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BRIEF COMMUNICATION

Responses of *Vigna radiata* to foliar application of 28-homobrassinolide and kinetin

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Abstract

Effects of 28-homobrassinolide (HBR) and kinetin (KIN) on photosynthesis, nitrogen metabolism, and the seed yield were studied. The leaves of 25-d-old plants of *Vigna radiata* (L.) Wilczek were sprayed with 0.01, 1.0 or 100 μM aqueous solution of KIN, or 0.0001, 0.01 or 1.0 μM that of HBR. KIN and especially HBR increased the activities of nitrate reductase and carbonic anhydrase, chlorophyll and total protein contents and net photosynthetic rate in the leaves, and pod number and seed yield, at harvest.

Additional key words: brassinosteroid, carbonic anhydrase, leaf protein, mung bean, net photosynthetic rate, nitrate reductase, seed yield.

Brassinosteroids (BRs) are regarded essential for growth and development of the plants (Li and Chory 1999) and their exogenous application improves the productivity potential of economically important crops by activating cell elongation, vascular differentiation and/or proton pump (Rao *et al*. 2002, Hayat *et al*. 2003). Plants supplemented with BRs exhibited increased activities of carbonic anhydrase (CA) and nitrate reductase (NR) (Hayat *et al*. 2001a,b), phosphoenolpyruvate carboxylase, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), the contents of soluble proteins (Braun and Wild 1984a,b, Yang *et al*. 1992), and resistance to various stresses (Rao *et al*. 2002).

The cytokinins may also be employed to improve photosynthetic rate (Chernyad'ev 1993, Metwally 1997) as it regulates the content of chlorophyll and the activity of RuBPCO (Chernyad'ev 1994, Singh *et al*. 2001). Keeping in view, the close relation between the actions of the two hormones, we tempted to study their impact on photosynthesis and nitrogen metabolism.

Seeds of *Vigna radiata* (L.) Wilczek cv. T-44 were obtained from National Seed Corporation, New Delhi, India. They were surface sterilized with 0.01 % mercuric chloride solution, followed with repeated washings, by using double distilled water. The seeds were then sown in earthen pots (25 cm diameter) filled with sandy loam soil and farmyard manure, mixed in a ratio of 9:1. A uniform basal dose (40, 295 and 73 mg) of N, P and K, in the form of urea, single superphosphate and muriate of potash, was applied at the time of sowing to each pot.

28-homobrassinolide (HBR) and kinetin (KIN) were obtained from *Godrej Agrovet Ltd*., Mumbai, India and *Sigma Chemicals Co*., St. Louis, USA, respectively. The plants of 25 d-age were sprayed with 0.01, 1.0 or 100 μM KIN or 0.0001, 0.01 or 1.0 μM HBR at the rate of 5 cm^3 $plant^{-1}$. Control plants were sprayed with double distilled water only. Each treatment was replicated thrice. The pots were lined, in the net house, according to simple randomized block design. The following parameters were studied, 10 and 20 d after the spray.

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Abbreviations: BRs - brassinosteroids; CA - carbonic anhydrase; Chl - chlorophyll; DAS - days after sowing; HBR - homobrassinolide; KIN - kinetin; NR - nitrate reductase; P_N - net photosynthetic rate.

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Nitrate reductase (E.C. 1.6.6.1) activity was determined in fresh leaves of the plants by the procedure adopted by Jaworski (1971). This method is based on the reduction of nitrate to nitrite, whose value was estimated colorimetrically. Carbonic anhydrase (E.C. 4.2.1.1) activity was assayed by the method of Dwivedi and Randhawa (1974). 200 mg fresh leaf was cut into small pieces, in 0.2 M cystein, at 4 °C. These pieces were transferred to test tube with phosphate buffer and 0.2 M sodium bicarbonate and bromothymol blue were added. CO_2 liberated during catalytic action of enzyme on $NaHCO_3$ was estimated by titrating the reaction mixture against HCl, using methyl red as an indicator. Total chlorophyll content was estimated following the method of Mackinney (1941). Net photosynthetic rate (P_N) at each stage of growth was measured in fully expanded leaves by a portable photosynthetic system (*LI-COR-6200*, Lincoln, USA). Each observation was replicated thrice. Protein content in the leaves was estimated by the method of Lowry *et al.* (1951). Treatment means were compared by analysis of variance using statistical package *SPSS*. Each sampling date was analysed separately. Least significant difference (LSD) was calculated at 0.05 level of probability.

