

Effect of brassinolide on certain enzymes of sorghum grown in saline soils of Karaikal

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Abstract

The effect of brassinolide on the activities of certain enzymes like IAA oxidase, protease and ribonuclease of two sorghum varieties ('CSH-5' and 'CSH-6') grown in four saline experimental sites of Karaikal viz. Varchikudy, Pogalam, Kilavur and Mallavur, was studied. Brassinolide-treatment resulted in lowered IAA oxidase, protease and ribonuclease activities. The study revealed that brassinolide was more effective in lowering the enzyme activities in Mallavur than other saline sites (Pogalam, Kilavur and Varchikudy).

Keywords: Brassinolide, IAA oxidase, protease, ribonuclease, sorghum

INTRODUCTION

Plant growth regulators play a very positive role in stress alleviation of various plants [1]. Brassinosteroids (BRs) are a novel group of phytohormones with significant growth promoting nature [2] and are ubiquitous in plant kingdom as they were reported in all plants tested (alga, bryophyte, pteridophyte, gymnosperms, dicots and monocots) [3]. BRs 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 resistance to plants against biotic and abiotic stresses [4]. BRs confer tolerance to a wide range of abiotic stresses in *Arabidopsis thaliana* and *Brassica napus* [5]. Krishna [6] also reported that BRs play a very prominent role in reducing the biotic and abiotic stresses in plants. In case of *Eucalyptus camaldulensis*, treatment of seeds with 24-epibrassinolide resulted in increased seed germination under saline conditions [7]. BRs increased the tolerance to high temperature in brome grass, where it has also been demonstrated that the tolerance in plants to high temperature due to the application of BRs is being associated with induction of *de novo* polypeptide protein (heat shock protein) synthesis [8]. Brassinolide was found to alleviate the negative impact of saline stress by monitoring the antioxidative system of two varieties of sorghum plants grown in two different saline soil of Karaikal [9]. Thus Xia *et al.* [10] aptly stated that BRs induce plant tolerance to a wide spectrum of stresses.

Sorghum vulgare Pers. is one of the five major cereal crops widely grown in the tropical and sub tropical parts of the world. It is the staple food for a large number of people and also a main source of fodder, feed and industrial raw material. It is a rain fed crop and poor monsoon and extended dry conditions play a devastating

influence on the crop performance [11]

Malibari *et al.* [12] reported that salinity stress is one of the critical environmental stresses that affect that ultimate yield of crops all around the world. Salinity usually effects the dry matter production, ionic relations, metabolic variations, physiological processes and water contents of the soil which leads to reduced growth of the plants. Karaikal is a part of the Union Territory of Puducherry. It falls in the Nagapatinam district of Tamil Nadu and lies in the east coastal belt of Bay of Bengal which usually experiences erratic rain fall. The *Tsunami* of 2004 caused many changes in the already poor soil texture of the land. After this massive deluge, both soil and water sources were highly enriched with salts of different chemical nature. The coastal soils have turned out to be more saline due to the process of secondary salinisation. The saline water consists of excess of neutral soluble salts mostly chlorides and sulfates of Na, Ca, and Mg. The present study was done to find out the effect of brassinolide, a potential plant growth regulator on activities of certain enzymes like IAA oxidase, protease and ribonuclease of sorghum plants grown in four experimental saline sites viz., Varchikudy, Pogalam, Kilavur and Mallavur of Karaikal, a *tsunami* hit area of Puducherry Union Territory of India.

MATERIALS AND METHODS

Chemicals and plant material

Brassinolide (double) is a commercially available brassinosteroid which is manufactured by Bahar Agrochem & Feeds Pvt. Ltd, Ratnagiri, Maharashtra, India, Ltd. and is marketed by Godrej Agrovet Ltd., Hyderabad, Andhra Pradesh, India. Brassinolide (Double) consists of 0.1% of brassinolide, 2.0% of emulsifier and 97.9 % of solvent IPA.

Seeds of sorghum (*Sorghum vulgare*. Pers) varieties 'CSH-5' and 'CSH-6' were purchased from National Seeds Corporation, Coimbatore, India. CSH-5 is a hybrid variety of 2077A × CS3541 and is a kharif crop (sown in early summer for harvesting in autumn). CSH-6 is a hybrid variety of 2219A × CS3541 and is also a kharif (sown in early summer for harvesting in autumn) crop.

