

Review Article

Brassinosteroids Application in Ornamental Crops: A Review

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Abstract

Brassinosteroids (BRs) comprises of a novel group of steroidal hormones, having structural similarity with animal and insect steroids. They were first identified from rapeseed pollen and since then their presence has been documented in numerous plant species. Usually, they occur endogenously at very low concentration and mediate multiple plant functions both at cellular and molecular levels. Widespread research and studies were conducted involving several agricultural and horticultural crops to ascertain their efficiency as a phytohormone. Findings on effects of exogenous application of BRs on ornamental group of plants have shown that they increase vegetative growth by influencing photosynthesis, promote early flowering, flower quality and yield, prolonged shelf-life of flowers, help in multiplication by promoting rooting in cuttings and corm production. Apart from assisting in growth and developmental processes, another major advantage of BRs application is their protective mechanism against abiotic stresses such as drought, heat, cold, salinity, heavy metal etc. Under the influence of BRs, plants have been reported to tackle these stresses by boosting the production of antioxidants along with reducing the impacts of harmful free radicals and through modifications in various gene expressions. In spite of having all these beneficial effects, their use as a growth regulator have not been commercial. Thus, there is a need for standardizing their concentrations as per the crop requirement and mode of application.

Keywords: Brassinosteroids, epibrassinolide, ornamental crops, flowering, abiotic stress.

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Introduction

Plant growth regulators (PGRs) are organic compounds that promote, inhibit or modify physiological processes in plants when applied exogenously at low concentration. They differ from phytohormones in not being synthesized by plants. Five PGRs, viz. auxins, gibberellic acids, cytokinin, abscisic acids, and ethylene are well known for their function in plants. In the recent past, several new classes of PGRs such as brassinosteroids, salicylic acid, methyl jasmonates, polyamines etc. have been identified which play significant role in plant functioning. Among these growth regulators, Brassinosteroids (BRs) are the ones that have been most extensively researched for their uses in plant growth and development [1].

Brassinosteroids are polyhydroxylated steroidal hormone produced endogenously in plants and recognized as the sixth generation phytohormone. BRs satisfy all the prerequisites to be considered as plant hormones i.e., natural in occurrence, mobility and activity in extremely low concentrations [2]. Being identified as key regulators of plant growth and development, they control important functions, such as promotion of cell elongation and division, photomorphogenesis, fertility, seed germination, senescence, retardation of abscission, promotion of ethylene biosynthesis and protection from environmental stresses. At first, it was identified as a growth promoting substance from the rapeseed (*Brassica napus*) pollen extract and was called 'Brassin' [3]. Later on, this new growth promoter was named as Brassinolide (BL) and its structure was determined [4]. Further, more numbers of steroid hormones were discovered and Brassinolides, along with its related compounds were collectively named brassinosteroids (BRs) [5].

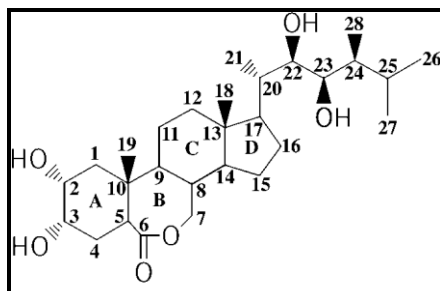
Occurrence of Brassinosteroids in plant kingdom

Since the discovery of brassinolide (BL), nearly 70 natural BRs have been identified from diverse group of plants including dicotyledons, monocotyledons, gymnosperms and algae. BRs generally occur at quite low levels endogenously and its concentration varies from plant to plant and tissues to tissues. These have been detected in various plant organs such as pollen, anthers, seeds, leaves, stems, roots, flowers, and grain. Pollen and immature seeds are generally considered as the richest source of BRs, while other vegetative plant parts like shoots and leaves have

low levels of BRs. Among naturally occurring BRs, Castasterone (CS) and BL are the most important BRs because of their wide distribution as well as their potent biological activity [6].

