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Application of brassinolide mitigates saline stress of certain metabolites of sorghum grown in Karaikal

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Abstract
Effect of brassinolide on the metabolite contents (free proline, soluble proteins and RNA) of two sorghum varieties ('CSH-5' and 'CSH-6') grown in two saline experimental sites of Karaikal viz. Varchikudy and Mallavur, was studied. Brassinolide application resulted in substantial elevated levels of free proline, soluble proteins and RNA of the two varieties of sorghum plants grown in two saline experimental sites of Karaikal. The study revealed that brassinolide was more effective in more saline site –II (Mallavur) than less saline site –I (Varchikudy) thus indicating its ability to counteract the negative impact of saline stress.

Keywords: Brassinolide, enzymes, metabolites, sorghum

INTRODUCTION

The devastating effect of saline stress on plant growth is an important research theme. Malibari *et al*. [1] 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 [2]. Karaikal is a part of the Union Territory of Puducherry. It falls in the Nagapatinum 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.

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 [3]. Plant growth regulators play a very positive role in stress alleviation of various plants [4]. Brassinosteroids (BRs) are a novel group of phytohormones with significant growth promoting nature [5, 6]. BRs are considered as plant 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 [7]. BRs confer tolerance to a wide range of abiotic stresses in *Arabidopsis thaliana and Brassica napus* [8], *Eucalyptus camaldulensis* [9] and brome grass [10].Thus Xia *et al*. [11] aptly stated that BRs induce plant tolerance to a wide spectrum of stresses. The present study was done to find out the effect of brassinolide, a potential plant growth regulator on metabolite contents like free proline, soluble proteins and RNA of sorghum plants grown in two experimental saline sites viz., Varichikudy and Mallavur of Karaikal, a *tsunami* hit area of Puducherry Union Territory of India.

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MATERIALS AND METHODS

Chemicals and plant material

Brassinolide (double) is a commercially available brassinosteroid which is manufactured by Bahar Agrochem & Feeds Pvt. Ltd, Ratnagiri, Maharastra, 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, Coimbature, 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 two different sites *viz*. Varichikudy and Mallavur of Karaikal) and compost in a 10: 1 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). On 60th day leaf material was homogenized using 70% (v/v) ethanol and stored in deep freezer for further biochemical analysis.

Metabolites

Free proline

Five hundred mg of leaves were homogenized with 10 ml of

3% (w/v) sulphosalicyclic acid and filtered through Whatman No 2 filter paper. The filtrate was used for free proline estimation using ninhydrin reagent employing the method of Bates *et al*. [12].

Soluble proteins

Soluble proteins in the ethanol homogenate were precipitated by adding 20% (w/v) tricholoroacetic acid. The precipitate was dissolved in 1% (w/v) sodium hydroxide. The method of Lowry *et al*. [13] was used for protein estimation.

RNA

RNA in the alcohol homogenate was separated by Ogur and Rosen [14] method and RNA was estimated by the procedure of Schneider [15].

The values were presented as Mean ± S.E. of 5 replicates. All the data processed by ANOVA one way revealed that the mean values of different activities are significant at 5% level of significance.

RESULTS AND DISCUSSION

Brassinolide application as foliar spray increased the levels of free proline in the two varieties *viz*. 'CSH-5' and 'CSH-6' of sorghum plants grown in both the experimental saline soils sites of Karaikal (Varchikudy and Mallavur) compared to the control plants (Table 1) and the levels of free proline in the stressed but untreated controls was more in Site I (Varichikudy) than Site –II (Mallavur) in both the CSH-5 and CSH-6 varieties. But the application of brassinosteroids resulted in enhanced free proline in the site –II (Mallavur) than Site-I (Varichikudy) in the two varieties of sorghum studied. Brassinolide at 3 μM was most effective in increasing the levels of free proline in both the varieties grown in two experimental saline soils sites of Karaikal. 24-Epibrassinolide increased the accumulation of free proline which is a predominant osmolyte in *Spirulina platensis* of Cyanophyta under NaCl stress [16]. Similarly Farooq *et al*. [17] reported that exogenously applied 28-homobrassinolide used both as seed priming and foliar spray produced profound changes that improved the drought tolerance in fine grain aromatic rice by improving the production of free proline. The results obtained in the present work are in tune with the research done by Ozdemir *et al*. [18] who revealed that 24- epibrassinolide increased proline content in rice under salinity stress.

