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Effect of 28-homobrassinolide on the nitrate reductase, carbonic anhydrase activities and net photosynthetic rate in *Vigna radiata*

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The foliage of fifteen day-old *Vigna radiata* seedlings was sprayed with water (control) or with a 10^{-10} M, 10^{-8} M or 10^{-6} M aqueous solution of 28-homobrassinolide (HBR). Samples of the treated plant material, collected at 30/50 days after sowing (DAS), were assessed for the activities of carbonic anhydrase (CA) and nitrate reductase (NR); leaf chlorophyll content (Chl); stomatal conductance (gs); carboxylation efficiency (CE), and net photosynthetic rate (PN). 28-homobrassinolide generated a significant impact on all these characteristics and on seed yield at harvest. Among the treatments, 10^{-8} M proved best, in which case the values for the above six physiological parameters in 30 days-old plants increased by, respectively, 31, 29, 27, 28, 29 and 33% over the control. Moreover, the harvest weight of the seeds of these plants was 27% larger than in the control.

Key words: Brassinosteroids, carbonic anhydrase, nitrogen, nitrate reductase, photosynthetic rate, seed, yield, *Vigna radiata*

Introduction

Brassinosteroids (BRs) are steroidal plant hormones, actively involved in various physiological processes and are essential for plant growth and development (BISHOP and YOKOTA 2001, SASSE 2003). BRs have pleiotropic effects and can induce a broad spectrum of cellular responses including stem elongation, pollen tube growth, leaf bending and epinasty, root inhibition, induction of ethylene biosynthesis, proton pump activation, xylem differentiation and the regulation of gene expression (LI and CHORY 1999, MUSSIG et al. 2002, SASSE 2003). BRs have been identified in 27 families of higher plants and three families of lower plants. They are distributed in various plant parts including roots (SASSE 2003). BRs are also employed in agricultural production. Previous studies have demonstrated that BRs influence seed germination, plant growth, nitrogen fixation, senescence, and leaf abscission and enhance tolerance to cold stress, salt stress, and diseases (CLOUSE and SASSE 1998, KHRIPACH et al. 2000, RAO et al. 2002, HAYAT et al. 2003). In the present study, dilute aqueous solutions, of 28-homobrassinolide were applied to the foliage, the site of active metabolism, to influence plant growth and productivity.

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Material and methods

The seeds of *Vigna radiata* (L.) Wilczeck cv. T 44 were obtained from the National Seed Corporation Ltd., New Delhi. *Vigna radiata* belongs to the family Fabaceae. Healthy seeds were surface sterilized with 0.01% mercuric chloride followed by inoculation with *Rhizobium* sp. The seeds were then sown in earthen pots (25 cm^2), filled with a mixture of sandy loam and farmyard manure in a ratio of 9:1. Fifteen day-old seedlings were sprayed with water (control) or with a 10^{-10} M, 10^{-8} M or 10^{-6} M aqueous solution of 28-homobrassinolide (HBR). Each treatment was replicated thrice. The plants were sampled at 30 and 50 d after sowing (DAS) to study various characteristics. Shoot dry mass was determined after dehydrating the plants at 80 °C for 24 h. Nitrate reductase (NR) activity was determined in fresh leaf samples, following the method of JAWORSKI (1971). Leaf nitrogen was estimated following the procedure of LINDNER (1944). Chlorophyll content was quantified by the procedure of MACKINNEY (1941). The net photosynthetic rate and stomatal conductance were measured using a LI-6200 portable photosynthetic system (LI-COR, NE, USA). The carboxylation efficiency was calculated by employing a formula given by TIWARI et al. (1998). The CA activity in the leaves was measured following the procedure described by DWIVEDI and RANDHAWA (1974). The data were analyzed for variance by one-way ANOVA. Mean values were tested by least significant difference at the 0.05 level of probability using the statistical package SPSS 7.5.

