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Photosynthetic efficiency of plants of *Brassica juncea*, treated with chlorosubstituted auxins

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Abstract

The leaves of 29-d-old plants of *Brassica juncea* Czern & Coss cv. Varuna were sprayed with 10^{-6} or 10^{-8} M aqueous solutions of indole-3-yl-acetic acid (IAA) or its substituted derivatives 4-Cl-IAA, 7-Cl-IAA, and 4,7-Cl_2-IAA. All the auxins improved the vegetative growth and seed yield at harvest compared with those sprayed with de-ionised water (control). 4-Cl-IAA was most prominent in its effect, generating 21.6, 39.7, 61.0, 35.0, 65.5, and 56.2 % higher values for dry mass, leaf chlorophyll content, carbonic anhydrase (E.C. 4.2.1.1) and nitrate reductase (E.C. 1.6.6.1) activities, net photosynthetic rate, and carboxylation efficiency, respectively, in 60-d-old plants. It also enhanced the seed yield by 31.1 % over the control. The order of response of the plants to various auxins was 4-Cl IAA $\geq$ 7-Cl IAA > 4,7-Cl_2 IAA = IAA > control.

Additional key words: carbonic anhydrase; carboxylation efficiency; chloroindole auxins; chlorophyll; net photosynthetic rate; nitrate reductase; pod; seed yield.

Introduction

Among the auxins, indole-3-acetic acid (IAA) is ubiquitous in plants (Davies 1995). However, chlorosubstituted auxins are of rare occurrence in plants. 4-chloroindole-3-acetic acid (4-Cl-IAA) has been identified in the extracts of *Vicieae* and also its methyl ester in the immature seeds (Gandar and Nitsch 1967, Marumo *et al.* 1968) and the shoot, root, and the cotyledons of 3-d-old etiolated seedlings (Schneider *et al.* 1985) of *Pisum*. Similarly, it is also reported in *Pinus sylvestris* (Ernstsen and Sandberg 1986). Chlorosubstituted auxins have been tested in various bioassays. Monochloroindole acetic acids (4-Cl-IAA and 7-Cl-IAA) and dichloroindole-acetic acid (4,7-Cl_2-IAA) have many times larger auxin-like activity (Reinecke *et al.* 1995) but is dependent on the plant system tested (Reinecke *et al.* 1998). They stimulate the growth of excised tissue (Marumo *et al.* 1974, Katekar and Geissler 1982), rooting and ethylene production in pea cuttings (Ahmad *et al.* 1987), and the synthesis of specific enzymes in detached cotyledons (Hirasawa 1989) and seedlings (Ahmad and Hayat 1999) of pea, and imbibed wheat grains (Ahmad *et al.* 2001).

With such a varied action in view, the effects of these substituted auxins were tested on photosynthesis and some related processes including seed yield, at harvest in *Brassica juncea*.

Materials and methods

The seeds of *Brassica juncea* Czern & Coss cv. Varuna were obtained from National Seed Corporation, New Delhi, India. The healthy seeds were surfaced sterilised with 0.01 % mercuric chloride solution followed by repeated washings with double distilled water. The seeds were sown in earthen pots (25 cm diameter) filled with sandy loam soil and farmyard manure in a ratio of 9 : 1. Pots were irrigated regularly with tap water. The aqueous solutions (10^{-8} or 10^{-6} M) of IAA, 4-Cl-IAA, 7-Cl-IAA, or 4,7-Cl_2-IAA were applied to the leaves of 29-d-old

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Abbreviations: CA – carbonic anhydrase; CE – carboxylation efficiency; Chl – chlorophyll; Cl-IAA – chloro-indole-3-yl-acetic acid; DM – dry mass; FM – fresh mass; IAA – indole-3-yl-acetic acid; NRA – nitrate reductase; P_N – net photosynthetic rate; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase.

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plants. Control plants were sprayed with double distilled water only. Each treatment was replicated five times. Dry mass per plant (dehydrated at 80 °C for 24 h), chlorophyll (Chl) (*a*+*b*) contents, carbonic anhydrase (CA) and nitrate reductase (NR) activities, carboxylation efficiency (CE), and net photosynthetic rate (P_N) in the leaves were assessed in 60-d-old plants. The pod number and seed yield were recorded at harvest. The Chl content was estimated following the method of Mackinney (1941). The procedures of Dwivedi and Randhawa (1974) and Jaworski (1971) were used for measuring the activities of CA and NR in leaf extracts, respectively. P_N in intact leaves was measured by the *LI-6200* portable photosynthesis system (*LI-COR*, Lincoln, NE, USA). The CE was calculated by adopting the formulae used by Tiwari *et al.* (1998). The means of various parameters were separated using Duncan's multiple range tests at a significance level of 0.05 (Gomez and Gomez 1984).