The seeds were sown in earthen pots containing 10 kg of saline soil (collected from four different sites viz. Varchikudy, Pogalam, Kilavur and Mallavur of Karaikal) and compost in a 10: 1

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ratio. Plants were grown in under natural day length. Brassinolide (double) was supplied to the plants as foliar spray at 2 different concentration levels viz., 2.0 and 3.0 μM on 35th, 45th and 55th DAS (days after sowing). Two sets of water treated controls were also maintained. On 70th DAS, enzyme studies were conducted. The leaves were harvested in the early hours of morning, washed with distilled water, the surface water was blotted off and the leaves were kept in an ice-box, which were later used for enzyme studies.

IAA oxidase (E.C.1.11.1.8)

IAA oxidase (IAAO) was extracted by the method of Hillman and Galastan [13]. Seedlings were homogenized in chilled phosphate buffer (pH = 6.1). The assay mixture contained IAA, enzyme extract and phosphate buffer. The reaction was terminated by adding 10% (w/v) trichloroacetic acid. The residual IAA was estimated by Salper reagent and quantified by the use of IAA standard graph.

Protease (E.C.3.4.22.44)

200mg of the seedlings was homogenized in a pre chilled mortar and pestle using 10ml of chilled 0.2M sodium acetate buffer (pH=5.2). The supernatant was used as enzyme extract and protease activity was estimated of the amount of protein present according to Lowry *et al.* [14] method.

Ribonuclease (E.C.3.1.27.5)

The seedlings was ground with potassium acetate buffer (pH = 6.5) and centrifuged. The supernatant was used as enzyme extract. Ribonuclease (RNase) activity was estimated by Corbishley *et al.* [15] method.

The values were presented as Mean \pm S.E. of 5 replicates. ANOVA - one way revealed that the mean values of different activities of 28-homoBL are significant at 5% level of significance over control. The values were calculated employing SPSS 16.0 statistical software.

RESULTS

Brassinolide application resulted in reduction in the activity of the enzyme IAAO in the two varieties of sorghum plants ('CSH-5' and 'CSH-6') grown in all the four saline experimental sites (Varchikudy, Pogalam, Kilavur and Mallavur) of Karaikal compared to the control plants (Table 1). The present experiments revealed that the activity of IAAO extracted from sorghum plants grown in saline soils of stressed but untreated controls was more in Mallavur than Varchikudy, Pogalam and Kilavur in both the CSH-5 and CSH-6 varieties. But the application of brassinolide resulted in reduced activity of IAAO in Mallavur than Pogalam, Kilavur and Varchikudy in the two varieties of sorghum studied. The maximum reduced activity was recorded in plants treated with 3 μM brassinolide in both the varieties and in all the experimental saline soil sites of Karaikal.

Table1. Effect of brassinolide on the activities of IAA oxidase, protease and ribonuclease of two varieties of sorghum grown in two experimental sites of Karaikal

