BRIEF COMMUNICATION

Photosynthetic response of *Vigna radiata* to pre-sowing seed treatment with 28-homobrassinolide

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Abstract

Surface sterilised seeds of mungbean (*Vigna radiata* L. Wilczek cv. T-44) were soaked in 0, 10^{-8}, 10^{-6}, or 10^{-4} M aqueous solution of 28-homobrassinolide (HBR) for 4, 8, or 12 h. The treated seeds were grown in sandy loam soil filled in earthen pots and sampled at 30, 40, and 50 d. Net photosynthetic rate, leaf chlorophyll content, carbonic anhydrase activity (E.C. 4.2.1.1), carboxylation efficiency, stomatal conductance, and seed yield at harvest were enhanced by the HBR treatment. The best combination was the pre-sowing seed treatment with 10^{-6} M HBR for 8 h.

Additional key words: carbonic anhydrase; carboxylation efficiency; chlorophyll; mungbean; net photosynthetic rate; seed yield; stomatal conductance.

Brassinosteroids (BRs) were initially isolated from pollen grains of *Brassica napus* (Grove *et al*. 1979). Their various forms, widely distributed in plant kingdom, have a distinct role in stem elongation, pollen tube growth, leaf bending, ethylene biosynthesis, and xylem differentiation by regulating transcription (Khripach *et al*. 2000). Plants supplemented with BRs exhibit healthy growth (Sakurai and Fujioka 1993), increased activities of carbonic anhydrase (CA) and nitrate reductase (Hayat *et al*. 2001), phosphoenolpyruvate carboxylase, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO), dark CO_2 fixation, concentration of soluble proteins, and surface area of the leaf (Braun and Wild 1984a,b, Yang *et al*. 1992). BRs-treated plants also exhibit higher resistance to stress and produce more seeds in crop plants (Hamada 1986, Cutler 1991, Hayat *et al*. 2000, Rao *et al*. 2002). One of BRs (28-homobrassinolide), fairly resistant to disintegration, was picked for study using mungbean as a test crop to characterise photosynthetic efficiency of the plants, grown from the seeds treated with HBR.

Seeds of *Vigna radiata* (L.) Wilczek cv. T-44 were procured from National Seed Corporation, New Delhi, India. The healthy seeds were surface sterilised with mercuric chloride solution (0.01 %) followed with repeated washing by double distilled water. The seeds were then soaked in water (control), 10^{-8}, 10^{-6}, or 10^{-4} M aqueous solution of 28-homobrassinolide (HBR) for 4, 8, or 12 h. The treated seeds were sown in earthen pots (25 cm diameter), filled with sandy loam soil plus farmyard manure, mixed in a ratio of 9 : 1. Pots were irrigated regularly with tap water. Each treatment was replicated five times. Net photosynthetic rate (P_N), leaf chlorophyll ($a+b$) content (Chl), carbonic anhydrase (CA) activity, stomatal conductance (g_s), and carboxylation efficiency (CE) were assessed in the leaves of 30, 40, and 50 d-old plants. Seed yield was recorded at harvest (65 DAS). Chl content was estimated according to Mackinney (1941). CA activity was determined by the procedure of Dwivedi and Randhawa (1974). P_N and g_s in intact leaves were measured by *LI-6200* portable photosynthesis system (*LI-COR*, Lincoln, NE, USA). The CE was computed by adopting the formulae used by Tiwari *et al*. (1998). The data were analysed for variance by two-way *ANOVA*. Mean values were tested by least significant difference

