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RESEARCH ARTICLE

INFLUENCE OF PEG IMPOSED WATER STRESS AND EXOGENOUS APPLICATION OF BRASSINOSTEROIDS ON METABOLITES IN RADISH

[1]Balaraju, P., [2]Ayodhya Ramulu, Ch., [3]Venkateshwarlu, M. and *[4]Ugandhar, T.

[1]Department of Botany, Govt. Degree and PG College Peddapally-505 172
[2]Regional Institute of Education, Unit IX, Sachiwalaya Marg, Bhubaneswar-751 022
[3]Department of Botany, University College Kakatiya University Warangal-506 009
[4]Department of Botany, SRR Govt. Arts and Science College Karimnagar-505 001

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ABSTRACT

Brassinosteroids (BRs) are plant hormones widely distributed throughout the plant kingdom in low concentrations and with structural homology to animal and insect steroids. BRs are involved in numerous physiological processes. The effect of 24-epibrassinolide (EBL) and 28-homobrassinolide (HBL) on the metabolites content of radish (*Raphanus sativus*) subjected to water (osmotic) stress being imposed by polyethylene glycol was studied. Brassinosteroids supplementation under desiccation stress was associated with elevated levels of soluble proteins, nucleic acids and carbohydrates. Brassinosteroids also enhanced the accumulation of the osmolyte free proline in radish seedlings challenged with drought stress. The results of present study demonstrate the protective role of brassinosteroids against PEG imposed water stress in radish seedlings.

INTRODUCTION

Brassinosteroid (BR) research began nearly thirty years ago when Mitchell *et al.* (1970) reported that organic extracts of *Brassica napus* pollen promoted stem elongation and cell division in plants. The announcement that the active component of these extracts was a unique steroid Grove *et al.* (1979.) stimulated international research interest on the chemistry and physiology of these very potent plant growth regulators, and by 1988 (when the first BR review appeared in this series; Mandava (1988), over 100 publications on BR-related topics were available. Survey of this literature Mandava (1988) led Mandava to conclude that “BRs may thus be regarded as a new group of plant hormones with a regulatory function in cell elongation and cell division” Although the case for BRs as endogenous plant growth regulators was strong Sasse (1991), there was not widespread acceptance of BRs as hormones and little or no attention was paid to these steroids in botany textbooks or general reviews of plant development. Radish (*Raphanus sativus* L.), belonging to the brassicaceae family, is an important annual root vegetable.

Corresponding author: Ugandhar, T.
Department of Botany, SRR Govt. Arts and Science College Karimnagar-505 001

Radishes are good source of ascorbic acid, folic acid and potassium, vitamin B6, riboflavin, magnesium, copper and calcium. It also a rich source of two important medicinal compounds: glucosinolates and isothiocynates (Curtis, 2003; Martınez-Villaluenga, 2008). Grusak and Dellapenna (1999) stressed the need of ‘divert research’ activities in improving the nutritional quality of plants with respect to nutrient content and composition.

Water stress is the most important abiotic stress limiting the plant growth. Water stress leads to a series of morphological, physiological and molecular changes which adversely affect plant growth. Drought stress is primarily manifested as osmotic stress resulting in the disruption of homeostasis and ion distribution in the cell (Wang *et. al.,* 2003). Phytohormones have been implicated in modulating the plant response to desiccation stress. Brassinosteroids (BRs) were discovered in 1970 by Mitchell and his co-workers and were later extracted from the pollen of *Brassica napus* L. (Grove *et al.,* 1979). BRs 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 (BRs) are a new group of polyhydroxy steroidal phytohormones which regulate broad spectrum of physiological processes including seed germination, plant growth, vascular differentiation and photomorphogenetic process (Rao *et. al.,* 2002; Sasse 2003). One of the most promising roles of BRs is their ability to confer resistance to wide array of abiotic stresses (Bajguz and Hayat 2009). The osmotic solution is used to impose water stress reproducibly under *in vitro* conditions. Polyethylene glycol (PEG) widely used to induce water stress, is a non-ionic water polymer which is not expected to penetrate into plant tissue rapidly (Macar *et al.,* 2009). As PEG does not enter the apoplast, water is withdrawn from the cell including cell wall and thus PEG solutions mimic dry soils more closely than solutions of low—MR osmotica which infiltrate the cell wall with solution (Verslues *et al*., 1998). The present study is undertaken to understand the effect of application of 24-epibrassinolide (EBL) and 28-homobrassinolide (HBL) on the metabolites of radish (*Raphanus sativus* L.) under water stress as imposed by PEG is being investigated.

