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SHORT COMMUNICATION

INFLUENCE OF BRASSINOSTEROIDS ON GERMINATION AND SEEDLING GROWTH OF SORGHUM UNDER WATER STRESS

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The effect of 28-homobrassinolide and 24-epibrassinolide on germination and seedling growth of *Sorghum vulgare* pers var. CSH-15 R (susceptible to water stress) was studied under water stress. Both the brassinosteroids were very effective in ameliorating the impact of water stress on seed germination and seedling growth. The stress alleviation by brassinosteroids was associated with enhanced levels of soluble proteins and free proline. Brassinosteroids treatment were found to enhance the activity of catalase and reduce the activity of peroxidase.

***Key words*: Brassinosteroids, germination, seedling growth, sorghum, water stress.**

Water stress is a serious problem in many parts of the world and is also fairly wide spread, and thus influences the overall productivity of agricultural systems around the world. The retardation in plant growth under water stress is attributed to reduced accumulation of dry biomass due to inhibition of physiological processes (Singh *et al*. 2000). As water availability is reduced, many plants show reduction in leaf turgor, leaf water potential, stomatal opening and growth rate (Colom and Vazzana 2001). *Sorghum vulgare* Pers. is one of the five major cereals of the world, and is extensively grown as rain fed crop in tropical and subtropical environments. Poor monsoon and extended dry condition during critical growth period have a devastating influence on the crop performance.

Brassinosteroids, a newly identified endogenous plant hormones, have significant growth promoting influence (Clouse and Sasse 1998, Rao *et al*. 2002). The ability of brassinosteroids to confer resistance to plants against abiotic stresses is gaining much attention. Available data indicate that exogenous application of brassinosteroid is effective during stress, rather than under optimal conditions (Sasse 1997). The ability of brassinosteroids to alleviate chilling stress in seedlings of maize (He *et al*. 1991), cucumber (Katsumi 1991) and rice (Wang and Zang 1993) have been reported. The brassinosteroids induced tolerance to high temperature stress in brome grass (Wilen *et al*. 1995) and moisture stress in wheat (Sairam, 1994) have also been reported. In the present study, the effect of brassinosteroids on seed germination and seedling growth of sorghum subjected to water stress along with the changes in certain metabolites and activities of certain oxidizing enzymes have been investigated.

28-Homobrassinolide and 24-epibrassinolide were purchased from M/S Beak Technologies Inc., Brampton, Ontario, Canada. Seeds of sorghum (*Sorghum vulgare* Pers.) variety CSH-15 R (susceptible to water stress) were purchased from National Seeds Corporation, Hyderabad, India.

Sorghum seeds were surface sterilized with 0.5% (v/v) sodium hypochlorite solution for fifteen minutes and then washed with several changes of sterile distilled water. Thirty seeds were placed per sterile petriplates of 15 cm diameter, layered with Whatman No 1 filter

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paper. The petriplates were supplied with 5ml solutions: (i) distilled water (control), (ii) 20% (w/v) polyethylene glycol 6000 (PEG), (iii) 20% (w/v) polyethylene glycol 6000 (PEG) supplemented with 2µM/3µM brassinosteroids. The plates were kept in a dark room at 25 ± 1°C . Germination counts were taken under safe green light at the end of 24, 36 and 48 h. Emergence of radicle was taken as germination. On the 5th day seedling length, fresh and dry weights were recorded. six day old seedlings were used for estimation of soluble proteins and free proline and for assaying catalase and peroxidase activities.

For extraction of soluble proteins 200 mg seedlings were homogenized with 10 ml of 70% (v/v) ethyl alcohol. The ethanol homogenate was precipitated by adding 20% (w/v) trichloroacetic acid and the precipitate was dissolved in 1%(w/v) sodium hydroxide. Protein estimation was done following the method of Lowry *et al*. (1951). For extraction of free proline 500 mg of seedlings were homogenized with 10 ml of 3% (w/v) sulfosalicylic acid and filtered through Whatman No 2 filter paper. The filtrate was used for free proline estimation using ninhydrin reagent by employing the method of Bates *et al.* (1973).

For extraction of catalase and peroxidase enzymes 200 mg of seedlings were homogenized in chilled 0.1 M phosphate buffer (pH 7). The homogenate was centrifuged at 15000 g for 15 min at 4°C and the supernatant was used for assaying catalase and peroxidase activities. Catalase activity was assayed by the method of Barber (1980). Enzyme extract (0.5ml) was added to 2.0ml of hydrogen peroxide and 3.5ml of phosphate buffer (pH 7.0). The reaction was stopped by adding 10ml of 2% (v/v) concentrated sulphuric acid, and the residual hydrogen peroxide was titrated against 0.01M $KMnO_4$ until a faint purple colour persisted for atleast 15 seconds. The activity of the catalase was expressed as enzyme units. Peroxidase activity was assayed as per the method of Kar and Mishra (1976). To 0.5ml of enzyme extract, 2.5ml of 0.1M phosphate buffer (pH 7.0), 1.0ml of 0.01M pyrogallol and 1.0ml of 5mM H_2O_2 were added. After incubation, the reaction was stopped by adding 1.0ml of 2.5N H_2SO_4. The amount of purpurogallin formed was estimated by measuring the absorbance at 420nm. The peroxidase activity was expressed in absorbance units.

