

Effects of brassinosteroids on growth, nodulation, phytohormone content and nitrogenase activity in French bean under water stress

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Abstract

Effect of pre-treatments of 1 and 5 μ M epibrassinolide or homobrassinolide prior to water stress induction on changes in root nodulation and contents of endogenous abscisic acid (ABA) and cytokinin *trans*-zeatin riboside (ZR), and nitrogenase activity was investigated in the nodulated roots of *Phaseolus vulgaris* L. cv. Arka Suvidha. Brassinosteroids in the unstressed plants increased root nodulation, ZR content and nitrogenase activity, and also ameliorated their stress-induced decline in the nodulated roots. The ABA contents in the nodules of control or stressed plants were not altered by brassinosteroids treatment. There was an increase in pod yield by brassinosteroids treatment (5 μ M) in the irrigated control as well as stressed plants without influencing pod number or pod length. Among the brassinosteroids, epibrassinolide was relatively more effective.

Additional key words: abscisic acid, cytokinins, drought, *Phaseolus vulgaris*, yield.

Introduction

Brassinosteroids are naturally occurring compounds well documented for their role in plant growth and development (Clouse and Sasse 1998). Their growth regulatory activity is suggested to be the result of their influence on the metabolic processes associated with photosynthesis, and nucleic acid and protein biosynthesis (Arteca 1995, Sasse 1997). In addition, brassinosteroids have also been reported to counteract stress effects in plants (Clouse and Sasse 1998, Khripach *et al.* 2000). The mechanism by which brassinosteroids exert such an influence is not well understood.

Root nodulation, an important developmental event in leguminous crops, is sensitive to drought (Bordeleau and Prevost 1994, Ramos *et al.* 1999, Serraj *et al.* 1999). As endogenous hormones play an important role in the organogenesis and initial growth of nodules in roots, attempts have been made to increase root nodulation by

growth regulator treatments (Singh *et al.* 1989, Fedorova *et al.* 1991). Although potential of brassinosteroids in the improvement of root nodulation and yield have been reported in groundnut (Vardhini and Rao 1999), studies are lacking with regards to their similar effects under water stress conditions. In the present investigation we studied the effects of two brassinosteroids, epibrassinolide and homobrassinolide on root nodulation and yield in French bean cv. Arka Suvidha (relatively drought susceptible) under water stress. In addition, realizing the importance of plant hormones such as cytokinins and ABA in nodule development and also in signal transduction pathways in plants experiencing stress, we studied the influence of brassinosteroids on these hormones content together with nitrogenase activity in the nodulated roots.

Received 30 September 2003, accepted 30 March 2004.

Abbreviations: ABA - abscisic acid; ZR - *trans*-zeatin riboside.

Acknowledgements: The authors are thankful to the Director of the institute for providing the necessary facilities, and to Mr. H.L. Jayaram and Mr. K.R. Earanna for technical help. (IIHR contribution No. 103/2003).

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Materials and methods

Seedlings of French bean (*Phaseolus vulgaris* L.) cv. Arka Suvidha were grown in plastic pots containing garden soil and farmyard manure (2:1) under natural day length in a polyhouse of Indian Institute of Horticultural Research, Bangalore. At flowering stage, the seedlings were given foliar sprays of 0, 1 and 5 μM of epibrassinolide and homobrassinolide (*Sigma-Aldrich*, St. Louis, MO, USA) containing 0.05 % of dispersant *Sandovit* (*Syngenta India Ltd*, Mumbai, India). After 5 d of brassinosteroids application, water stress treatments were imposed by withholding water supply for of 4 and 8 d. Soil moisture contents as measured gravimetrically were 31.2 % in the control, and 24.3 and 19.1 % in the 4- and 8-d stressed pots. Experiment was conducted in completely randomized design with 4 replications in each treatment. After imposition of stress, plants were irrigated to saturation level. During the experimentation, the average relative humidity was 71.1 %, and maximum and minimum temperatures varied between 30.2 and 19.1 °C, respectively. For observations on nodule number, nodulated root mass and root length, the plants were removed gently from the soil, ensuring intactness of the root system followed by its washing. After drying the root system by keeping between the filter papers, the number of nodules was counted, and mass of nodulated roots and root length were recorded. Simultaneously, the nodulated root samples from control and brassinosteroid treated plants were drawn for estimating the contents of