Varieties /Experimental sites	Treatments	IAA oxidase activity* ^a	Protease activity* ^b	Ribonuclease activity* ^c
CSH-5/ Varchikudy	Control-I	3.19 \pm 1.02	08.46 \pm 0.08	0.626 \pm 0.08
	Control-II	3.21 \pm 1.11	08.00 \pm 0.09	0.645 \pm 0.10
	2 μM BL	2.66 \pm 1.67	05.92 \pm 0.08	0.354 \pm 0.06
	3 μM BL	1.66 \pm 1.10	04.63 \pm 0.02	0.322 \pm 0.02
CSH-5/ Pogalam	Control-I	3.45 \pm 1.02	08.78 \pm 0.06	0.676 \pm 0.10
	Control-II	3.87 \pm 1.45	07.34 \pm 0.09	0.645 \pm 0.12
	2 μM BL	2.71 \pm 1.81	05.70 \pm 0.08	0.474 \pm 0.08
	3 μM BL	1.98 \pm 1.20	04.86 \pm 0.08	0.318 \pm 0.04
CSH-5/ Kilavur	Control-I	3.65 \pm 1.21	08.93 \pm 0.09	0.706 \pm 0.60
	Control-II	3.22 \pm 1.34	08.02 \pm 0.06	0.685 \pm 0.12
	2 μM BL	2.43 \pm 1.35	06.21 \pm 0.09	0.414 \pm 0.06
	3 μM BL	1.59 \pm 1.22	05.54 \pm 0.10	0.382 \pm 0.07
CSH-5/ Mallavur	Control-I	4.66 \pm 1.22	10.98 \pm 0.05	0.706 \pm 0.09
	Control-II	4.33 \pm 1.24	09.86 \pm 0.07	0.685 \pm 0.12
	2 μM BL	2.31 \pm 1.27	02.34 \pm 0.09	0.274 \pm 0.04
	3 μM BL	1.34 \pm 1.00	02.10 \pm 0.08	0.212 \pm 0.08
CSH-6/ Varchikudy	Control-I	3.29 \pm 1.12	08.64 \pm 0.04	0.676 \pm 0.09
	Control-II	3.41 \pm 1.00	08.20 \pm 0.07	0.655 \pm 0.10
	2 μM BL	2.69 \pm 1.08	05.72 \pm 0.08	0.494 \pm 0.02
	3 μM BL	2.00 \pm 1.12	05.03 \pm 0.05	0.402 \pm 0.06
CSH-6/ Pogalam	Control-I	3.50 \pm 1.22	09.98 \pm 0.05	0.686 \pm 0.09
	Control-II	3.61 \pm 1.02	08.88 \pm 0.08	0.645 \pm 0.12
	2 μM BL	2.88 \pm 1.08	06.34 \pm 0.06	0.474 \pm 0.04
	3 μM BL	2.08 \pm 1.12	06.10 \pm 0.04	0.392 \pm 0.08
CSH-6/ Kilavur	Control-I	3.21 \pm 1.22	08.98 \pm 0.05	0.696 \pm 0.09
	Control-II	3.32 \pm 1.00	08.86 \pm 0.07	0.665 \pm 0.10
	2 μM BL	2.36 \pm 1.00	05.34 \pm 0.08	0.484 \pm 0.04
	3 μM BL	1.95 \pm 1.20	04.10 \pm 0.09	0.462 \pm 0.02
CSH-6/ Mallavur	Control-I	4.70 \pm 1.28	10.62 \pm 0.10	0.716 \pm 0.08
	Control-II	4.42 \pm 1.22	09.16 \pm 0.05	0.705 \pm 0.10
	2 μM BL	2.11 \pm 1.02	02.54 \pm 0.01	0.264 \pm 0.02
	3 μM BL	1.26 \pm 1.22	02.20 \pm 0.12	0.222 \pm 0.02

BL = Brassinolide

*The presented values are Mean \pm SE. ANOVA one way classification revealed that the differences are significant at 5% level of significance.

a = IAA oxidase activity is expressed in terms of the amount of IAA destroyed in $\mu\text{g g}^{-1}\text{Fr. Wt./20 min}$.

b = Protease activity is expressed in terms of the amount of protein destroyed in $\mu\text{g g}^{-1}\text{Fr. Wt. /30 minutes}$.

c = RNase activity is expressed in absorbance units which indicated the amount of nucleotides formed due to depolymerization of RNA.

Brassinolide application resulted in reduction in the activity of the enzyme protease in the two varieties of sorghum plants ('CSH-5' and 'CSH-6') grown in all four saline experimental sites (Varchikudy, Pogalam, Kilavur and Mallavur) of Karaikal compared to the control plants (Table 1). The present experiments revealed that the activity of protease extracted from sorghum plants, in the stressed but untreated controls was more in Mallavur than Varichikudy, Pogalam and Kilavur in both the CSH-5 and CSH-6 varieties. But the application of brassinolide resulted in reduced activity of protease in Mallavur than Varichikudy, Pogalam and Kilavur in the two varieties of sorghum studied. The maximum reduced activity was recorded in plants treated with 3 μ M brassinolide in both the varieties and in all the experimental saline soil sites of Karaikal.