The spray of either KIN or HBR increased the activity of CA, irrespective of the sampling time. HBR was more efficient than KIN where the CA activity increased with an increase in the concentration of HBR from 0.0001 to 0.01 μM but a further increase (1.0 μM) possibly became supra-optimal. Maximum increase in CA activity (43 % relative to control), was noted in 35 d-old plants treated with 0.01 μM HBR at 25 d-stage (Table 1). The elevation in the activity of CA induced by KIN was in support of Sugiharto *et al.* (1992) and Hayat *et al.* (2001b). Sugiharto *et al.* (1992) also suggested that cytokinins acted either at the level of transcription and/or the stabilization of the transcripts as they increased the level of CA-mRNA. However, the exact mode of action of HBR is not known but Hayat *et al.* (2001a) are of the opinion that HBR could have acted in a way similar to that of KIN.

NR activity increased with the exogenous application of KIN and HBR (Table 1) but the latter was a better inducer of NR activity than the former. BRs have also earlier been claimed to be the elevator of the NR activity (Hayat *et al.* 2001a, Hayat and Ahmad 2003). A distinct increase in the content of the proteins, in the treated leaves (Table 1) may be assigned as a possible reason for increased NR activity, which supports the findings of Saroop *et al.* (1998). Moreover, the observed positive correlation between the content of the proteins and that of the enzyme (r^2 = 0.915) gives additional support to the above belief.

High protein content, increased NR and CA activities in the leaves of the plants (Table 1), sprayed with KIN or HBR, is possibly an expression of the impact of the hormones on the transcription and/or translation, as reported earlier (Kalinich *et al.* 1985).

Table 1. Leaf carbonic anhydrase (CA) activity [mol(CO_2) kg^{-1} s^{-1}], nitrate reductase (NR) activity [nmol(NO_2) g^{-1} h^{-1}], protein content [%(d.m.)], chlorophyll (Chl) content [g kg^{-1}(f.m.)] and net photosynthetic rate (P_N) [μmol(CO_2) m^{-2} s^{-1}] in *Vigna radiata* plants, sprayed with water (control), kinetin (KIN) or 28-homobrassinolide (HBR) at 25 d after sowing and sampled at 35 and 45 DAS. LSD for *P* = 0.05, mean ± SE, * = values significantly different from control.

Parameter	Treatment	[μM]	35 DAS	45 DAS
CA	Control		1.92 ± 0.16	1.65 ± 0.14
	KIN	0.01	2.28 ± 0.19*	1.95 ± 0.16*
		1.0	2.52 ± 0.24*	2.05 ± 0.20*
		100.0	2.48 ± 0.25*	1.98 ± 0.15*
	HBR	0.0001	2.56 ± 0.23*	2.05 ± 0.18*
		0.01	2.76 ± 0.19*	2.13 ± 0.21*
		1.0	2.32 ± 0.20*	1.93 ± 0.17*
LSD			0.089	0.065
NR	Control		524.0 ± 6.2	380.0 ± 6.5
	KIN	0.01	641.0 ± 9.3*	464.0 ± 5.8*
		1.0	709.0 ±10.4*	498.0 ± 6.3*
		100.0	659.0 ± 7.8*	455.0 ± 5.5*
	HBR	0.0001	702.0 ± 8.3*	465.0 ± 6.2*
		0.01	758.0 ± 9.1*	526.0 ± 5.8*
		1.0	653.0 ± 7.5*	467.0 ± 4.9*
LSD			33.0	21.0
Protein	Control		15.90 ± 1.4	13.76 ± 1.2
	KIN	0.01	17.19 ± 1.6*	14.34 ± 1.1*
		1.0	18.62 ± 1.7*	16.19 ± 1.3*
		100.0	18.48 ± 1.8*	15.73 ± 1.3*
	HBR	0.0001	18.27 ± 1.7*	15.53 ± 1.5*
		0.01	19.24 ± 1.5*	17.78 ± 1.4*
		1.0	17.82 ± 1.7*	14.75 ± 1.3*
LSD			1.11	0.85
Chl	Control		1.45 ± 0.13	1.26 ± 0.11
	KIN	0.01	1.61 ± 0.15*	1.36 ± 0.12*
		1.0	1.83 ± 0.16*	1.53 ± 0.14*
		100.0	1.77 ± 0.15*	1.47 ± 0.11*
	HBR	0.0001	1.82 ± 0.17*	1.49 ± 0.12*
		0.01	1.87 ± 0.15*	1.52 ± 0.14*
		1.0	1.62 ± 0.14*	1.36 ± 0.11*
LSD			0.09	0.05
P_N	Control		19.23 ± 1.5	17.11 ± 1.6
	KIN	0.01	20.37 ± 1.4*	18.72 ± 1.8*
		1.0	22.79 ± 1.9*	19.84 ± 1.7*
		100.0	21.20 ± 1.8*	18.93 ± 1.8*
	HBR	0.0001	23.61 ± 2.1*	21.66 ± 2.1*
		0.01	25.22 ± 2.4*	21.00 ± 1.5*
		1.0	20.93 ± 1.9*	19.47 ± 1.7*
LSD			0.53	0.93