ABA and cytokinin, *trans*-zeatin riboside (ZR) and assaying nitrogenase activity. The samples for phytohormone analysis were macerated in chilled 80 % methanol and filtered. The filtrate was evaporated *in vacuo* at 35 °C, the residue dissolved in water and pH was adjusted to 3.0. For ABA analysis, the aqueous acidic extract was partitioned twice against equal volumes of chilled diethyl ether. The ether phase was separated and dried over anhydrous sodium sulphate. The extract was kept over night at 4 °C, filtered and after evaporating ether, the residue was dissolved in 20 mM tris buffer, pH 7.8 for ABA analysis. The aqueous phase left after ether partitioning was used for cytokinin analysis. The aqueous phase pH was adjusted to 8.0 and partitioned thrice with equal volumes of water-saturated n-butanol. The butanol phase was separated, evaporated *in vacuo* at 35 °C and the residue was dissolved in tris buffer (20 mM, pH 7.8) for cytokinin analysis. The contents of ABA (Weiler 1982) and ZR (Barthe and Stewart 1985) were determined by ELISA employing laboratory raised polyclonal antibodies against their protein conjugates.

The nitrogenase activity was determined by acetylene to ethylene conversion assay of Hardy *et al.* (1968) employing gas liquid chromatograph (*Perkin Elmer*, CA, USA). Pod yield, pod length and pod number were recorded in the stress recovered plants at harvest.

Results and discussion

Water stress treatments negatively influenced the nodule number, mass of nodulated root and root length, with the impact of stress being greater on nodule number than the other parameters (Table 1). Similar decline in root nodulation under the conditions of water stress has been reported earlier in French bean (Ramos *et al.* 1999, Upreti and Murti 1999), groundnut and cowpea (Venkateswarlu *et al.* 1990), pea (Gonzalez *et al.* 1998) and soybean (Serraj *et al.* 1998). Exogenous brassinosteroids application prior to stress induction enhanced the nodule number and increased mass of nodulated roots and root length in the irrigated control plants, and also ameliorated the stress induced decline in these parameters; the effect being prominent at 5 μM concentration in 4-d stressed plants. Among the brassinosteroids, epibrassinolide was more effective than homobrassinolide. Vardhini and Rao (1999) reported brassinosteroid stimulation to root nodulation in the groundnut. Our results are in line with this, and further extend brassinosteroid potential in mitigating the stress effects in plants by checking stress-induced decline in root nodulation.

Water stressed plants also showed reduced pod

number, pod length and pod yield at harvest (Table 2). This is suggested to be the result of observed decline in root nodulation, as root nodules serve as an important source of fixed nitrogen for the normal growth and development of plants. Brassinosteroids treatment especially epibrassinolide (5 μM) showed an increase in pod yield in the unstressed control plants, and also ameliorated stress induced decline in pod yield without influencing the pod number or pod length.

Water stress treatments resulted in accumulation in ABA, and inhibited ZR content and nitrogenase activity in the nodulated roots, more so under 8-d stress (Table 3). ABA and cytokinins are well known for their role in signal transduction pathways in plants experiencing water stress (Davies and Zhang 1991, Itai 1999). Upreti *et al.* (1998) reported accumulation of ABA and decline in cytokinin content in the leaves of water stressed French bean cultivars. The present results in the nodulated roots follow a similar trend. The increased ABA is expected to inhibit nodule growth and promote senescence by inhibiting cortical cell differentiation process as revealed by Philips (1971), Bano *et al.* (1983), Williams and

Table 1. Effect of brassinosteroids on nodule number, mass of nodulated roots and root length in French bean. Means \pm SE, $n = 4$.