MATERIALS AND METHODS

24-epibrassinolide (EBL) and 28-homobrassinolide (HBL) were purchased from CID tech research Inc, Mississauga, Ontario, Canada. Seeds of radish (*Raphanus sativus* L.) Pusachekthi varieties were obtained from National Seed Corporation, Hyderabad. After preliminary experiments employing a range of concentrations of polyethylene glycol (PEG 6000), a concentration of 15% was selected as the drought stress concentration. The osmotic potential of 15% aqueous solution of PEG is −2.95 bars at 25°C. Seeds were surface sterilized with 0.5% (v/v) sodium hypochlorite solution from commercially available 4% NaClO and washed thoroughly with several changes of sterile distilled water. They were soaked for 24 h either in 1) Distilled water (control) 2) 0.5, 1.0 and 2.0 μM concentrations of EBL and HBL 3) 15% Polyethylene glycol (stressed control) 4) 15% Polyethylene glycol supplemented with brassinosteroids. The two brassinosteroids (EBL and HBL) were employed at 3 concentration levels viz., 0.5 μM, 1.0 μM and 2.0 μM. 20 seeds for each treatment were distributed in separate petri plates (15 cm diameter) provided with whatman No. 1 filter papers. The seeds were allowed to germinate in the dark at 25 ± 1°C. On the seventh day fresh seedlings material (200 mg) was homogenized with 70% (v/v) ethyl alcohol and stored in deep freezer. The alcoholic homogenate was used for the metabolites content estimation.

Nucleic Acids

DNA and RNA fractions in the ethyl alcohol homogenate were separated by the method of Ogur and Rosen (1950). DNA was estimated by the procedure of Burton (1968) employing diphenylamine reagent and RNA was quantified by the method of Schneider (1957) using Orcinol reagent.

Soluble Proteins

Soluble proteins in alcohol homogenate (extract in case of enzyme assay) were precipitated by using 20% (w/v) trichloroacetic acid. The precipitate was dissolved in 5 ml of 1% (w/v) sodium hydroxide and was centrifuged at 4000 rpm for 10 min. The supernatant was used for estimation of proteins by Lowry *et al.* (1951) method.

Carbohydrates

The alcohol homogenate was heated and centrifuged. The supernatant was used for the estimation of reducing sugars (Nelson, 1944). Non-reducing sugars were calculated by the formula given by Loomis and Shull (1937). The residue was used for the estimation of starch (Mc Cready *et al.,* 1950).

Free Proline

The amount of proline content was estimated as described by Bates and others (1973). Seedling material (0.5 g) was homogenized with 10 ml of 3% (w/v) sulfosalicylic acid and the homogenate was filtered through filter paper. The supernatant was taken for proline estimation.

RESULTS AND DISCUSSION

The decline in the levels of nucleic acids was found negated by the exogenous application of BRs. Even in BRs alone treatments also, the levels of nucleic acids were found higher than the untreated and unstressed controls. The growth promotion in radish seedlings by BRs in unstressed and water stressed conditions was found associated with enhanced levels of DNA and RNA (Figures 1(a) and (b)). It has been suggested by Key (1969) that phytohormones regulate the growth by affecting nucleic acid synthesis. The results obtained in the present study with BRs are in conformity with the observations made by Key. The increase in the levels of nucleic acids might be due to enhanced synthesis and reduced degradation. Mung bean seedlings, when treated with 24-epibrassinolide, are reported to exhibit elevated activity of RNA polymerase and lowered activity of RNase and DNase (Wu and Zhao, 1993). Similarly, BRs application markedly increased the DNA and RNA content in 3 week old maize plants under salinity stress (Khallal *et al.,* 2009). The ameliorative influence of BRs on salinity stress induced growth inhibition in rice plants was linked to elevated levels of nucleic acids (Anuradha and Rao, 2003). In radish seedlings under drought stress, there was remarkable decline in soluble proteins. Supplementation of BRs resulted in improvement in soluble protein content in radish seedlings growing under water stress. Brassinosteroids alone treatments also cause enhancement in soluble protein levels (Table 1).