Brassinolide application resulted in reduction in the activity of the enzyme RNase in the two varieties of sorghum plants ('CSH-5' and 'CSH-6') grown in all the saline experimental sites (Varchikudy, Pogalam, Kilavur and Mallavur) of Karaikal compared to the control plants (Table 1). The present experiments revealed that the activity of RNase extracted from sorghum plants, in the stressed but untreated controls was more in Mallavur than Varichikudy, Pogalam and Kilavur in both the CSH-5 and CSH-6 varieties. But the application of brassinosteroids resulted in reduced activity of RNase in Mallavur than Varichikudy, Pogalam and Kilavur in the two varieties of sorghum studied. The maximum reduced activity was recorded in plants treated with 3 μ M brassinolide in both the varieties and in all experimental saline soil sites of Karaikal.

DISCUSSION

The activity of IAAO was decreased by the supplementation of BRs to four varieties of sorghum seedlings grown under PEG-imposed water stress [11]. Thus BRs seem exhibiting IAA-sparing influence. Similar decrease in the IAAO activity in the mung bean epicotyls treated with BL was observed by Wu and Zhao [16]. On the other hand the gravitropic curvature of maize primary roots caused by BL was found promotive in the presence of IAA indicating the interactions of auxins and BRs Kim *et al.* [17].

Seed treatment of BRs to the four varieties of sorghum seedlings grown under PEG-imposed water stress exhibited reduced protease activity. The supplementation of BRs to wheat plants [18] and rice [19] resulted in enhanced soluble proteins under various stress conditions. The decrease in the protease activity might have been due to reduced protein degradation and *denovo* polypeptide synthesis [19]. Further Vardhini *et al.* [20] reported decreased protease activity in sorghum seedlings [20] grown under PEG – imposed water stress.

Elevated activity of RNA polymerase and lowered activity of RNase and DNase were observed in mung bean seedlings when treated with epibrassinolide [21]. The present study with whole plant system also revealed reduced RNase activity in brassinosteroid treated sorghum plants grown in saline soils of Karaikal.

CONCLUSIONS

BRs bind to the membrane proteins and scavenge the reactive oxygen species which are generated by heavy metal toxicity, thereby reducing the membrane destruction that from AOS (active oxygen species)-induced oxidative damage [22]. After binding to the membrane proteins BRs may enhance the enzyme and metabolic

activities, thus detoxifying heavy metals in plants. How ever further research is required to unravel the mechanism of BRs in alleviating the impact of abiotic stress in crop plants. Li *et al.* [66] emphatically stated that '*Treatment of seedlings with BL may be a useful management tool for afforestation projects in arid and semiarid areas*'.

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REFERENCES

- [1] Vardhini B.V., S. Anuradha, E. Sujatha, and S.S.R. Rao. 2010. Role of Brassinosteroids in Alleviating Various Abiotic and Biotic Stresses - A Review. In: N. A. Anjum, (Ed.), Plant Nutrition and Abiotic Stress Tolerance I. Plant Stress 4 (Special Issue 1), London: Global Science Books, pp.56-61.
- [2] Vardhini, B.V., E. Sujatha, and S.S.R. Rao. 2011. Effect of brassinolide on biochemical composition of radish (*Raphanus sativus*). *Bioinfolet* 8:404-406
- [3] Vardhini, B.V., S. Anuradha, and S.S.R. Rao. 2006. Brassinosteroids-New class of plant hormone with potential to improve crop productivity. *Indian J. Plant Physiol.* 11: 1-12.
- [4] Bajguaz, A. 2009. Isolation and characterization of brassinosteroids from algal cultures of *Chlorella vulgaris* Beijernick (Trebouxiophyceae). *J. Plant Physiol.* 166: 1946-1949.
- [5] Kagale, S., K. D. Uday, E.K. Joan, A.K. Wilfred, and P. Krishna. 2006. Brassinosteroid confers tolerance in *Arabidopsis thaliana* and *Brassica napus* to a range of abiotic stresses. *Planta* 225: 353-364.
- [6] Krishna, P. 2003. Brassinosteroid mediated stress responses. *J Plant Growth Regul* 22: 289-297.
- [7] Sasse, J.M., R. Smith, and I. Hudson. 1995. Effect of 24-epibrassinolide on germination of seeds of *Eucalyptus camaldulensis* in saline conditions. *Proc. Plant Growth Regul. Soc. Am.* 22:136-141.
- [8] Kulaeva, O.N., E.A. Burkhanova, A.B. Fedina, V.A. Khokhlova, G.A. Bokebayeva, H.M. Vorbrodt, and G. Adam. Effect of brassinosteroids on protein synthesis and plant cell ultrastructure under stress conditions, Brassinosteroids – Chemistry, Bioactivity and Application Cutler, H.G., Yokota, T., Adam, G. Eds., ACS Symposium, Ser 474, American Chemistry Society, Washington DC, 1991, pp 141-155.
- [9] Vardhini, B.V. 2011. Studies on the effect of brassinolide on the antioxidative system of two varieties of sorghum grown in saline soils of Karaikal. *The Asian and Australasian Journal of Plant Science and Biotechnology.* 5:31-34.
- [10] Xia, X-J., Y-J. Wang, Y-H. Zhau, Y. Tao, W-H, Mao, K. Shi, T. Asami, Z Chen, and J-Q. Yu. 2009. Reactive oxygen species