Chemical Structure of Brassinosteroids

The chemical structure of BRs is close to animal and insect steroid hormones. Natural BRs, have a common 5α -cholestane skeleton, and their structural variations come from the kind and orientation of oxygenated functions in rings A and B [7]. Oxidation and reduction during the biosynthesis leads to these structural modifications. Out of 70 BRs isolated, 65 are free and the remaining 5 are sugar and fatty acid conjugates. Depending on the alkyl-substitution on the C-24 in the side chain, the unconjugated ones are classified as C27, C28 or C29 BRs.



The structure of brassinolide, a commonly occurring BR [8]

Biosynthesis of BRs and its Mutants

Brassinosteroids are synthesized from different plant sterols. The biosynthetic pathway of brassinolide was first studied using feeding experiment on cell culture of *Catharanthus roseus* [9] and it involves three steps. At first campesterol (which is the precursor of brassinolide) is converted to campestenol, which is further converted to castasterone through either early or late C-6 pathway. In the final step, castasterone is oxidized to produce brassinolide [10]. Both early and late C-6 pathways are known to operate in several plants including *Arabidopsis*, *Catharanthus roseus*, etc. But in plants like tomato, the late C-6 oxidation pathway may be the major pathway leading to active BR production [11]. The co-occurrence of brassinosteroids belonging to the different pathways in the same plant species indicates that these pathways could be ubiquitous in plants [12].

The major breakthrough in the brassinosteroids research was identification of mutants involved in BR biosynthesis and signalling pathway using model plant *Arabidopsis thaliana* [13]. These mutants show drastic phenotypic effects such as dwarf stature, dark-green leaves, delayed flowering, reduced fertility and are blocked during biosynthesis pathway at different steps. When these mutants were treated with exogenous BRs, their phenotypic effect reverted back to wild-type [14], suggesting that brassinosteroids might have an essential role in plant growth and development.

Brassinosteroids Responses in Ornamental Crops

Ornamentals includes various groups of plants such as trees, shrubs, climbers, annuals, foliage plants, turfgrasses, etc. which are primarily grown for beautification and display purpose. Unlike other horticultural crops, here either flowers, leaves or whole plant is considered as the economic part. These group of plants also show remarkable response to application of different growth regulators. While the role of traditional growth regulators is well recognised, use of recently identified ones such as BRs is gaining momentum gradually. An array of plant growth related functions such as seed germination, vegetative growth, flowering and flower quality, post-harvest life, response to biotic and abiotic stresses etc. are known to be influenced by BRs application. Among different types of BRs available in the market, epibrassinolide (EBL) is most commonly used and applied in the form of seed/corm treatment and foliar spraying. Some responses of the ornamental plants to the application of BRs are discussed here under:

Plant Propagation

Exogenous application of BRs at lower concentrations stimulated root elongation in both wild type and BR-deficit mutants of *Arabidopsis thaliana*, but it was inhibited at higher levels. And BRs and auxin stimulated root growth largely additively [15]. Similar effect was observed in rooting of rosehip (*Rosa canina L.*) genotypes by hardwood cuttings, where highest values in terms of root length, root number, fresh and dry root weight of SRK 26 genotype

were obtained from combined treatment of 24-epibrassinolide (24-epiBL) (0.50 ppm) with IBA (2000 ppm) and highest viable cutting rate was obtained from 24-epiBL (0.50 ppm) application [16]. Application of 24-epiBR and 28-homobrassinolide (28-homoBL) not only stimulated root formation, but also root growth in geranium stem cuttings and their positive effect was also reflected in enhancement of fresh and dry weight of the shoot system [17].

In gladiolus, foliar sprays of BR (10 ppm), followed by GA₃ (150 ppm) at 3rd and 6th leaf stage significantly increased number of corms per plant, corm size, corm weight, and propagation coefficient [18]. Application of BRs in micropropagation of orchids was reported for the first time [19], where successful initiation of protocorm-like bodies (PLBs) and *in vitro* regeneration was achieved using shoot tip sections of *Cymbidium elegans* and 4.0 μM 24-epiBL supplemented basal medium. Contrastingly, [20] reported that 24-epiBL and 28-homoBL couldn't initiate induction of PLBs when used in PGR-free Teixeira *Cymbidium* (TC) medium.