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Abbreviations: BRs – brassinosteroids; CA – carbonic anhydrase; CE – carboxylation efficiency; Chl – chlorophyll; DAS – days after sowing; g_s – stomatal conductance; HBR – 28-homobrassinolide; P_N – net photosynthetic rate; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase.
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Table 1. Leaf carbonic anhydrase (CA) activity [mol(CO_2) kg^{-1}(FM) s^{-1}], chlorophyll (Chl) content [g kg^{-1}], carboxylation efficiency (CE) [mol m^{-2} s^{-1}], stomatal conductance (g_s) [mol m^{-2} s^{-1}], net photosynthetic rate (P_N) [μmol(CO_2) m^{-1} s^{-1}], and seed yield per plant [g] in *Vigna radiata* plants, raised from the seeds soaked in water (control), 10^{-8}, 10^{-6}, or 10^{-4} M HBR for 4, 8, or 12 h and sampled at 30, 40, and 50 d after sowing (DAS). LSD for p = 0.05, S = soaking, NS = non-significant.

	Treatment	30 DAS			40 DAS			50 DAS		
	[h]	4	8	12	4	8	12	4	8	12
CA	Control	1.82	1.86	1.83	1.92	2.00	1.96	1.68	1.62	1.69
	10^{-8} M	1.93	1.99	1.95	2.05	2.13	2.11	1.82	1.91	1.89
	10^{-6} M	2.15	2.29	2.32	2.34	2.51	2.48	2.09	2.20	2.18
	10^{-4} M	1.97	2.05	2.02	2.07	2.14	2.16	1.85	1.96	1.94
LSD		S = 0.08			S = 0.07			S = 0.08		
		HBR = 0.11			HBR = 0.10			HBR = 0.12		
Chl	Control	0.95	1.02	1.00	1.14	1.18	1.16	0.85	0.90	0.88
	10^{-8} M	1.03	1.09	1.15	1.32	1.44	1.42	1.02	1.13	1.09
	10^{-6} M	1.16	1.34	1.32	1.51	1.71	1.68	1.19	1.30	1.27
	10^{-4} M	1.10	1.32	1.21	1.38	1.46	1.41	1.09	1.18	1.16
LSD		S = 0.11			S = 0.09			S = NS		
		HBR = 0.15			HBR = 0.16			HBR = 0.11		
CE		0.039	0.039	0.041	0.042	0.044	0.045	0.032	0.034	0.034
		0.044	0.044	0.052	0.048	0.050	0.050	0.039	0.040	0.043
		0.052	0.05č	0.054	0.056	0.057	0.060	0.044	0.047	0.048
		0.045	0.048	0.048	0.048	0.052	0.052	0.041	0.044	0.044
LSD		S = NS			S = NS			S = NS		
		HBR = 0.006			HBR = 0.005			HBR = 0.006		
g_s	Control	0.317	0.320	0.320	0.430	0.425	0.430	0.370	0.365	0.363
	10^{-8} M	0.323	0.339	0.346	0.450	0.481	0.493	0.385	0.393	0.390
	10^{-6} M	0.371	0.414	0.420	0.493	0.520	0.529	0.438	0.460	0.462
	10^{-4} M	0.373	0.375	0.381	0.472	0.503	0.492	0.410	0.425	0.439
LSD		S = NS			S = NS			S = NS		
		HBR = 0.020			HBR = 0.029			HBR = 0.019		
P_N	Control	13.59	13.98	14.06	15.08	15.44	15.38	11.45	12.07	12.17
	10^{-8} M	14.77	15.51	17.75	16.73	17.23	16.95	13.25	14.62	14.85
	10^{-6} M	17.29	18.78	18.09	19.01	20.45	20.14	14.74	16.08	15.95
	10^{-4} M	15.68	16.50	16.61	17.18	18.11	17.80	14.02	14.90	15.10
LSD		S = 0.84			S = 0.64			S = 0.66		
		HBR = 1. 45			HBR = 1.69			HBR = 0.88		
Seed yield	Control							3.79	3.89	3.80
	10^{-8} M							3.85	4.03	4.10
	10^{-6} M							4.29	4.72	4.63
	10^{-4} M							4.05	4.19	4.25
LSD								S = 0.13		
								HBR = 0.30		

at 0.05 probability level (Gomez and Gomez 1984).