- are involved in brassinosteroid-induced stress tolerance in cucumber. *Plant Physiol.* 150: 801-814.
- [11] Vardhini, B.V., and S. S. R. Rao, 2003. Amelioration of osmotic stress by brassinosteroids on seed germination and seedling growth of three varieties of sorghum. *Plant Growth Regul.* 41: 21-31.
- [12] Malabari, A.A., M. A. Zidan, M. M. Heikel and S. EL-Shamary. 1993. Effect of salinity on some crop plants. 1. Germination and growth. *Pak. J. Bot.* 25: 156-160.
- [13] Hillman, W.S. and A.W. Galaston. 1957. Inductive control of indole acetic acid oxidase by red and near infrared light. *Plant Physiol.* 32:129-139.
- [14] Lowry, O.H., N. J. Rosenbrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with folin-phenol reagent. *J. Biol. Chem.* 193: 265-275.
- [15] Corbishley, P.T., J.P. Johnson and R. Williams. 1984. Esterases: Serum Ribonuclease. In *Methods of enzyme analysis* (3rd edn. Berymeyer, J. and Grabi, M., eds), Florida : Verlag Chemie , pp 134-148.
- [16] Wu, Deng-Ru. and Yu-Ju. Zhao. (1991) Effects of epibrassinolide on endogenous IAA and its oxidase in epicotyls of mung bean seedlings. *Acta. Phytophysiol. Sin.* 74: 327-332.
- [17] Kim, S. K., S.C. Chang, E. J. Lee, W. S. Chung, Y.S. Kim, S. Hwaong and S. Lee. 2000. Involvement of brassinosteroids in the gravitropic responses of primary roots of maize. *Plant Physiol.* 123:997-1004.
- [18] Ozdemir, F., M. Bor, T. Demiral and I. Turkan. 2004. Effects of 24-epibrassinolide on seed germination, seedling growth, lipid peroxidation, proline content and antioxidative system of rice (*Oryza sativa* L) under salinity stress. *Plant Growth Regul.* 42: 203-211.
- [19] Sairam, R. K. 1994. Effect of homobrassinolide application on plant metabolism and grain yield under irrigated and moisture - stress conditions of two wheat varieties. *Plant Growth Regul.* 14:173-181.
- [20] Vardhini, B.V., E. Sujatha and S. S. R. Rao. 2011. Alleviation of water stress by supplementation of brassinosteroids on the activities of certain enzymes of four varieties of sorghum. *Journal of Phytology.* 3:38-43.
- [21] Wu, Deng-Ru and Yu-Ju. Zhao. 1993. Effect of epibrassinolide on the metabolism of nucleic acids in epicotyls of mung bean seedlings. *Acta Phytophysiol. Sin.* 19: 49-51.
- [22] Cao, S. , Q. Xu, Y. Cao, K. Qian, K. An, Y. Zhu, H. Binzeng, H. Zhao and B. Kuai. 2005. Loss-of- function mutation in *DET2* gene lead to an enhanced resistance to oxidative stress in *Arabidopsis*. *Physiol. Plant.* 123:57-66.
- [23] Li, K.R., H.H. Wang, G. Han, Q-J. Wang and J. Fan. 2008. Effects of brassinolide on the survival, growth and drought resistance of *Robinia pseudoacacia* seedlings under water-stress. *New Forests* 35: 255-266.