The leaves of the plants, receiving KIN treatment, had

Table 2. Number of pods and seed yield in *Vigna radiata* plants, sprayed with water (control), kinetin (KIN) or 28-homobrassinolide (HBR) at 25 d after sowing and sampled, at harvest. LSD for $P = 0.05$, mean ± SE, * - values significantly different from control.

Treatment	$[\mu M]$	Number of pods [plant^{-1}]	Seed yield [g plant^{-1}]
Control		27.12 ± 2.1	3.73 ± 0.31
KIN	0.01	28.56 ± 2.3*	3.83 ± 0.35*
	1.0	31.47 ± 2.9*	4.23 ± 0.38*
	100.0	30.22 ± 2.7*	4.16 ± 0.39*
HBR	0.0001	32.89 ± 3.1*	4.46 ± 0.43*
	0.01	34.96 ± 3.2*	4.71 ± 0.49*
	1.0	30.77 ± 2.9*	3.88 ± 0.37*
LSD		1.42	0.24

higher chlorophyll content, compared with control (Table 1) which is in agreement with Ray and Choudhuri (1981), and Ghosh and Biswas (1991). This effect of HBR was quite prominent than that of KIN (Table 1). A cumulative effect of the increased activities of NR and CA and higher protein and Chl contents of the leaves, receiving KIN or HBR treatment, was further reflected as high net photosynthetic (P_N) rate (Table 1). It could either be an expression of increased availability of CO_2, generated by high CA activity (Table 1) and/or induced activity of Rubisco, whose activity is known to be elevated by BR treatment (Braun and Wild 1984b). Moreover, the activity of CA was positively correlated with P_N ($r^2 = 0.848$). Various other plants, fed with KIN (Borzenkova 1976) or HBR (Hayat *et al.* 2000, Fariduddin *et al.* 2003) are also reported to express high P_N.

It is quite natural that the number of flowers and fruits is the main determinant factor of plant productivity where cytokinins have been implicated to rescue their premature drop (Nagel *et al.* 2001). This improvement might have been caused by re-directing the movement of assimilates (Dybing 1994). In the present study, not only KIN but also HBR delayed the senescence and abscission of leaves (data not included). Similarly, the senescence was slowed down by BRs in the leaves and fruits of citrus (Sugiyama and Kuraishi 1989, Iwahori 1990) and hypocotyl segments of mungbean seedlings (Zhao *et al.* 1987). The observed increase in the number of pods, over the control (Table 2) and similar results of Peterson *et al.* (1990), and Hayat *et al.* (2001b) was obviously the outcome of the effect of the hormones in checking the pre-mature drop of the reproductive organs. Moreover, additional quantity of assimilates is made available to the flowers and fruits because the treatments with KIN or HBR enhanced the P_N of the leaves (Table 1). The observed increase in seed yield (Table 2) and that of Takahashi *et al.* (1994) is, therefore, an expression of the cumulative effect of the hormones on the leaf metabolism, re-directed mobilization of metabolites, delayed senescence and abscission of plant organs.

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