Brassinolide application as foliar spray increased the levels of soluble proteins in the two varieties *viz*. 'CSH-5' and 'CSH-6' of sorghum plants grown in both the experimental saline soils sites of Karaikal (Varchikudy and Mallavur) compared to the control plants (Table 1). The present study revealed that the sorghum plant levels of soluble proteins, in the stressed but untreated controls was more in Site I (Varichikudy) than Site –II (Mallavur) in both the CSH-5 and CSH-6 varieties. But the application of brassinosteroids resulted in enhanced growth parameters in the site –II (Mallavur) than Site-I (Varichikudy) in the two varieties of sorghum studied. Brassinolide at 3 μM was most effective in increasing the levels of soluble proteins in both the varieties grown in two experimental saline soils sites of Karaikal. Nilovskaya *et al*. [19] (2001) reported that application of epibrassinolide increased protein content in barley plants. Sasse [20] suggested that BRs can stimulate the synthesis of particular proteins associated with growth. Supplementing the Petri plates with BRs increased seedling growth and amount of soluble proteins in sorghum under osmotic stress imposed by PEG [21]. BRs were found to play a dominant role in the protection of the translational machinery and heat shock protein synthesis following thermal stress [22].

Brassinolide application as foliar spray increased the levels of RNA in the two varieties *viz*. 'CSH-5' and 'CSH-6' of sorghum plants grown in both the experimental saline soils sites of Karaikal (Varchikudy and Mallavur) compared to the control plants (Table 1). The present study revealed that the sorghum plant levels of RNA, in the stressed but untreated controls was more in Site I (Varichikudy) than Site –II (Mallavur) in both the CSH-5 and CSH-6 varieties. But the application of brassinosteroids resulted in enhanced RNA contents in the site –II (Mallavur) than Site-I (Varichikudy) in the two varieties of sorghum studied. Brassinolide at 3 μM was most effective in increasing the levels of nucleic acids in both the varieties grown in two experimental saline soils sites of Karaikal. Phytohormones influence the growth by regulating nucleic acid synthesis [23]. The growth promotion in groundnut by BRs was found to be associated with enhanced nucleic acids [24]. The results obtained in the present study with brassinosteroids are in confirmity with the observations made by Key [23] with regard to the then known phytohormones. The promotion of growth by BR under salt stress conditions was associated with enhanced levels of nucleic acids and soluble proteins [25] . The increase in the levels of RNA might be due to enhanced synthesis and reduced degradation.

Table 1. Effect of brassinolide on the metabolites of two varieties of sorghum grown in two experimental sites of Karaikal.

Variety/experimental site	Treatment	Free Proline (mg g^{-1} Fr.Wt.)*	Soluble Proteins (mg g^{-1} Fr.Wt.)*	RNA (mg g^{-1} Fr.Wt.)
CSH-5/site-I	Control	3.01 ± 0.82	6.62 ± 0.48	6.99 ± 0.37
	2 μM BL	4.00 ± 0.52	7.68± 0.79	8.51 ± 0.28
	3 μM BL	5.66 ± 0.75	8.26± 0.88	9.64 ± 0.68
CSH-5/site-II	Control	2.33 ± 0.21	5 .38 ± 0.96	5.80 ± 0.47
	2 μM BL	4.33 ± 0.54	8.41 ± 0.89	8.99 ± 0.27
	3 μM BL	5.96 ± 0. 51	9.68 ± 0.58	10.34 ± 1.09
CSH-6/site-I	Control	3.65 ± 0.16	6.14 ± 0.69	7.61 ± 0.88
	2 μM BL	4.22 ± 0.47	8.02 ± 0.37	8.01± 0.99
	3 μM BL	5.43 ± 0.35	8.79 ± 0.69	9.38 ± 0.19
CSH-6/site-II	Control	2.66 ± 0.21	5.98 ± 0.43	5.33 ± 0.49
	2 μM BL	4.33 ± 0.53	8.97 ± 0.37	8.90 ± 1.09
	3 μM BL	5.83 ± 0.28	9.01± 0.59	10.46 ± 1.06

BL= brassinolide; site I=Varchikudy; site II=Mallavur.
* Mean ± S.E (N=5). ANOVA one way revealed that the mean values of different activities are significant at 5% level of significance.

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