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BRIEF COMMUNICATION

Carbonic anhydrase, photosynthesis, and seed yield in mustard plants treated with phytohormones

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Abstract

The leaves of 30-d-old plants of *Brassica juncea* Czern & Coss cv. Varuna were sprayed with 10^{-6} M aqueous solutions of indole-3-yl-acetic acid (IAA), gibberellic acid (GA_3), kinetin (KIN), and abscisic acid (ABA) or 10^{-8} M of 28-homobrassinolide (HBR). All the phytohormones, except ABA, improved the vegetative growth and seed yield at harvest, compared with those sprayed with deionised water (control). HBR was most prominent in its effect, generating 32, 30, 36, 70, 25, and 29 % higher values for dry mass, chlorophyll content, carbonic anhydrase (E.C. 4.2.1.1) activity, and net photosynthetic rate in 60-d-old plants, pods per plant, and seed yield at harvest, over the control, respectively. The order of response to various hormones was HBR > GA_3 > IAA > KIN > control > ABA.

Additional key words: abscisic acid; *Brassica juncea* L.; chlorophyll content; gibberellic acid; indole-3-yl-acetic acid; kinetin; net photosynthetic rate; pod and seed number; seed mass and yield.

Various phytohormones exhibit specialised effects on the properties of the membrane and the transport of substances through them, according to their physiological functions. In addition to other functions, auxins affect the proton pump (Aducci *et al.* 1985), gibberellins alter membrane permeability to ions (Crozier and Turnball 1984), cytokinins favour membrane selectivity by involving calcium (Olah *et al.* 1983), and abscisic acid regulates water movement by modifying K^+/Na^+ selectivity (Knight *et al.* 1995). These and other related factors may have a direct impact on the leaf chlorophyll (Chl) content (Ghorbanli *et al.* 1999, Singh *et al.* 1999). Depending on the plant interaction with the phytohormone, its content increased by auxin application and decreased by GA_3. Assimilatory pattern modified by phytohormones (Chatterjee *et al.* 1976) may establish a canopy structure most suited for optimal interception of radiant energy favouring higher biological yield (Hayat *et al.* 2000) in mustard. The goal of this experiment was to maximise the biological yield by improving light-harvesting assembly to ensure higher net photosynthetic rate (P_N) through the application of various phytohormones to the plants.

The seeds of *Brassica juncea* Czern & Coss cv. Varuna were obtained from National Seed Corporation Ltd., New Delhi, India. The healthy seeds were surface sterilised with 0.1 % mercuric chloride solution followed by washings with double distilled water and sown in earthen pots (25 cm diameter), lined on its inner surface with polythene sleeves, filled with acid washed sand (Hewitt 1966). Each pot was supplied with 200 cm^3 of full nutrient solution (Hoagland and Arnon 1950) on alternate days, up to day 30. Thereafter, the quantity of nutrient solution was increased to 500 cm^3. De-ionised water (250 cm^3) was also given to each pot, daily. Based on our studies (unpublished), 10^{-6} M aqueous solutions of IAA, GA_3, KIN, ABA, and 10^{-8} M of HBR were sprayed on 30-d-old plants with the precaution that the phytohormone must not fell in the pot and must stay on the plants. Control plants were sprayed with double distilled water. Each treatment was replicated five times. Dry mass per plant (recorded at 80 °C), chlorophyll (Chl) *a* and *b* contents and Chl *a/b* ratio, carbonic anhydrase (CA) activity, and P_N in the leaves were assessed in 60-d-old plants. The yield characteristics were recorded at harvest. The Chl content was estimated following the

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Abbreviations: ABA - abscisic acid; CA - carbonic anhydrase; Chl - chlorophyll; DAS - days after sowing; GA_3 - gibberellic acid; HBR - 28-homobrassinolide; IAA - indole-3-yl-acetic acid; KIN - kinetin; P_N - net photosynthetic rate.

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method of Mackinney (1941). The procedure of Dwivedi and Randhawa (1974) was used for measuring the activity of CA. P_N in intact leaves was measured by *LI-6200* portable photosynthesis system (*LI-COR*, Lincoln, NE, USA). The results were statistically analysed following the procedures described by Gomez and Gomez (1984).

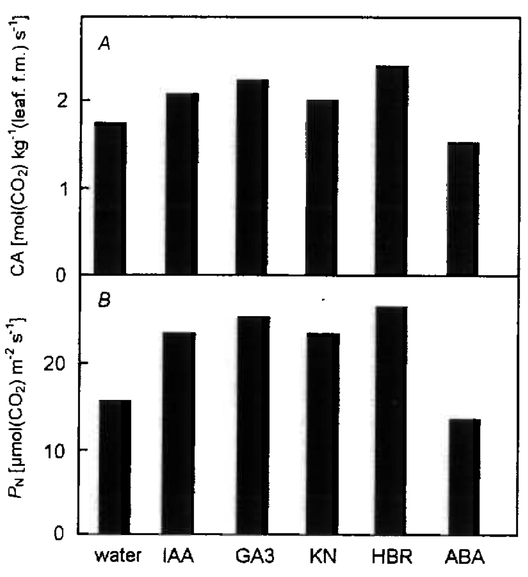

Fig. 1. Effect of phytohormones on leaf carbonic anhydrase (CA) activity (*A*) and net photosynthetic rate, P_N (*B*) in 60-d-old mustard plants.