Sasse, (1990) suggested that BRs can stimulate the synthesis of particular proteins associated with growth. Supplementing the culture media with 24-epibrassinolide increased cell division rate and soluble protein content in Chinese cabbage protoplasts (Nakajima *et. al.,* 1996.). Sairam (1994) also obtained enhanced protein levels in wheat plants by 28-homobrassinolide under moisture stress. Similarly, the alleviating influence of BRs on salinity stress induced inhibition of growth in rice was found associated with elevated levels of proteins (Anuradha and Rao, 2001). Exogenous application of BRs to stressed seedlings reduced the inhibitory effect of drought stress and decrease in the contents of reducing sugars, non-reducing sugars and starch was significantly restored (Table 1). Brassinosteroids alone applications also enhanced the carbohydrate fractions in radish plants. Schilling *et al.,* (1991) reported that homobrassinolide increased sucrose content in sugar beets grown under drought stress.

Table 1. Effect of Brassinosteroids alone treatments and in combination with water stress on carbohydrate and protein profile of radish seedlings

Treatment	Reducing Sugars (mg^{-1} g FW)	Non Reducing Sugars (mg^{-1} g FW)	Starch (mg^{-1} g FW)	Protein (mg^{-1} FW)
Control	0.99 ±0.007g	1.69 ±0.041f	2.29 ±0.051g	4.72 ±3.280e
0.5µM EBL	1.14 ±0.056f	1.72 ±0.029e	2.54 ±0.058e	5.21 ±1.854cd
1µM EBL	1.52 ±0.068d	1.80 ±0.022d	2.66 ±0.042c	5.91 ±1.974c
2µM EBL	1.78 ±0.022b	1.99 ±0.044b	2.84 ±0.045a	6.72 ±2.067b
0.5µM HBL	1.23 ±0.033e	1.72 ±0.028e	2.42 ±0.074f	5.53 ±1.154c
1µM HBL	1.65 ±0.023c	1.82 ±0.034c	2.61 ±0.043d	6.11 ±1.274b
2µM HBL	1.83 ±0.032a	2.06 ±0.097a	2.81 ±0.050b	7.93 ±1.867a
15% PEG *	0.45 ±0.017n	0.72 ±0.006m	1.13 ±0.042n	1.91 ±0.807j
PEG+0.5µM EBL	0.54 ±0.015m	0.84 ±0.009l	1.30 ±0.041l	2.45 ±1.435i
PEG+1µM EBL	0.63 ±0.021k	0.96 ±0.010i	1.63 ±0.051j	3.87 ±2.969g
PEG+2µM EBL	0.80 ±0.037i	1.05 ±0.027h	1.93 ±0.026i	4.28 ±0.910ef
PEG+0.5µM HBL	0.57 ± 0.023l	0.85 ±0.009k	1.24 ±0.030m	2.16 ±1.435i
PEG+1µM HBL	0.69 ±0.007j	0.95 ±0.027j	1.62 ±0.043j	3.14 ±2.609h

The values are means ±SE (*n* = 5); mean followed by the same alphabet in a column is not significantly different at *p*=0.05 according to Post Hoc test.
*15% PEG is equivalent to osmotic potential of -2.95 bars at 25° C.