Treatments	Conc. [μ M]	Stress [d]	Nodule number		Nodulated root mass [g plant ⁻¹]		Root length [cm]	
			control	stressed	control	stressed	control	stressed
Control		0	37.0 \pm 2.23	-	1.90 \pm 0.19	-	22.4 \pm 2.52	-
		4	39.3 \pm 3.44	23.0 \pm 1.42	1.57 \pm 0.21	1.20 \pm 0.15	22.6 \pm 1.61	15.3 \pm 0.65
		8	46.0 \pm 1.63	18.0 \pm 1.19	1.87 \pm 0.08	0.97 \pm 0.07	21.3 \pm 0.87	11.8 \pm 1.31
Epibrassinolide	1	0	51.0 \pm 2.92	-	1.97 \pm 0.73	-	23.4 \pm 1.34	-
		4	54.0 \pm 4.02	33.7 \pm 1.85	2.07 \pm 0.17	1.60 \pm 0.22	25.5 \pm 3.07	19.1 \pm 0.36
		8	58.0 \pm 1.71	20.3 \pm 2.45	2.13 \pm 0.51	1.23 \pm 0.08	23.4 \pm 2.85	17.8 \pm 0.68
	5	0	55.0 \pm 2.53	-	1.90 \pm 0.45	-	27.6 \pm 0.52	-
		4	72.3 \pm 4.23	50.3 \pm 2.23	2.90 \pm 0.28	2.43 \pm 0.13	29.4 \pm 1.21	25.5 \pm 0.74
		8	78.3 \pm 2.98	42.7 \pm 3.76	3.07 \pm 0.92	1.50 \pm 0.49	30.2 \pm 2.63	22.2 \pm 1.56
Homobrassinolide	1	0	46.7 \pm 1.68	-	1.77 \pm 0.73	-	23.1 \pm 0.97	-
		4	43.0 \pm 1.96	29.0 \pm 2.39	1.90 \pm 0.10	1.63 \pm 0.42	20.1 \pm 1.61	11.1 \pm 1.02
		8	48.7 \pm 2.51	22.7 \pm 1.34	2.20 \pm 0.44	1.13 \pm 0.37	22.3 \pm 1.08	14.6 \pm 0.96
	5	0	52.0 \pm 2.09	-	1.70 \pm 0.12	-	20.4 \pm 1.32	-
		4	64.0 \pm 2.12	45.3 \pm 2.55	2.60 \pm 0.36	2.00 \pm 0.11	27.5 \pm 1.02	23.0 \pm 0.34
		8	62.0 \pm 3.29	35.7 \pm 3.31	2.70 \pm 0.88	1.30 \pm 0.07	26.3 \pm 2.64	20.3 \pm 0.66

Table 2. Effect of brassinosteroids on pod yield, pod number and pod length in French bean. Means \pm SE, $n = 4$.

Treatments	Conc. [μ M]	Stress [d]	Pod yield [g plant ⁻¹]		Pod number		Pod length [cm]	
			control	stressed	control	stressed	control	stressed
Control		0	139.6 \pm 5.08	-	29.3 \pm 2.13	-	16.5 \pm 1.75	-
		4	119.7 \pm 4.51	79.3 \pm 4.02	18.7 \pm 1.92	12.5 \pm 0.95	15.2 \pm 1.92	10.4 \pm 1.87
		8	126.5 \pm 2.09	64.1 \pm 3.16	24.4 \pm 3.11	10.7 \pm 1.87	15.9 \pm 2.27	8.9 \pm 1.32
Epibrassinolide	1	0	129.8 \pm 3.15	-	25.3 \pm 1.15	-	16.0 \pm 1.93	-
		4	126.7 \pm 6.02	95.3 \pm 4.24	28.5 \pm 2.49	11.7 \pm 1.29	14.7 \pm 0.79	9.0 \pm 0.83
		8	135.3 \pm 1.83	68.9 \pm 4.57	22.7 \pm 3.01	9.3 \pm 0.64	16.3 \pm 1.44	9.7 \pm 1.62
	5	0	164.2 \pm 3.26	-	24.0 \pm 1.54	-	17.1 \pm 2.16	-
		4	157.6 \pm 4.39	128.7 \pm 3.32	25.3 \pm 1.78	10.7 \pm 1.76	17.3 \pm 1.67	10.5 \pm 1.25
		8	160.8 \pm 3.73	89.6 \pm 2.81	23.7 \pm 2.06	9.3 \pm 1.91	16.4 \pm 1.45	9.3 \pm 1.71
Homobrassinolide	1	0	119.8 \pm 2.54	-	22.4 \pm 2.91	-	16.8 \pm 1.76	-
		4	126.7 \pm 4.12	85.4 \pm 2.32	25.3 \pm 0.93	11.5 \pm 1.53	17.6 \pm 1.07	10.5 \pm 2.46
		8	120.4 \pm 3.23	67.7 \pm 2.71	21.7 \pm 1.58	8.7 \pm 1.82	15.8 \pm 2.52	9.3 \pm 1.06
	5	0	151.3 \pm 3.77	-	20.4 \pm 1.89	-	15.9 \pm 1.23	-
		4	145.8 \pm 2.91	111.5 \pm 5.50	23.7 \pm 1.65	10.5 \pm 0.85	16.5 \pm 1.71	9.7 \pm 1.18
		8	148.7 \pm 5.36	75.6 \pm 2.11	24.7 \pm 1.46	10.3 \pm 0.53	15.7 \pm 1.59	10.3 \pm 2.02