Results and discussion

The leaves of 30 and 50 day-old plants, sprayed with 28-homobrassinolide (HBR) at day 15, photosynthesized at a significantly higher rate than the control (Tab. 1). Moreover, a maximum increase of 33 and 30 %, over the control, at 30 and 50 DAS was recorded in the plants treated with 10^{-8} M HBR. Similarly, BRAUN and WILD (1984a) reported enhancement in the photosynthetic parameters in BR-treated wheat and mustard plants because of the activation of CO_2 fixation. However, we are of the opinion that, like other hormones, HBR might be acting at multiple points. One of them, which has been approached from selected angles in the present study, may be the facilitated diffusion of CO_2 followed by its reduction. A clear-cut response of the plants to applied HBR is evident from an increase in the stomatal conductance (Tab. 1), facilitating the diffusion of carbon dioxide into the stomatal cavity. The next important stage is the entry of CO_2 across the membrane of the chloroplast, which is catalyzed by the enzyme carbonic anhydrase (CA). Finally, the CO_2 is reduced by RuBPCO in the chloroplast stroma. The values of carbonic anhydrase (Tab. 1) and of RuBPCO (BRAUN and WILD 1984b, YU et al. 2004) are elevated by BRs. The response of the plants, in terms of CA activity, was best at 10^{-8} M HBR exhibiting, respectively, a 31 and 26% increase over the control, at 30 and 50 DAS. The exact nature by which HBR exerts its influence on enzyme activity is not understood but KHRIPACH et al. (1999) suggested that BRs affect gene expression and/or membrane functions. The former would be corroborated by the observed (Tab. 1) increase in the activity of a further enzyme, nitrate reductase (NR). Comparable observations have also been reported in rice (MAI et al. 1989), maize (SHEN et al. 1990) and wheat (SAIRAM 1994), treated with BRs. This specific protein is responsible for the initiation of nitrogen metabolism and, consequently, for the level of protein synthesis. KALINICH et al. (1985) have reported higher protein content in the plants of *Phaseolus vulgaris* and *Phaseolus aureus*, treated with BRs and assigned the im-

pact of the hormone on the process of protein synthesis, as a possible reason. Moreover, high nitrogen content (Tab. 1) may be an additional factor involved in increased NR activity, as nitrate is known to induce functional NR, essential for nitrate reduction (SAROOP et al. 1998) by producing žnitrate sensing' proteins of an unknown nature which possibly bind with the regulatory regions at the NR-genes and turn on the expression of NR-mRNA (REDINBAUGH and CAMPBELL 1991). However, the total nitrate-reducing capacity in a plant system depends on three important factors (a) the availability of nitrate (the substrate) in the cytoplasm (b) the level of nitrate reductase and (c) its activity level (CAMPBELL 1999).

The increase in the level of CO_2 in the stroma is also associated with an accelerated rate of its reduction which is evident from the increased carboxylation efficiency (CE), in response to HBR, over the control (Tab. 2). The leaves of the plants treated with HBR not only photosynthesized at a faster rate, with higher chlorophyll content but also possessed an extended period of metabolic activity because HBR delayed senescence (data not included), as compared to the control. The overall effect of HBR was reflected by the higher dry mass of the plants and increased pod bearing capacity, resulting in improved seed yield at harvest time.

Tab. 1. Net photosynthetic rate (PN) [μ mol (CO_2) m^{-2} s^{-1}], stomatal conductance (gs) [mol m^{-2} s^{-1}], carbonic anhydrase (CA) activity [mol (CO_2) $kg^{-1}s^{-1}$], nitrate reductase (NR) activity [n mol (NO_2) h^{-1} g^{-1}] and nitrogen (N) content [% of DM] in *Vigna radiata* plants, sprayed with water (control), 28-homobrassinolide (HBR: 10^{-10}, 10^{-8} or 10^{-6} M) at 15 d, after sowing (DAS) and sampled at 30 and 50 DAS. LSD for p = 0.05

	PN		gs		CA Activity		NR Activity		Nitrogen	
	30 DAS	50 DAS	30 DAS	50 DAS	30 DAS	50 DAS	30 DAS	50 DAS	30 DAS	50 DAS
Control	14.50	12.00	0.367	0.325	1.80	1.55	412	300	3.14	2.09
10^{-10}	17.80	13.75	0.445	0.400	2.19	1.82	495	345	3.55	2.25
10^{-8}	19.31	15.16	0.471	0.438	2.36	1.96	535	376	3.82	2.39
10^{-6}	17.00	13.18	0.417	0.371	2.07	1.70	470	351	3.57	2.26
LSD	0.90	1.00	0.017	0.016	0.09	0.15	28	15	0.12	0.10

Tab. 2. Carboxylation efficiency (CE) [mol $m^{-2}s^{-1}$], leaf chlorophyll content (Chl) [g kg^{-1} of FM], dry mass (g) of shoot, pod number $plant^{-1}$ and seed yield (g) $plant^{-1}$ in *Vigna radiata* plants, sprayed with water (control), 28-homobrassinolide (HBR: 10^{-10}, 10^{-8} or 10^{-6} M) at 15 d, after sowing (DAS) and sampled at 30 and 50 DAS. LSD for p = 0.05

	CE		Chl		DM		Pod number	Seed yield
	30 DAS	50 DAS	30 DAS	50 DAS	30 DAS	50 DAS		
Control	0.041	0.033	1.31	1.41	2.30	6.13	26.00	3.65
10^{-10}	0.049	0.040	1.52	1.66	2.51	6.63	31.60	3.95
10^{-8}	0.053	0.042	1.67	1.71	2.95	7.00	33.60	4.64
10^{-6}	0.047	0.036	1.43	1.65	2.56	6.64	30.14	3.90
LSD	0.003	0.002	0.09	0.10	0.15	0.19	1.55	0.30

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