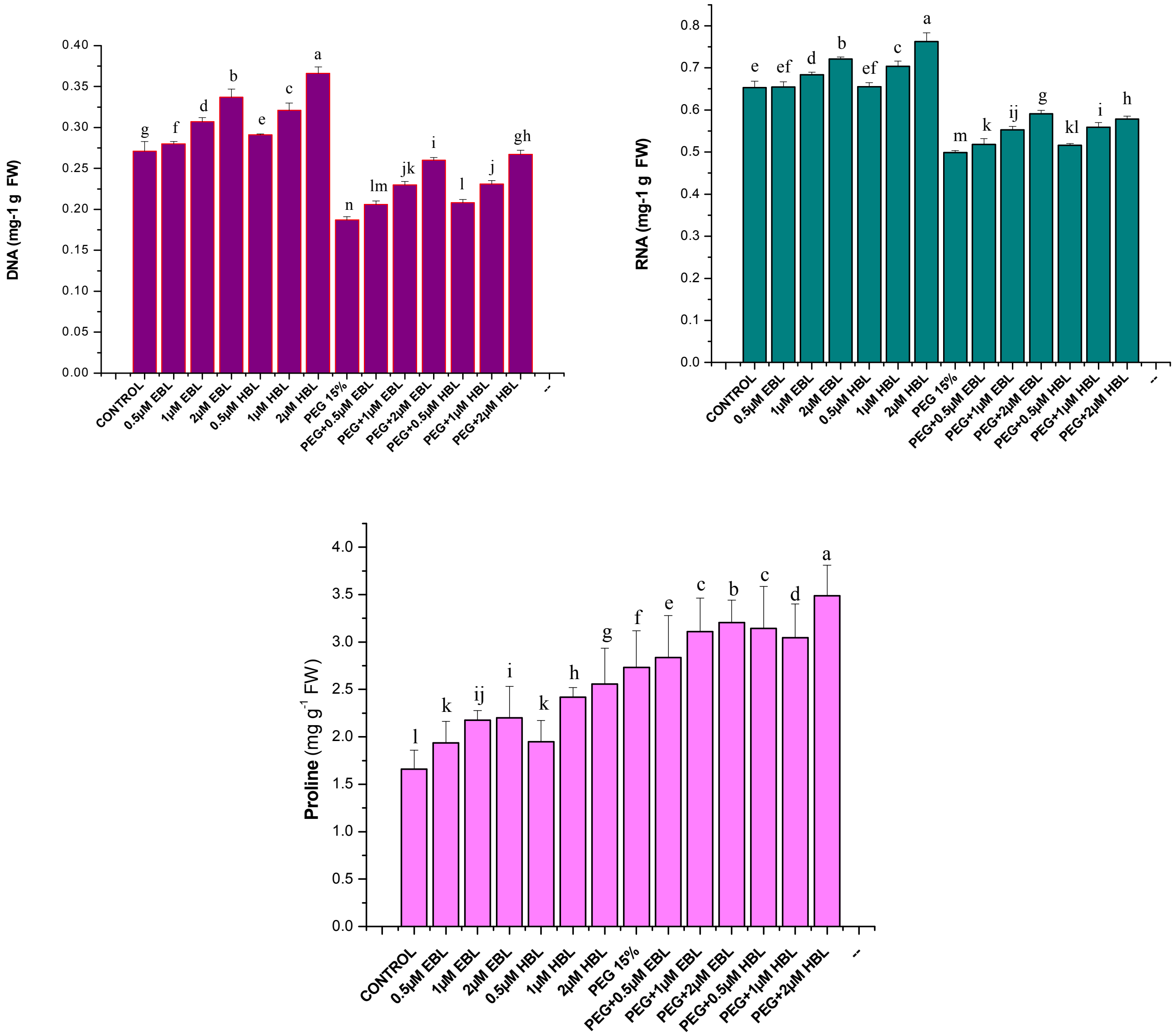

Figure 1. Effect of BRs on the levels of DNA (a), RNA (b) and Free Proline (c) levels of radish seedlings grown under PEG imposed water stress (OP = −2.95 bars). Vertical bars represent the means ±SE (*n* = 5); mean followed by the same alphabet in a column is not significantly different at *p*=0.05 according to Post Hoc test

The increase in carbohydrate levels as observed in this study might be due to enhanced photosynthetic capacity of the plants as influenced by the 28-HBL and 24-EBL application. In Fact, an increase in CO_2 fixation and levels of reducing sugars was reported in wheat and mustard plants by the application of brassinolide (Braun and Wild, 1984). Similarly, Vardhini *et al.,* (2011) reported increased carbohydrate fractions like reducing sugars and starch in the storage roots of radish by foliar supplementation of 28-HBL and 24-EBL. Osmolytes play significant protective role in plant responses to water stress and resistance. Under water stress, proline concentration can reach up to 80% of the total amino acid pool. In the present study, osmotic stress imposed by PEG caused substantial increment free proline levels and furtherance of proline content was found in radish seedlings exposed to desiccation stress (Figure 1(c)). The biochemical defense system against abiotic stress involves the amino acid proline (an osmolyte) which acts as cellular protectors largely accumulated in several plant species in response to abiotic stress, and scavenge ROS (Ashraf and Foolad, 2007). Farooq *et al.,* (2009) also observed that application of BRs increased the free proline levels in rice under drought stress. Anjum *et al.,* (2011) reported that *Zea mays* showed the enhanced chlorophyll levels under drought stress upon brassinolide (BL) application. The findings of present investigation suggest that both the BRs (EBL/HBL) at 2 μM concentration are playing a positive role in combating the PEG-6000 induced drought stress by enhancing the levels of metabolites and osmolytes. BRs alone treatments are analysed promising results that are substantial improvement rather than control treatment.