The data were analyzed by one-way ANOVA, followed by Post Hoc Test (Multiple Comparisons). The differences were considered significant if P was at least =0.05. The mean values have been compared and lower case alphabets are used in the table to highlight the significant differences between the treatments.

28- Homobrassinolide and 24-epibrassinolide used in the study reversed the inhibitory effect of water stress on seed germination of sorghum (Table 1). 28-Homobrassinolide at 3µM conc showed maximum effect in ameliorating the inhibitory effect of water stress. The percentage of germination with brassinosteroid application under water stress almost reached to that of the control seeds at the end of 24 h and even exceeded the control seeds at the end of 36 and 48 h. Brassinosteroids application reduced the impact of water stress and improved the growth of seedlings (Table 1). Greater alleviation of water stress was found with 3µM 28-homobrassinolide supplemented treatment. Earlier brassinosteroids were found to ameliorate the impact of drought stress on germination and seedling growth in spring wheat (Puroska *et al*. 2000), salinity stress in *Eucalyptus camaldulensis* (Sasse *et al.* 1995) as well as in rice (Anuradha and Rao 2001).

Soluble protein content in sorghum seedlings under water stress increased above the control levels by the addition of brassinosteroids (Table 2). Enhancement of proteins in water stressed wheat seedlings by brassinosteroids application has also been reported by Sairam (1994). Increase in proline levels as induced by water stress was further enhanced by brassinosteroid supplementation. The involvement of proline as compatible solute in osmotic adjustment under water stress is well established (Yadav *et al.* 1997).

Under water stressed condition, the activity of catalase decreased as compared to unstressed control seedlings (Table 2). Brassinosteroid application caused increase in catalase activity. Catalase is an H_2O_2 scavenging enzyme, which removes toxic H_2O_2 during development, which is otherwise lethal. The higher

Table 1. Effect of brassinosteroids on seed germination and seedling growth of sorghum seeds under water stress

Treatment	Seed germination %			Seedling length		
	24 h	36 h	48 h	Length (cm)	Fresh weight (mg)	Dry weight (mg)
Control	16.63a	41.10b	93.30ab	17.2a	145.26c	37.96c
20% PEG	—	18.86d	53.30d	8.8c	114.36d	24.56d
20% PEG+2µM HBL	13.30b	43.30ab	95.53ab	17.5a	166.20ab	48.80ab
20% PEG+3µM HBL	16.63a	54.43a	100.00a	18.5a	173.43a	52.80a
20% PEG+2µM EBL	9.96c	38.86c	91.06cv	16.1b	163.66ab	47.80ab
20% PEG+3µM EBL	15.50b	51.06a	98.86a	17.6a	167.36ab	49.10ab

The values are mean (N=5). Values in the same column, followed by the same letter are not statistically different at P = 0.05.
— = Not Germinated

Table 2. Effect of brassinosteroids on the soluble proteins, free proline, catalase and peroxidase activity in sorghum seeds under water stress

Treatment	Soluble proteins (mg g^{-1} fr wt)	Free Proline (mg g^{-1} fr wt)	Catalase activity@ (Enzyme units)	Peroxidase activity# (Absorbance units)
Control	3.45c	3.44d	21.43c	0.567b
20% PEG	2.29d	3.93c	16.83d	0.673a
20% PEG+2µM HBL	4.13ab	4.33b	24.30b	0.360c
20% PEG+3µM HBL	4.53a	4.90a	27.30a	0.234d
20% PEG+2µM EBL	4.04ab	4.41b	22.70bc	0.354c
20% PEG+3µM EBL	4.40a	4.76a	26.40a	0.264d

The values are mean (N=5). Values in the same column, followed by the same letter are not statistically different at P = 0.05.
@ Catalase activity is expressed in terms of enzyme units.
# Peroxidase activity is expressed in absorbance units which indicates the amount of purpurogallin formed.

activity of catalase in brassinosteroid treated seedlings of sorghum might have resulted in better scavenging of H_2O_2 leading to improved seedling growth. Water stress increased the peroxidase activity of sorghum seedlings (Table 2). But the supplementation of brassinosteroids resulted in reduced activity of peroxidase in sorghum seedlings. The lower peroxidase activity might be an indicator of removal of stressful condition by brassinosteroids. 28-Homobrassinolide treated seedlings exhibited lower peroxidase activity compared to other treatments. Similar reduction of peroxidase activity in 24-epibrassinolide treated hypocotyls of light grown cucumber seedlings (Xu and Zhao 1989) has also been reported.

The present study clearly demonstrated the abilities of brassinosteroids to alleviate the water stress on seed germination and seedling growth. Khripach *et al*. (2000) predicted a great role for brassinosteroids in 21st century in agriculture. One of the potential areas could be improving the growth and economic yield of crop plants growing under hostile habitats.

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