Indian J. Plant Physiol., Vol. 11, No. 1, (N.S.) pp. 1-12 (Jan.-Mar., 2006)

Mini Review

BRASSINOSTEROIDS – NEW CLASS OF PLANT HORMONE WITH POTENTIAL TO IMPROVE CROP PRODUCTIVITY

B. VIDYA VARDHINI, S. ANURADHA AND S. SEETA RAM RAO*

Department of Botany, University College of Science, Osmania University, Hyderabad –500007

Received on 26 Dec., 2003, Revised on 10 Feb., 2006

SUMMARY

Brassinosteroids are a new group of plant growth substances with significant growth promoting activity. In addition, brassinosteroids also confer resistance to crop plants against environmental stresses. The article reviews the studies employing brassinosteroids on crop plants such as vegetables, fruits, cereals, oil seeds and others. It also highlights the constraints faced earlier in large scale field application of brassinosteroids and also presents the current scenario enlisting the commercially available brassinosteroids. The potentiality of brassinosteroids in enhancing crop productivity is discussed.

Key words: **Brassinosteroids, crop productivity, plant growth, stress tolerance**

INTRODUCTION

Brassinosteroids are a new group of plant hormones with growth promoting activity (Mandava 1988). The ability of certain pollen extracts to promote growth led to the discovery of this group of substances in plants. Collective efforts initiated by the scientists at various agricultural research stations (ARS) of USDA resulted in the isolation of an active factor from the pollen grains of rape plant (*Brassica napus* L.) which was named as brassinolide (Grove *et al*. 1979). Subsequently castasterone, another steroidal substance was isolated from insect galls of chestnut (*Castanea crenata*). As the first steroidal plant growth regulator was isolated from *Brassica napus*, a generic name 'brassinosteroids' has been given to this new group of phytohormones. Brassinosteroids are polyhydroxy steroids. They have a common α-cholestane skeleton and their structural varieties come from the kind and the orientation in the A/B rings and side chain (Yokota 1997). Brassinosteroids can be classified as either C_{27}, C_{28} or C_{29} BRs according to the number of carbons in the structure. Till now, 65 brassinosteroiods and 5 sugar fatty acid conjugates have been detected in the plant kingdom (Bajguz and Tretyn 2003). However brassinolide, 24-epibrassinolide and 28-homobrassinolide are the three bioactive brassinosteroids, being widely used in physiological studies. Brassinosteroids have been reported from 60 species which includes 51 angiosperms (12 monocots and 39 dicots), 6 gymnosperms, 1 pteridophyte (*Equisetum arvense* strobillus), 1 bryophyte (*Marchantia polymorpha*) and 1 alga (*Hydrodictyon reticulatum*) [Rao *et al*. 2002., Bajguz and Tretyn 2003] and are provisionally considered ubiquitous in plant kingdom (Sasse 1999). Brassinosteroids satisfy all the prerequisites to be considered as plant hormones i.e. natural in occurrence, mobility and activity in extremely low concentrations (Rao *et al.* 2002). Brassinosteroids are considered as plant hormones with pleiotropic effects as they influence wide array of developmental processes such as growth, seed germination, rhizogenesis , flowering, senescence, abscission and maturation (Sasse 1999).

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* Corresponding author, E-mail: ssrrao2002@rediffmail.com

The studies conducted with brassinosteroid biosynthetic mutants provided compelling evidence for the essentiality of this new group of growth regulators for the normal growth and development. The growth of BR-deficient dwarf mutant of *Arabidopsis* such as *cpd*$_1$ *dwf 1* were rescued by brassinolide (Bishop *et al*. 1999). The growth of brassinosteroid deficient dwarf mutant *lkb* of *Pisum sativum* was restored with the exogenous application of brassinolide (Nomura *et al*. 1997). The dwarf tomato mutant *dumpy* (dpy), a dwarf with abnormal leaf phenotype (curled with dark ruggose leaves) with suppression of axillary shoots was found rescued to wild type with the foliar application of brassinosteroids (Bishop and Yokota 2001). Rinrei, is a dwarf mutant of faba bean (*Vicia faba*) characterized by dark green leaves and reduced plant height, contributed by a single recessive gene mutant and the gene was tentatively identified as *brassinosteroid deficient dwarf* (*bdd*) (Fukuta *et al*. 2002). The phenotype of Rinrei was restored to that of wild type by the application of brasssinolide (Fukuta *et al.* 2002).