REFERENCES

Anjum, SA., Wang, LC., Farooq, M., Hussain, M., Xue, LL. and Zou, CM. 2011. Brassinolide application improves the drought tolerance in maize through modulation of enzymatic antioxidants and leaf gas exchange. J. Agron. Crop Sci. 197, 177-185.

Anuradha, S. and Rao, SSR. 2001. Effect of Brassinosteroids on Salinity Stress Induced Inhibition of Seed Germination and Seedling Growth of Rice (*Oryza sativa*)," *Plant Growth Regulation*, 33: 151-153.

Anuradha, S. and Rao, S.S.R. 2003. "Application of Brassinos- teroids to Rice Seeds (*Oryza sativa*) Reduced the Impact of Salt Stress on Growth, Prevented Photosynthetic Pig- ment Loss and Increased Nitrate Reductase Activity," *Plant Growth Regulation*, Vol. 40, No. 1, 2003, pp. 29-32. http://dx.doi.org/10.1023/A: 1023080720374

Ashraf, M. and Foolad, M.R. 2007. "Roles of Glycine Betaine and Proline in Improving Plant Abiotic Stress Resis- tance," *Environmental and Experimental Botany*, Vol. 59, No. 2, 2007, pp. 206-216. http://dx.doi.org/10.1016/ j.envexpbot.2005.12.006

Bajguz, A. 2009. Isolation and characterization of brassinosteroids from algal cultures of chlorella vulgaris Beijernick (Trebouxiophyceae). *J. Plant Physiol.* 166: 1946-1949.

Bajguz, A. and Hayat, S. 2009. Effects of brassinosteroids on the plant responses to environmental stresses. *Plant Physiol. Biochem.* 47, 1–8.

Bates, L., Waldren, R.P. and Teare, I.D. 1973. "Rapid Determination of Free Proline for Water-Stress Studies," *Plant and Soil*, Vol. 39, No. 1, pp. 205-207.

Braun, P. and A. Wild. 1984. The influence of brassinosteroid – A growth promotion steroidal lactone, on development and carbon-di-oxide fixation capacity of intact wheat and mustard seedlings; in Advances in Photosynthesis Reseach. Proc. 6th Congr. Photosynthesis (ed) C Sybesma (Hague, Nijhoff) pp 461-464.

Burton, K. 1968. "Determination of DNA Concentration with Diphenyl Amine," In: L. Grossman and M. Meidave, Eds., *Methods in Enzymology*, Academic Press, New York, p. 163.

Curtis, IS. 2003. The noble radish: past and future. *Trends plant Sci*. 8, 305-307.

Farooq, M., Wahid, A., Basra, S.M.A. and Din, I.U. 2009. "Im- proving the Water Relations and Gas Exchange with Brassinosteroids in Rice under Drought Stress," *Journal of Agronomy and Crop Science*, Vol. 195, No. 4,pp. 262-269.

Grove, MD., Spencer, GF., Rohwedder, WK., Mandava, NB. and Worley, JF. 1979. Brassinolide, a plant growth-promoting steroid isolated from *Brassica napus* pollen. *Nature* 281:216–17

Grove, M. D., G. F. Spencer., W. K. Rohwededer, N. B. Mandava, J.F. Worlet, , J.C. Warthen Jr., G.L. Steffens, J.L. Flippen- Andersen and J.C. Cook Jr. 1979. Brassinolide, a plant promoting steroid isolated from Brassica napus pollen. *Nature* 281:121-124.

Key, T.L. 1969. Hormones and nucleic acid metabolism. *Annu. Rev. Plant Physiol.* 20: 449-447.

Khallal, S.M.E1, Hathout, T.A., Ashour, A.A. and Kerrit, A.A. 2009. "Brassinolide and Salicylic Acid Induced Growth, Biochemical Activities and Productivity of Maize Plants Grown under Salt Stress," *Research Journal of Agricul- tural and Biological Sciences*, Vol. 5, No. 4, pp. 380-390.

Loomis, W.E. and C.A. Shull, 1937. Methods in plant physiology. New York, Mc Graw Hill Book Co.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Ran, R.J. – dall 1951. "Protein Measurement with the Folin Phenol Re- agent," *Journal of Biological Chemistry*, Vol. 193, No. 1, pp. 265-275.