Plants from the HBR-treated seeds exhibited a better state of metabolism than control (Table 1). At harvest the seed bearing capacity of these plants was also high (Table 1). The photosynthesising tissues of C_3 and C_4 plants contain CA that makes CO_2 available to RuBPCO by catalysing reversible hydration of CO_2 (Badger and Price 1994). The distribution pattern of CA is, therefore, very much comparable with that of RuBPCO (Tsuzuki *et al*. 1985). Moreover, CA is also assigned some unexplained role in electron transport chain (Stemler 1997) and the regulation of pH at thylakoids (Reed and Graham 1981). However, the foliage of the plants resulting from the HBR-treated seeds possessed more CA than the control, which increased up to day 40 followed with a slight decline (Table 1). It was also associated with the observed increase in the values for leaf Chl content (Table 1) which finds the support of Sairam (1994), Bhatia and Kaur (1997), and Hayat *et al.* (2000). This expression of the plants to HBR may be explained on the basis of the findings of Kalinich *et al.* (1985): BRs had an impact on the transcription and translation leading to an increase in protein content in *Phaseolus vulgaris* and *Phaseolus aureus*.

Plants from HBR-treated seeds exhibited higher CE than the control (Table 1). This state has possibly been generated because the plants contain more Chl (Singh *et al*. 1999) and lower stomatal resistance facilitating free exchange of gases (Arteca and Dong 1981). The comulative effect of more Chl and more CA could have improved the availability of CO_2 under high g_s (Table 1), and its rate of reduction by RuBPCO whose activity is generally enhanced by brassinolides (Braun and Wild 1984a). At last, the interaction of these basic determinants favoured an improvement in P_N (Table 1). Higher P_N under the influence of BRs was reported also in other crops (Yang *et al.* 1992, Sairam 1994, Hayat *et al.* 2000). Moreover, P_N was positively correlated with CA, Chl, or g_s (Fig. 1, and Ohki 1978 and Ahmad *et al.* 2001).

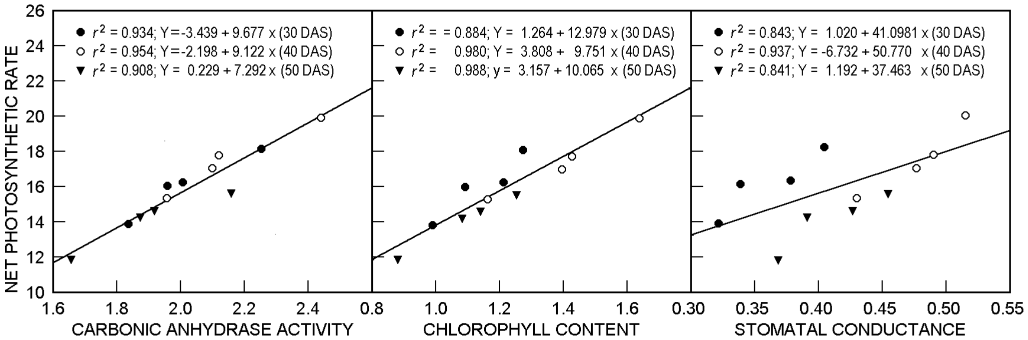

Fig. 1. Correlations between net photosynthetic rate (P_N) and carbonic anhydrase (CA) activity, chlorophyll (Chl) content, or stomatal conductance (g_s).

The HBR-generated increase in photosynthetic activity of the leaves with delayed senescence (Menon and Srivastava 1984, Fariduddin 2002) and abscission (Iwahori *et al.* 1990) should have naturally favoured vegetative growth. Once the plants are healthy they produce better yields at harvest (Table 1). Among the various concentrations and durations of soaking, 8 h-treatment with 10^{-6} M HBR proved best. The other concentrations were either very low or supra-optimal for most of the parameters.

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