Inhibitor studies employing brassinozole, a specific inhibitor of brassinolide in plants, further strengthened the view that brassinosteroids are essential for growth and development in plants (Asami *et al*. 2000). Now there is consensus that brassinosteroids represent the sixth group of phytohormones (Sasse 2003). Studies employing brassinisteroids insensitive mutant of *Arabidopsis* led to the discovery of BRI as brassinosteroid-receptor. *BRI 1* is a leucine rich repeat receptor kinase(LRRRK) known to recognize protein ligands [Li and Chory 1997]. *BRI 1* and BAK (BRl *1*) associated kinase exists as inactive monomers (Fellner 2003). Brassinosterods binding via a BR-binding protein stabilizes heterodimer formation, activates receptor kinases and initiates BR signalling cascade (Fellner 2003). This results in regulation of the activity of downstream elements of the signal transduction pathway, the details of which are further to be elucidated.

Similar to all established phytohormones brassinosteroids regulate growth by influencing of nucleic acid and protein synthesis. The induction of mungbean epicotyls growth was found associated with increase in RNA polymerase activity and elevated levels of RNA (Kalinch *et al*. 1985; Wu and Zhao 1993). 24 - Epibrassinolide stimulated growth of Chinese cabbage mesophyll protoplast was associated with enhanced protein levels (Nakajima *et al*. 1996). Inhibitors of nucleic acid synthesis (actinomycins) and protein synthesis (cyclohexamide) were found to set aside the epibrassinolide- stimulated growth in mungbean epicotyls (Wu and Zhou 1993).

Agricultural uses of brassinosteroids

Immediately after the discovery of brassinolide, the application of this substance for improving crop productivity employing vegetable crops like radish, lettuce, bush bean and pepper was started. Since the publication of the articles “New chemicals promise larger crops” by Maugh (1982) to “Twenty years of brassinosteroids: Steroidal plant hormones warrant better crops for the XXI Century” by Khripach *et al*. (2000), several publications appeared in the literature reflecting the great potentialities of this new group of substances to improve crop productivity. However, the saga of brassinosteroids during these twenty years has its webs and flows marked by reluctant conservatism on one side and unlimited enthusiasm on the other.

There are several reasons for the cautious approach adopted in the large scale field application of brassinosteroids. One of the important constraints was the prohibitive costs of the new chemicals and it is true that the academic grade brassinosteroids are highly expensive. Bhushan Mandava who is one of the pioneer in this field of research predicted in early eighties that 1 gram of synthetic brassinosteroid would cost between $ 5 to $10 once commercial production starts and also expressed the view that this cost is not unreasonable, since a gram would cover as much as 5 acres (Maugh 1981). However, all through these years, the academic grade of brassinosteroids were not in the reach of scientists for large scale field works. However, prompted by the success stories, several agrochemical industries in Japan, USA, Belarus, Cuba and India recently started producing the synthetic analogues on commercial scale (Table 1) and now the costs of brassinosteroids have been brought down to affordable levels. The shadow of ‘Flop story’ of triacontanol also put a rider on the commercial production of brassinosteroids . The euphoria generated

Table 1. Commercially available brassinosteroids

Trade name	Country	Recommended crops
Biobras-6	Cuba	Potato, sugarcane and onion
Biobras-16	Cuba	Sugarcane , maize and onion
Bountee	India	Tea
Brassinosteroid 1105	USA	Soybean
Cherkaz	Belarus	Barley
Combine	India	Grapes
DAA-6	Cuba	Potato, tomato and sugarcane
Double	India	All major crop plants
DI-31	Cuba	Rice
Epin	Belarus	Plum, sloepruce and cherry
MH_5	Cuba	Coffee
ME	Cuba	Rice
Ts 303	Japan	Rice, potato wheat, rape seed and beet
Tskamim	Belarus	Barley
Yaponiya	Belarus	Barley

in the late 70's on the 'unlimited potential' of triacontanol in boosting crop yields were blasted off with the realization of inconsistency in its activity. However, in case of brassinosteroids, the consistency in improvement in agricultural production has been proved by the extensive field work results from Russia and Japan. The phobia to the suffix 'steroid' is another stigma for the entry of brassinosteroids to commercial market. However in 1980's itself, the fear of 'side effects' was annulled by Mandava and others. The clinical studies proved that only at a very high concentration of around 1000mg /kg in rats, brassinosteroids bring some undesirable side effects and that concentration is several thousand folds more than the reachable concentrations in living organisms (Khripach 1999). Infact brassinosteroids are natural and ubiquitously present in plant kingdom. They are not xenobiotic substances and exhibit no negative impact on human health.