Sircardi-de-Mallorca (1984), and Cho and Harper (1993). ABA has also been reported to play a role in the auto regulation of nodulation. Cytokinins are vital for nodule initiation and maintenance of their early growth (Taller 1992). Their decline is implicated in impaired root nodulation (Upreti and Murti 1999). Further exogenous cytokinin application has been shown to increase root nodulation (Lorteau *et al.* 2001, Fli and Vessey 2003). Studies have further revealed cytokinins inductive to nodule expressing genes (Fang and Hirsch 1998). There are several reports to account for water stress induced

decline in nitrogenase activity. Besides, ABA has been shown as inhibitory (Bano *et al.* 1983) and cytokinins as stimulatory (Garg *et al.* 1992) to nitrogenase activity in root nodules. Brassinosteroids pretreatment did not alter the ABA content in nodulated roots of either control or stressed plants indicating that the improvement of root nodulation by brassinosteroids could be unrelated of ABA. There was, however, an increase in ZR content and nitrogenase activity in the nodulated roots of both control and stressed plants following brassinosteroid treatments, this influence being apparent with epibrassinolide (5 μ M)

(Table 3). This increase in ZR is expected to favour nodulation. The positive influence of brassinosteroid on nitrogenase activity has also been reported by Vardhini and Rao (1999).

From the results presented, it was apparent that water stress induced decline in root nodulation is associated with increase in ABA and decline in cytokinins contents,

and reduced nitrogenase activity in the nodulated roots. Brassinosteroids especially the epibrassinolide (5 μM) have potential to improve root nodulation and pod yield in the irrigated and water stressed plants; the effect that could be mediated through an influence on cytokinin content and nitrogenase activity in the nodulated roots.

Table 3. Effect of brassinosteroids on abscisic acid and *trans*-zeatin riboside contents and nitrogenase activity in nodulated roots in French bean. Means \pm SE, $n = 4$.

Treatments	Conc. [μM]	Stress [d]	ABA [ng g ⁻¹ (f.m.)]		ZR [pg g ⁻¹ (f.m.)]		Nitrogenase activity [nmol(C ₂ H ₄) g ⁻¹ (f.m.) min ⁻¹]	
			control	stressed	control	stressed	control	stressed
Control		0	25.70 \pm 4.07	-	26.53 \pm 1.42	-	1.40 \pm 0.021	-
		4	32.78 \pm 7.83	57.17 \pm 4.26	24.04 \pm 2.08	18.23 \pm 1.46	1.25 \pm 0.031	0.87 \pm 0.058
		8	27.42 \pm 2.05	85.76 \pm 3.42	23.12 \pm 1.79	13.02 \pm 2.58	1.36 \pm 0.036	0.53 \pm 0.047
Epibrassinolide	1	0	27.62 \pm 3.81	-	27.43 \pm 2.64	-	1.51 \pm 0.068	-
		4	24.92 \pm 2.24	50.51 \pm 2.58	24.82 \pm 2.78	21.70 \pm 1.82	1.49 \pm 0.054	1.09 \pm 0.018
		8	25.26 \pm 1.88	97.04 \pm 1.86	24.11 \pm 1.53	17.56 \pm 2.16	1.46 \pm 0.060	0.72 \pm 0.044
	5	0	27.52 \pm 3.11	-	44.84 \pm 0.82	-	1.99 \pm 0.094	-
		4	27.21 \pm 2.82	50.51 \pm 3.15	40.36 \pm 1.56	32.43 \pm 3.05	1.92 \pm 0.053	1.84 \pm 0.069
		8	26.25 \pm 2.36	91.04 \pm 3.89	39.24 \pm 2.14	27.38 \pm 2.22	1.79 \pm 0.064	1.15 \pm 0.051
Homobrassinolide	1	0	29.50 \pm 1.46	-	26.74 \pm 1.52	-	1.40 \pm 0.080	-
		4	29.04 \pm 1.89	58.73 \pm 1.96	26.48 \pm 1.10	18.36 \pm 1.47	1.44 \pm 0.025	1.00 \pm 0.020
		8	27.42 \pm 0.73	77.63 \pm 2.79	23.19 \pm 1.94	15.58 \pm 1.03	1.51 \pm 0.057	0.67 \pm 0.022
	5	0	32.78 \pm 2.98	-	33.83 \pm 1.18	-	1.69 \pm 0.055	-
		4	28.37 \pm 1.72	58.53 \pm 1.98	29.79 \pm 1.57	26.89 \pm 1.15	1.61 \pm 0.019	1.27 \pm 0.026
		8	28.81 \pm 3.28	83.81 \pm 2.62	28.11 \pm 1.38	19.22 \pm 1.79	1.66 \pm 0.038	0.84 \pm 0.029

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