Physiological and Growth Response

As mentioned earlier, BRs have a significant impact on plant growth and development as they promote photosynthesis and related processes. Plants of daisy cv. Dwarf Pink sprayed with a combination of BR (0.5ppm) and gibberellic acid (150ppm) recorded maximum vegetative growth with respect to plant height, number of leaves, internodal length and leaf area [21]. Similar response was seen in chrysanthemum cv. Yellow Gold [22], where effects of BR treatment on different growth parameters were at par with gibberellic acid treatment. Effects of brassinosteroids alone or in combination with gibberellin or auxin on the development and foliar anatomy of *Tabebuia alba* was studied [23]. They observed a positive interaction between GA₃ and brassinolide when treated together, increasing the petiole and stem growth rate of *Tabebuia alba* seedlings. [24] found that seed priming and foliar application of 24-epibrassinolide for marigolds improved their growth traits and photosynthesizing pigments. Growth response of some flowering annuals such as *Petunia hybrida*, *Tagetes erecta*, and *Calendula officinalis* to plant and human steroid, suggested that 24-epibrassinolide at 1 mg/L increased leaf area, while homobrassinolide at the same concentration had the highest impact on the carotenoid level, chlorophyll a content, and total chlorophyll content [25]. In *Jasminum sambac*, exogenous application of 24-epibrassinolide at 2 μM proved to be the best treatment for morpho-physiological and bio-chemical traits like number of leaves, chlorophyll contents, leaf area, fresh and dry weight ratio, number of flowers per plant, photosynthetic rates, transpiration rate and stomatal conductance compared with other treatments [26].

Flowering, Flower Quality and Yield attributes

Regulation of flowering time in flower crops is essential as growers can harvest the flowers as per the need and demand in the market in order to get good price for their produce. Role of BRs in flower regulation was unknown, until recent discovery of BR mutants, where flowering is delayed, suggesting control of BRs on early flowering. This response was observed in tuberose cv. Prajwal, where foliar spray of 1ppm 28-homobrassinolide recorded early flowering, highest number of florets, floret weight per spike and length of spike [27]. Foliar application of 24-epibrassinolide at 10-8 M concentration promoted not only vegetative parameters, but also significantly increased number of flowers, mean diameter of flowers along with fresh and dry weight of flowers in African marigold [28]. Longer scapes, thicker leaves, prolonged flowering days and decrease abortions in lotus was observed by the application of 24-epihomobrassinolide [29].

In a study on rose scented geranium (*Pelargonium graveolens*), 24-epibrassinolide increased economic yield of the plant (herbage yield) by higher chlorophyll levels coupled with an increase in photosynthesis [30]. Further, significant increase in the essential oil content in geranium plants was observed when applied at 3 μM concentration along with slight increase in the content of geraniol and a marginal decrease in citronellol. Similar observation was recorded when EBL application @ 3 μM, increased the essential oil contents and its quality in *J. Sambac* [26]. [31] studied antioxidant capacity and pigment synthesis of *Calendula officinalis* L. influenced by benzyl adenine (BA) and epibrassinolide (EBL). Here, they found highest phenol content at combined action of 5 mg/L BA × 1 mg/L EBL, highest antioxidant at 0 mg/L BA × 5 mg/L EBL and highest flavonoid content at 10 mg/L BA × 10 mg/L EBL. Corm priming and foliar spraying of 1 μM EBL in gladiolus, recorded highest levels of corm sprouting and flower spike emergence along with highest floret numbers, flower spike fresh and dry weight, and vase life [32].

Post-harvest life of Flowers

The post-harvest life of ornamentals is directly related to its economics as it influences its longevity. Flowers with prolonged shelf-life are more preferred by the consumers. Post-harvest life of ornamentals can be manipulated using various storage methods, use of chemical preservatives and certain growth regulators. PGRs such as cytokinin and