Results and discussion

CA is a zinc containing protein with a molecular mass of 180 kDa (Lawlor 1987). CA is the other most abundant soluble protein in the chloroplasts of C_3 plant after RuBPCO (Reed and Graham 1981, Okabe *et al.* 1984). Its role in C_3 photosynthetic carbon assimilation is, however, speculative. The enzyme catalyses reversible hydration of CO_2 and maintains a constant supply of CO_2 for RuBPCO in the stroma. IAA stimulates activities of photosynthetic carboxylases (Pandey *et al.* 2000). Otherwise, the rate of CO_2 generation from its reduced form HCO_3^- is relatively very slow that obviously restricts the activity of RuBPCO at the ambient concentration of inorganic carbon. CA is also expected to facilitate the diffusion of CO_2 across the chloroplast membrane by catalysing hydration of dissolved CO_2 as it enters the more alkaline environment of the stroma (Majeau and Coleman 1994). The CA activity is naturally under the regulation of transcription and/or translation (Okabe *et al.* 1980). However, either process was somehow influenced by the auxins that increased the activity of CA (Fig. 1*A*). A maximum increase of 61 % over the control was noted in the leaves sprayed with 10^{-8} M 4-Cl-IAA. Increased CA activity, in association with elevated leaf Chl contents (Table 1), lower stomatal resistance (Arteca and Dong 1981), and higher rate of phosphorylation (Tamas *et al.* 1973) boosted P_N of the auxin-treated plants (Chatterjee *et al.* 1976 and Fig. 1*B*). P_N was maximum in the plants sprayed with 10^{-6} M 4-Cl-IAA and the increase over the control was about 65 %. The observed signifi-

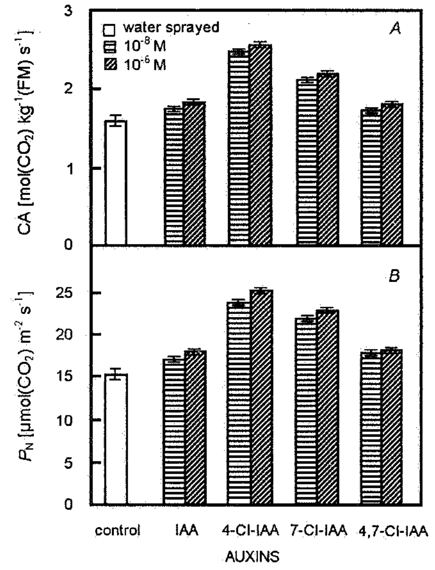

Fig. 1. Effects of leaf applied chloroindole auxins on carbonic anhydrase activity, CA (*A*) and net photosynthetic rate, P_N (*B*) of 60-d-old *Brassica juncea* plants.

Table 1. Effect of leaf applied chloroindole auxins on dry mass (DM), carboxylation efficiency (CE), chlorophyll (Chl) (*a*+*b*) content, and nitrate reductase activity (NR) of 60-d-old *Brassica juncea* plants, and pod number and seed yield per plant at harvest. Different superscripts within column represent the values significant at $p = 0.05$.

Treatment	DM [g]	CE [mol m^{-2} s^{-1}]	Chl (*a*+*b*) [g kg^{-1}]	NR [µmol kg^{-1}(leaf FM)]	Pod number	Seed yield [g]
Control	2.31^{c}	0.048^{e}	1.325^{c}	290.29^{f}	208.12^{d}	7.32^{d}
10^{-8} M IAA	2.43^{c}	0.043^{e}	1.411^{c}	310.17^{e}	222.32^{c}	8.05^{c}
10^{-6} M IAA	2.46^{bc}	0.069^{abc}	1.451^{ab}	320.19^{de}	224.11^{c}	8.11^{c}
10^{-8} M 4-Cl-IAA	2.81^{a}	0.073^{ab}	1.752^{a}	380.27^{b}	260.05^{ab}	9.43^{a}
10^{-6} M 4-Cl-IAA	2.85^{a}	0.075^{a}	1.852^{b}	393.27^{a}	265.17^{a}	9.60^{a}
10^{-8} M 7-Cl-IAA	2.69^{ab}	0.065^{bcd}	1.629^{b}	354.71^{c}	240.16^{bc}	8.92^{ab}
10^{-6} M 7-Cl-IAA	2.72^{a}	0.064^{d}	1.711^{c}	356.23^{c}	245.53^{abc}	8.63^{bc}
10^{-8} M 4,7-Cl_2-IAA	2.48^{bc}	0.063^{d}	1.450^{c}	317.18^{de}	224.32^{c}	8.21^{bc}
10^{-6} M 4,7-Cl_2-IAA	2.49^{bc}	0.061^{d}	1.431^{c}	324.13^{d}	228.19^{c}	8.52^{bc}