Brassinosteroids improve the resistance of the plants against environmental stresses such as water stress, salinity stress, low temperature stress and high temperature stress (Rao *et al*. 2002). The ability of brassinosteroids to confer resistance to plants against abiotic stresses has been their added advantage for usage in agriculture for improving crop productivity. Kumaro and Takatsuto (1999) rightly stated "the role of brassinosteroids in protecting plants against environmental stresses will be an important research theme for clarifying the mode of action of brassinosteroids and may contribute greatly to the usage of brassinosteroids in agricultural production". The blending of two traits (growth promotion and stress tolerance) as imparted by brassinosteroids will have great economic bearing in future agriculture for enhanced crop production. The work relating to the application of brassinosteroids on crop improvement is given in Table 2. The results of the studies concerning the effect of brassinosteroids on the performance of plants under stress conditions are incorporated in Table 3.

Table 2. Effect of brassinosteroids on crop plants

	Crop	Compound	Response (Increase)	References
Vegetable Crops				
	Lettuce	Brassinolide	Growth & yield	Meudt *et al.* 1983
		Biobras-16		Alfonso and Nunez 1996
	Radish	Brassinolide Epibrassinolide Homobrassinolide	Storage root yield	Sujatha 2001
	Bush bean	Brassinolide	yield	Meudt *et al.* 1983
	Pepper	Brassinolide	yield	Meudt *et al.* 1983
	Garlic	Biobras-6	yield	Nunez *et al.* 1994
	Spinach	Epibrassinolide DA-6	growth & yield	Liang *et al.* 1998
	Tomato	Brassinolide Epibrassinolide	fruit yield	Vardhini and Rao 2001
		Homobrassinolide	acceleration of ripening	Vardhini and Rao 2002
		Biobras-6	yield	Nunez 2000
		Biobras-16	yield	Augustin 2001
	Potato	Brassinosteroids	tuber yield	Nunez *et al.* 1995
	Onion	Biobras-6 Biobras-16	Bulb yield	Nunez *et al.* 1998
	Soybean	Epibrassinolide	Growth & yield	Nakaseko and Yoshida 1989
	Yam	DA-6	Tuber yield	Labrada 1997
Cereals				
	Wheat	Brassinolide	Yield	Braun and Wild 1984
		Epibrassinolide	Yield	Ramraj *et al.* 1997
		Homobrassinolide	Growth & yield	Sairam 1994
	Rice	Brassinolide	Grain yield	Yokota and Takahashi 1986 Krishnan *et al.* 1996
		Epibrassinolide Homobrassinolide	Yield	Ramraj *et al.* 1997
		Biobras-6	Yield	Franco *et al.* 2002
	Maize	Brassinolide	Yield	Takahashi *et al.* 1995
		Epibrassinolide	Growth & yield	Ikekawa and Zhao 1991
	Barley	Biobras-16	Growth & yield	Almenares *et al.* 1999

	Crop	Compound	Response (Increase)	References
		Epibrassinolide	Growth & yield	Lapa *et al.* 1998 Prusakova *et al.* 1995
Fruit crops				
	Grapes	Epibrassinolide	Yield	Ikekawa and Zhao 1991
		Brassinolide	Reduced abscission of young flowers, early maturation	Xu *et al.* 1994a
	Orange	Epibrassinolide	Growth & yield	Takahashi *et al.* 1985
	Citrus	Brassinolide	Retardation of abscission increased fruit lets	Iwahari *et al.* 1990
	Strawberries	Brassinosteroids	Reduced abscission of leaf and fruit lets	Pipattanawong *et al.* 1996
	Watermelon	Epibrassinolide	Yield	Ikekawa and Zhao 1991
	Banana	Biobras-16	*In vitro* multiplication	Rodriguez *et al.* 1998
Oil seeds				
	Groundnut	Brassinolide	Growth & Yield	Vardhini and Rao 1998
		Epibrassinolide & Homobrassinolide	Nodulation and nitrogenase activity	Vardhini and Rao 1999
	Mustard	Homobrassinolide	Yield	Hayath *et al.* 2000, 2001
	Lens	Homobrassinolide	Yield	Hayath and Ahmed 2003
Other crops				
	Tobacco	Brassinolide	Leaf yield	Yokota and Takahashi 1986
		Epibrassinolide	Leaf yield	Ikekawa and Zhao 1991
	Sugarcane	Biobras-6 & Biobras-16	Cane micro propagation	De 1aFa-CF *et al.* 1998
	Sugarbeet	Brassinosteroids	Yield	Schilling *et al.* 1991
	Lavender	Brassinosteroids	Increase in lavender oil yield	Youssef and Talaat 1998
	Cotton	Homobrassinolide	Yield	Ramraj *et al.* 1997
	Chickpea	Epibrassinolide	Proteins and yield	Ramos 1997

Table 3. Effect of brassinosteroids on plant growth under abiotic and biotic stresses

