Brassinosteroids and Gibberellic Acid act synergistically to influence plant growth and development

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ABSTRACT

Plants treated with brassinolide alone or where brassinolide was combined with gibberellic (BL+GA_3) exhibited a notable increase in the size of both epidermal and mesophyll cells, this was also true for leaf size but such changes were not observed in the Control plants. A combination of Gibberrelic acid (GA_3) and brassinolide exhibited the highest stem elongation as well as petiole growth rates. Lone brassinolide application stimulated petiole growth but no significant increase in stem growth as compared to the Control. Brassinolide alone had no significant effect on petiole growth, but the combination of brassinolide with GA_3 increased petiole growth. There was a synergistic interaction between gibberellic acid and brassinolide in stimulating significant increase in stem growth in comparison to the other treatments where these plant growth regulators were used separately. Brassinolide treated plants at 150 mg.$L^{-}1$ triggered a small increase in petiole growth when compared to the control. A combination of Gibberrelic acid (GA_3) and brassinolide (BL) triggered a significant development of lateral buds. The herbage yield in terms of the leaf number, leaf weight and total leaf area was higher owing to brassinolide (BL) treatment in comparison to the control. Biochemical analysis of the leaf in this study showed that plants treated with brassinolide contained more sugars (both reducing and non-reducing) as well as starch in comparison to the control plants. The higher levels of starch and sugars in the leaf of plants treated with brassinolide positively correlated with higher chlorophyll levels as well as photosynthesis.

KEYWORDS: Plant hormones, Brassinosteroids, Stem elongation; photosynthesis; Gibberellic acid; etc.

1.0 INTRODUCTION

Brassinosteroids (BR) are a group of polyhydroxy lactones with a common 5 α-cholestane skeleton. This class of plant hormones has been extracted from a variety of plant species from pollen grains, anthers, seeds, stems, leaves, roots, flowers, and other organs. Plants produce numerous steroids and sterols, some of which are recognized as hormones in animals (Geuns, 1978; Jones and Roddick, 1988). Brassinolide (BL) is the most bioactive form of the growth-promoting plant steroids termed brassinosteroids (BRs). Grove et al. (1979) purified 4 mg of BL from 40 kg of bee-collected rape pollen to determine its structure, which shows similarity to animal steroid hormones. Brassinosteroids are important plant growth regulators in multi-processes at the nanomolar (nM) concentration, including cell division, cell elongation, vascular differentiation, reproductive development and modulation of gene expression (Bajguz, 2007). They also influence various developmental processes like germination of seeds, rhizogenesis, flowering, senescence, abscission and maturation. The highest brassinosteroid concentrations were measured in pollen and immature seeds (Bajguz and Tretyn, 2003). Brassinosteroids are considered ubiquitous in plant kingdom as they are found in almost all the phyla of the plant kingdom like alga, pteridophyte, gymnosperms, dicots and monocots (Bajguz, 2009). Brassinosteroids are considered also as a new group of plant growth hormones that perform a

variety of physiological roles like growth, seed germination, rhizogenesis, senescence, and resistance to plants against various abiotic stresses (Rao et al., 2002). Examples of brassinosteroids are Castasterone (CS), which is the most widely distributed brassinosteroid (53 species), followed by brassinolide (BL) (37 species). In pollen grains of adult *H. annuus* plants, four BRs (BL, CS, dolichosterone, norcastasterone) have been identified, and a conversion of CS to BL in metabolically active plant cells. Brassinosteroids are a novel group of phytohormones with significant growth promoting nature. Brassinosteroids are considered as growth regulators with pleiotropic effects, as they influence diverse physiological processes like growth, germination of seeds, rhizogenesis, senescence etc. and also confer abiotic stress resistance in plants. Brassinosteroids occur in gymnosperms, monocotyledonous, dicotyledonous plants, and in algae. Brassinosteroids give resistance to plants against various abiotic and biotic stresses (Sasse, 2003). Yokota et al. (1998) confirmed that brassinosteroids are obligatory plant constituents, with the highest concentrations occurring in the reproductive organs and in growing tissue such as pollen, immature seeds, shoots, etc. The highest measured concentration is about 10^{-1} nmol $^{g-1}$ fresh weight (brassinolide in the pollen of *Brassica napus* and *Vicia faba*) and the lowest is about 10^{-7} nmol $^{g-1}$ (homocastasterone in immature seeds and sheats) of Chinese Cabbage *Brassica campestris var. pekinensis*. It has been observed that the *Arabidopsis* mutations abolishing the biosynthesis of Brassica that result in a dwarf phenotype can be restored to a wild-type phenotype by externally provided brassinolide and intermediates of brassinosteroid biosynthesis (Szekeres et. al., 1996). Characterization of Brassinosteroids-insensitive mutants showing a similar dwarf phenotype has subsequently identified key genes in BR signaling (Clouse et al., 1996).