All the parameters studied on day 60 were significantly enhanced by all the phytohormones, except ABA. HBR proved best, enhancing plant dry mass, leaf Chl *a*, *b*, and (*a*+*b*) contents, Chl *a*/*b* ratio, CA activity, and P_N by 39.6, 50.3, 32.4, 13.6, 35.8, and 68.8 %, respectively, over the control (Table 1, Fig. 1). IAA and KIN also induced a significant response but the values were equal. Similarly, pod number per plant and seed yield at harvest were significantly enhanced by all the phytohormones studied, except ABA. A maximum increase of 29.1 % more seeds over the control was recorded with HBR. However, the plants sprayed with ABA yielded 5.6 % less seeds.

Higher P_N in the leaves may successfully be attained by the exogenous application of aqueous solution of IAA (Turner and Bidwell 1965, Chatterjee *et al.* 1976), GA$_3$ (Gale *et al.* 1974, Erkan and Bangerth 1980), KIN/benzyladenine (Treharne *et al.* 1970), or BR/HBR (Braun and Wild 1984, Luo *et al.* 1986, Yang *et al.* 1992, Hayat *et al.* 2000). However, ABA is assigned some important regulatory roles in protecting the plants from stress (Markhart *et al.* 1979, Davies and Jones 1991) but brings about stomata closure resulting in a negative impact on the amount of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) and P_N (Davies and Jones 1991, Makheev *et al.* 1992). Similar conclusions may be derived from our observations (Fig. 1*B*) where all the phytohormones studied, except ABA, enhanced P_N of mustard leaves. HBR was best increasing P_N by 29 % over the control. The higher P_N of the plants receiving phytohormones may be explained by the observed increase in Chl contents (Table 1), higher P_N and lower stomatal resistance (Feierabend 1969, Treharne *et al.* 1970, Hoad *et al.* 1977, Arteca and Dong 1981), and elevated amount of photophosphorylation in isolated chloroplast (Tamas *et al.* 1973).

Table 1. Effect of phytohormones on dry mass, yield characteristics, seed yields, and leaf chlorophyll (Chl) content in 60-d-old mustard plants. NS = non-significant. For abbreviations see title page of the paper.

Parameter	Control	IAA	GA$_3$	KIN	HBR	ABA	C.D. at 5 %
Dry mass [g plant^{-1}]	2.25	2.65	2.77	2.50	2.98	1.97	0.15
Pod number [plant^{-1}]	216.65	250.15	259.15	250.45	271.50	194.15	8.35
Seed number [pod^{-1}]	12.50	12.53	12.62	12.52	12.60	12.43	NS
100 seed mass [mg]	320.15	321.45	324.15	322.00	323.00	320.45	NS
Seed yield [g plant^{-1}]	7.24	8.10	8.64	8.25	9.35	6.83	0.38
Chl *a* [mg kg^{-1}]	502	635	662	581	755	401	80
Chl *b* [mg kg^{-1}]	753	899	952	851	997	623	70
Chl (*a*+*b*) [mg kg^{-1}]	1255	1535	1615	1432	1752	1025	130
Chl *a*/*b*	0.67	0.71	0.70	0.69	0.76	0.64	0.07

The activity of CA was increased by the application to the leaves of all the phytohormones, except ABA (Fig. 1*A*) which may be because of its *de novo* synthesis by involving transcription and/or translation (Okabe *et al.* 1980). CA is found in abundance in the photosynthesising tissue of both C$_3$ and C$_4$ plants and regulates the availability of CO$_2$ to RuBPCO by catalysing the reversible hydration of CO$_2$ (Badger and Price 1994). This makes the distribution patterns of CA and RuBPCO comparable to each other (Tsuzuki *et al.* 1985). Moreover, CA should also have some role in electron transport chain (Stemler 1997) and regulation of pH in chloroplasts exposed to rapidly changing irradiance because of its association with thylakoids (Reed and Graham 1981). Antisense expression has been employed to identify the specific role(s) of chloroplast located CA in C$_3$ plants

(Majeau *et al.* 1994, Price *et al.* 1994). In antisense plants only a 5 % decline in P_N was found by a 1 % decrease in CA activity (Williams *et al.* 1996). In contrast, an inhibition of CA activity in ethoxyzolamide infiltrated C_3 plant leaf pieces resulted in 80-90 % inhibition of P_N at low CO_2 concentrations. Thus some more essential role could be assigned to CA than that noted in antisense studies (Badger and Pfanz 1995).

The leaves of the plants, treated with HBR, GA_3, IAA, and KIN, photosynthesised at a faster rate than the control (Fig. 1*B*) and were possibly withheld for a longer duration because of delayed senescence (Menon and Srivastava 1984) and abscission (Iwahori *et al.* 1990). Thus availability of photosynthates in larger quantities for a longer duration may have contributed favourably in improving pod bearing capacity of the plants leading to an increase in seed yield at harvest (Table 1). The order of response to various phytohormones is similar for all the parameters studied (HBR > GA_3 > IAA > KIN > control > ABA).

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