Stress	Plant	Compound	Effect	Reference
Abiotic stress				
Drought/water stress	Sugar beet	Homobrassinolide	Increased growth & yield	Schilling *et al.* 1991
	Wheat	Homobrassinolide	Increased yield	Sairam 1994, 1996
		Epibrassinolide	Enhanced growth and yield	Prusakova *et al.* 2000
	Chick pea	Epibrassinolide	Enhanced growth	Singh *et al.* 1993
	Sorghum	Epibrassinolide	Increased growth	Xu *et al.* 1994b,c
		Brassinolide Epibrassinolide Homobrassinolide	Alleviation of stress impact on seedling growth	Vardhini and Rao 2003
	Maize	Brassinolide	Increased tolerance	Li and Staden 1998
	Mustard	Brassinosteroid	Increased oil yield	Kumawat *et al.* 1997
	Jack pine	Brassinosteroid	Enhanced photosynthesis and growth	Rajasekaran and Blake 1999
Salinity stress	Mung bean	Epibrassinolide	Alleviation of stress	Zhao *et al.* 1987
	Sugarcane	DAA-6	Enhanced growth & yield	Gonzalez and Gianza 1997
	Groundnut	Epibrassinolide & Homobrassinolide	Alleviation of stress	Vardhini and Rao 1997
	Rice	Brassinosteroid	Improved tolerance	Takeuchi 1992
		Brassinolide Epibrassinolide Homobrassinolide	Enhanced seed germination & seedling growth	Anuradha and Rao 2001
			Amelioration of stress, restoration of pigment levels, increase in nitrate reductase	Anuradha and Rao 2003
		Brassinolide	Growth recovery	Hamada 1986
	Eucalyptus	Epibrassinolide	Enhanced seed germination	Sasse *et al.* 1995
	Barley	Epibrassinolide Homobrassinolide	Alleviation of stress	Khrustaleva *et al.* 1995
			Improvement of growth	Kuleava *et al.* 1991
	Wheat	Epibrassinolide	Growth recovery	Shakirova and Bezrukova 1998

Stress	**Plant**	**Compound**	**Effect**	**Reference**
High temperature	Brome grass	Epibrassinolide	Alleviation of stress and production of heat shock proteins	Wilen *et al*. 1995
	Wheat	Brassinosteroids	Enhanced photosynthetic rate and nitrate reductase activity	Kuleava *et al.* 1991
	Rape	Epibrassinolide	Increased stress tolerance	Dhaubhadel *et al.* 1999
Chilling stress	Tomato	Epibrassinolide	Increased stress tolerance	Dhaubhadel *et al*. 1999
	Maize	Brassinolide	Increased stress tolerance	He *et al*. 1991
	Tomato	Brassinolide	Alleviation of stress	Kamuro and Takatsuto 1999
	Rice	Epibrassinolide	Alleviation of stress and increased membrane stability	Wang and Zang 1993
		Brassinolide	Enhanced osmoregulation and grain yield	Hirai *et al*. 1991
		Brassinolide	Increased growth	Fujii and Saka 2002
	Cucumber	Brassinosteroids	Alleviation of stress	Katsume 1991
	Lilium Japonicum	Epibrassinolide	Regeneration of bulblets under stress	Ohshiro *et al.* 1997
	Citrus	TS303	Enhanced fruit set	Watanabe *et al.* 1998
Heavy metal stress	Barley	Brassiniosteroids	Enhanced resistance	Khripach *et al.* 2000
	Tomoto	Brassinosteroids	Reduced uptake of Cd and Zn	Khripach *et al.* 1999
	Sugarbeet	Brassinosteroids	Reduced uptake of Pb	Khripach *et al.* 1999
Herbicides	Rice	Brassinosteroids	Herbicide safener	Kim *et al.* 1993
Biotic stress	Tobacco	Brassinosteroid containing leaf extracts	Enhanced resistance against fungal pathogens	Roth *et al*. 2000
	Potato	Epibrassinolide	Enhanced resistance against mixed fungal phathogens	Volynets *et al.* 1997

Future Prospects

With the entry of reputed agrochemical companies in commercial production of brassinosteroids, the potentialities of brassinosteroids to improve agricultural yield can soon be visualized. The physiological and molecular mechanism of the action of brassinosteroids in enhancing productivity and improving resistance to environmental stresses, however, needs further elucidation. Futher research may also bring into focus many more significant roles to this group of steroids. New discoveries in this field may allow us to employ brassinosteroids as highly promising, environment-friendly natural substances suitable for wide application in plant protection and yield enhancement in agriculture. With availability of brassinosteroids at affordable prices along with the cost effectiveness, it is expected that, the chemical promise held by brassinosteroids to boost crop production will soon be accomplished.

ACKNOWLEDGEMENTS

The financial support to B.V.V. (SRA-Scientist pool) and S.A (RA) from Council of Scientific and Industrial Research, New Delhi, India is gratefully acknowledged.

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