Macar, T.K., Turan, O. and Ekmekcd, Y. 2009. "Effects of Wa-ter Deficit Induced by PEG and NaCl on Chickpea (*Cicer arietinum*) Cultivars and Lines at Early Seedling Stages," *Gazi University Journal of Science*, Vol. 22, No. 1, pp. 5-14. http://dx.doi.org/10.1104/pp.116.4.1403

Mandava, NB. 1988. Plant growthpromoting brassinosteroids. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 39:23– 52

Martı´nez-Villaluenga, C., Frı´as, J., Gulewicz, P., Gulewicz, K. and Vidal-Valverde, C. 2008. Food safety evaluation of broccoli and radish sprouts. *Food and Chemical Toxicol*. 46, 1635–1644.

Mc Cready, R.M., J. Guggloz, V. Silviora and H.S. Owens. 1950. Determination of starch and amalyase in vegetables. Application to peas. *Anal. Chem*. 29: 1156-1158.

Mitchell, JW., Mandava, NB., Worley, JF., Plimmer, JR. and Smith, MV. 1970. Brassins: a new family of plant hormones from rape pollen. *Nature* 225:1065–66

Nakajima, N., Atsubikoshida and Toyami, S. 1996. Effect of Brassinosteroids on Cell Division and Colony Formation of Chinese Cabbage Mesophyll Protoplasts" *Japanese Crop Science*, Vol.65, No. 1, pp. 114-118. http://dx.doi.org/ 10.1626/jcs.65.114

Nelson, N. 1944. A photometric adaption of the somagji method for determination of Glucose. *J. Biol. Chem.* 154: 375-380.

Ogur, M. and Rosen, G. 1950. "The Extraction and Estimation of Deoxypentose Nucleic Acid and Pentose Nucleic Acids," *Archive Biochemistry Biophysics*, Vol. 24, pp. 262- 276.

Rao, S.S.R., Vardhini, B.V., Sujatha, E. and Anuradha, S. 2002. "Brassinosteroids-New class of Phytohormones," *Current Science*, Vol. 82, No. 10, pp. 1239-1245.

Sairam, S.K. 1994. "Effects of Homobrassinolide Application on Plant Metabolism and Grain Yield under Irrigated and Moisture-Stress Conditions of Two Wheat Varieties," *Plant GrowthRegulation* Vol.14, No. 2, pp. 173-181. http://dx.doi.org/10.1007/BF00025220

Sasse, J. M. 2003. "Physiological Actions of Brassinosteroids: An Update," *Journal of Plant Growth Regulation*, Vol. 22, No. 4, pp. 276-288. http://dx.doi.org/10.1007/s00344-003-0062-3.

Sasse, JM. 1991. The case for brassinosteroids as endogenous plant hormones. See Ref. 27a, pp. 158–66.

Sasse, J. 1990. "Brassinolide-Induced Elongation and Auxin," *Physiologia Plantarum*, Vol. 80, No. 3, pp. 401- 408. http://dx.doi.org/10.1111/j.1399-3054.1990.tb00059.x

Schilling, G., Schiller, C. and Otto, S. 1991. Influence of brassinosteroids on organ relation and enzyme activities of sugar beet plants. In: Cultler HG, Yokota T, Adam G (eds.), Brassinosteroids- chemistry, bioactivity and application. ACS Symp Ser American Chemical Society, Washington. pp. 208–219.

Schneider, W.C. 1957. "Determination of Nucleic Acids in Tissues by Pentose Analysis," In: S. P. Colowick and W. O. Kaplan, Eds., *Methods in Enzymology*, Academic Press, New York, p. 680.

Vardhini, B.V., Sujatha, E. and Rao, S.S.R. 2011. Studies on the effect of brassinosteroids on the qualitative changes in the storage roots of radish. *Asian and Australasian Journal of Plant Science and Biotechnology*. 5:27-30.

Verslues, P.K., Ober, E.S. and Sharp, R.E. 1998. "Root Growth and Oxygen Relations at Low Water Potentials, Impact of Oxygen Availability in polyethylene Glycol Solutions," *Plant Physiology*, Vol. 116, No. 4, pp. 1403-1412.

Wang, W., Vinocur, B. and Altman, A. 2003. "Plant Responses to Drought Salinity and Extreme Temperatures towards Genetic Engineering for Stress Tolerance," *Planta*, Vol. 218, No. 1, pp. 1-14. http://dx.doi.org/10.1007/s00425-003-1105-5.

Wu, D.R. and Zhao, Y.J. 1993. "Effect of Epibrassinolide on the Metabolism of Nucleic Acids in Epicotyls of Mung Bean Seedlings," *Actaphysiologica Sinica*, Vol. 19, No. 1, pp. 49-51.

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