2.0 MATERIALS AND METHOD

Sweet pepper seeds were surface-sterilized and germinated on filter paper soaked with either distilled water in the case of Control plants or combined solutions of Brassinolide, BL, and Giberellic acid, GA_3 (BL+GA_3) or separate solutions of Brassinolide, BL, or Gibberellic acid, GA_3. Three weeks after germination, twenty (20) healthy pepper seedlings were selected for each treatment. Seedlings were raised in the greenhouse at one plant per pot in small earthen pots under same conditions. Each treatment was applied with a small hand sprinkler. Each treatment was replicated four (4) times and sprayed with 100 mL of 150 mg.L^{-}1 of each solution. Control plants were sprayed with only distilled water of the same volume. Foliar application was repeated at 28 days interval until two weeks before plant harvest. The chlorophylls were extracted in 80% (v/v) acetone and determined following the method of Arnon, et al. (1949). Total chlorophyll content was estimated by the method of Coombs et al. (1985). Hill reaction activity (HRA) was determined according to Cherry (1973). Biochemical analysis of leaves for the presence of reducing sugars was conducted after Nelson, (1944), while non-reducing sugars were determined using the method by Loomis and Shull (1937). For starch determination in leaf, the method after McCready et al. (1950) was employed.

3.0 RESULTS AND DISCUSSION

Generally, plants treated with brassinolide (BL) alone or where brassinolide was combined with gibberellic acid (BL+GA_3) exhibited a notable increase in the size of both epidermal and mesophyll cells, this was also true for leaf size but such changes were not observed in the Control plants. A combination of Gibberrelic acid (GA_3) and brassinolide (i.e. BL+GA_3) exhibited the highest stem elongation as well as petiole growth rates. Lone brassinolide application stimulated petiole growth but no significant increase in stem growth as compared to the Control. Brassinolide alone had no significant effect on petiole growth, but the combination of brassinolide with GA_3 increased petiole growth. These results suggest a synergistic interaction between gibberellic acid and brassinolide in stimulating significant increase in stem growth in comparison to the other treatments where these plant growth regulators were used separately. Brassinolide treated plants at 150 mg.L^{-1} triggered a small increase in petiole growth when compared to the control. Further, in this study, a combination of Gibberrelic acid (GA_3) and brassinolide (BL) triggered a significant development of lateral buds. The herbage yield in terms of the leaf number, leaf weight and total leaf area was concomitantly increased owing to brassinolide (BL) treatment in comparison to the control. The result of this study is similar to that reported by Swamy and Rao (2010) which stated that plants obtained from brassinosteroid treated stem cuttings exhibited better foliage growth as compared to that of the control and that increase in leaf area amounted to the enhancement in photosynthetic area and that might have contributed to increase in growth. Brassinosteroids are known to induce a broad spectrum of responses, including stimulation of longitudinal growth of young tissues via cell elongation and cell division (Hu et al., 2000) and vascular differentiation, which is a developmental process critical for plant growth. Brassinosteroids effectively stimulated the elongation and formation of lateral shoots and shoot buds. Similar results were reported by Pereira-Netto et al. (2006) in Eucalyptus and *Malus prunifolia*. Biochemical analysis of the leaf in this study showed that plants treated with brassinolide contained more sugars (both reducing and non-reducing) as well as starch in comparison to the control plants. The higher levels of starch and sugars in the leaf of plants treated with brassinolide positively correlated with higher chlorophyll levels as well as photosynthesis. This result is in harmony with that reported by Fariduddin et al. (2009) which highlighted that application of brassinosteroids increased the total chlorophyll content and hence net photosynthetic rate in *Brassica juncea*. Similarly, Bajguz and Czerpak (1998) and Swamy and Rao (2008) reported increase in photosynthesis due to brassinosteroid application in *Chlorella* and *Geranium*. Further, this result is similar to that observed by Vardhini and Rao (1998) that brassinosteroids substantially increased the growth of the plant which was associated with enhanced levels of DNA, RNA, soluble proteins, and carbohydrate. Gibberellic acid stimulated the elongation of plant internodes. Plants treated with gibberellic acid (GA_3), as the sole plant growth regulator, were at least three (3) folds greater in height in comparison to the control. Gibberellin stimulated elongation of internodes and proved to be necessary for meristem growth. The exogenous application of GA_3 increased sweet pepper plant height even after the end of hormone treatment and had a positive effect on petal elongation and inflorescence stalk

length. Sweet pepper plants treated with giberrellic acid (GA_3) increased the number of inflorescences per plant by 30% as compared to the Control. The application of gibberellic acid (GA_3) outwardly eliminated the deficiency of endogenous gibberellic acid (GA_3) and enabled the sweet pepper berry to be as large as the fruits in the control plants which had seeds. The results of this study show that exogenously applied gibberellic acid promoted increased plant height; this is in agreement with the work reported by many authors. On the basis of comparison of molecular changes in transcript and metabolite levels, low gibberellic acid (GA_3) levels affect plant growth by uncoupling growth from carbon availability.

4.0 CONCLUSION

Brassinosteroids are a class of phytohormones that has been found to influence many physiological processes in plants. Their wise application within recommended doses for different crop plants can positively influence plant growth and development and bring about the achievement of desired morphological and yield parameters. Their combinations with other classes of phytohormones, for example gibberellic acid, auxins, have been seen to be very useful. Brassinosteroids have been used successfully to improve the yields and quality of agricultural and horticultural crops, especially in relation to disease control and for improving crop resistance against biotic and abiotic stresses.

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