GA₃ are known to delay senescence in some cut flowers. BRs were also found to prolonged the vase life in several cut flowers. [33] investigated effect of 2,4-epibrassinolide treatment on the postharvest quality of fresh daylily (*Hemerocallis* sp.) flower buds. They found flower buds treated with 0.5mg/L EBR showed electrolyte leakage and MDA content, implying improve cell membrane integrity of flower buds. Further, it maintained high GA content, reduced ABA content and respiration rate, contributing to the maintenance of high percentage of buds with good visual quality, chlorophyll content and weight loss of daylily flower buds, suggesting EBR could benefit in delaying senescence and preserving the postharvest quality of daylily flower buds. In another study, EBL at lower concentration (3 µmol/L) significantly decreased the activity of ACC oxidase, enhanced different physiological parameters of the flowers including water uptake rate, chlorophyll and anthocyanins contents, and decreased the MDA production rate resulting in a significant increase in the vase life of lisianthus (*Eustoma grandiflorum*) cut flowers [34]. While at a higher dose (9µmol/L), it adversely affected the flower vase life and postharvest quality by enhancing the ACC oxidase enzyme activity and subsequent ethylene production. Fresh-cut lotus root slice treated with 80 nM 24-epibrassinolide (EBR) followed by storage at 8° C, reduced browning of cut surface in lotus root slices and promoted activity of antioxidant enzymes [35].

Biotic and Abiotic Stress Tolerance

In addition to their impact on many plants' physiological and developmental processes, BRs play a role in biotic and abiotic stress tolerance, including disease resistance and tolerance against drought, salt, heat, cold, hypoxia, and heavy metals [36]. Greenhouse roses are greatly affected by *Botrytis cinerea* which causes gray mold disease, a major post-harvest biotic stress in roses. Positive responses of BRs application against *Botrytis* infection have been reported in several studies [37, 38].

In pot marigold (*Calendula officinalis*), it was reported [39] that under water-stress conditions combined application of EBL and Ascorbic acid induced tolerance to water deficit, reduced the reactive oxygen species by increasing antioxidant enzymes activity and osmotic adjustment along with improved capitula and essential oil yield. Interaction of electrical conduction of the nutrient solution and spray of 24-epibrassinolide on *Calendula officinalis* under hydroponic conditions was studied [40]. They found maximum number of flowers was obtained in EC (4.5 dS.m⁻¹) and at EBL (0.5 µM), suggesting that EBL at 0.5 Mm led to reduction of damage resulting from increase in concentration of the nutrient elements. Exogenous application of 24-epibrassinolide at 10-8M to zinnia (*Zinnia elegans*) plants under Pb stress, improved chlorophyll a, b, total chlorophyll, total carotenoid content and flower characters along with increased activity of antioxidant enzymes such as SOD, CAT [41].

Turfgrasses are adversely affected by soil salinity which reduces their growth and development, resulting in poor aesthetic value and playing surfaces. BRs application in turfgrasses have proven to be beneficial as they reduce inhibitory effects of salt stress. In a study on perennial ryegrass (*Lolium perenne*) under salt stress condition [42], EBR at 10nM concentration improved salt tolerance by enhancing leaf relative water content (RWC), proline, soluble sugar, soluble protein content and antioxidants activity, while reducing electrolyte leakage, malondialdehyde (MDA), H₂O₂ content and accumulation of Na⁺ ions. In another study, [43] observed similar results, where EBL treatment alleviated detrimental effects of the salt stress by enhancing turfgrass quality (7–26%), and increasing shoot growth and clipping yield in perennial rye grass (*Lolium perenne*) and tall fescue (*Festuca arundinaceae*).

Conclusion and Future Perspectives

Considerable amount of research has been done on functioning of brassinosteroids and their efficacy as a plant growth regulator, during the past two decades. These cumulative efforts have proven that BRs significantly influence physiological and developmental processes of plants. In the current scenario of climate change, these might be helpful in providing a defence mechanism to plants against several abiotic stresses, however their role against biotic stresses needs more investigation. As BRs mediate these responses by regulating numerous genes, their actions at molecular level and interaction with the conventional phytohormones also require more examination. Although they have been tried and tested in wide group of plants, work on ornamental crops is insignificant and mostly of recent times. And from commercial point of view their usage is still restricted only to a few agricultural and horticultural crops. While showing inhibitory effects at higher dose, they perform satisfactorily under very low concentrations. On that account, crop specific scientific studies for standardization of dose, stage and mode of BRs application is need of the hour.

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Publication History

Received	22.08.2023
Revised	23.03.2023
Accepted	25.03.2023
Online	31.03.2023