BRs) from both higher and lower plants through newer techniques and assays.

Although, the precise information on various responses of BRs has been obtained, a little is accomplished towards the understanding of the mechanism involved. There are contradictory reports, which showed that BRs may function both in synergistic as well as additive manner, depending upon the species. It is a general opinion that BRs exhibit a different mode of action than auxin or other members of hormone family. Moreover, the site of action of these substances differs and needs clear explanation. How BRs affect enzymes such as peroxidase, ATPase, RNA and DNA polymerase still require indepth investigation. Recently, epochal findings on BR synthesis mutants and application of molecular genetics have provided the necessary components to provide a basis for conclusion how BR acts to influence a diverse array of plant developmental processes. These synthetic mutants are important tool for the study of the molecular basis of BRs synthesis. Moreover, the cloning of BR responsive genes *i.e.*, BRUT and TCH4; the identification and closing of genes encoding biosynthetic enzymes and the immanent closing of a gene etc., are certain aspects of BR's future studies. In addition, BR should also answer the questions concerning whether or not sterol hormones receptors predated the evolutionary divergence of the plants.

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