cant correlation between CA activity and P_N or CE (Fig. 2) and those found by Edwards and Mohamed (1973) and Ohki (1978) strengthen our concept of the impact of the auxins on P_N through the direct involvement of CA and CE.

Moreover, the elevated leaf NR activity (Table 1) might additionally support its photosynthetic efficiency. NR is responsible for the initiation of nitrate metabolism and consequently for the protein synthesis at various levels of the plant body. However, the rate of nitrate reduction is possibly a repercussion of co-ordinated action of the three main variable factors, by involving some form of signal transduction and/or sensors. The substrate (nitrate) concentration which essentially induces functional NR (Hewitt and Afridi 1959) produces a "nitrate sensing" protein of unknown nature that presumably binds with the regulatory regions of NR-genes and transcripts NR-mRNA and other regulators proteins involved in the metabolic response (Redinbaugh and Campbell 1991, Crawford 1995, Scheible *et al.* 1997a,b,c). The activity of NR is very much unsteady and depends on the presence or absence of irradiation (Knypl and Krystyna 1979), presence of hormones such as gibberellic acid and/or cytokinin (Roth-Bejerano and Lips 1970), auxin or its substituents (Ahmad 1988, 1994, Ahmad and Hayat 1999), 28-homobrassinolide (Hayat *et al.* 2001), and/or monochloroindole acetic acids. Exogenous addition of IAAs increased the enzyme activity by about 35 % over the control (Table 1). In our case, the treated plants might hold a large quantity of metabolites which is evident from their higher P_N and CE activities and dry mass (Table 1).

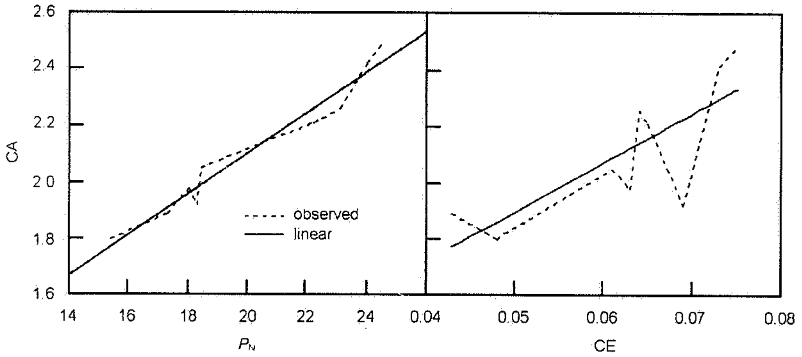

Fig. 2. Correlation coefficients between carbonic anhydrase activity, CA and net photosynthetic rate, P_N ($r = +0.978$, $y = -8.06 + 13.35\,x$) or carboxylation efficiency, CE ($r = +0.773$, $y = -0.0092 + 0.0338\,x$).

Among the various auxins tested, the order of response by various parameters was 4-Cl-IAA ≥ 7-Cl-IAA > 4,7-Cl_2-IAA = IAA > control (Table 1). The superiority of 4-Cl-IAA over IAA was also found in the elongation of *Avena* coleoptiles (Marumo *et al.* 1974), rooting, growth, ethylene synthesis (Ahmad *et al.* 1987), and induction of NR (Ahmad and Hayat 1999) in pea cuttings, and α-amylase activity in cotyledons of pea (Hirasawa 1989) and wheat seeds (Ahmad *et al.* 2001). These differential responses to various chloroindole auxins are presumably an expression of their varied degree of degradation and/or conjugation and other related properties in which they differ from each other and the IAA (Engvild 1994). Moreover, the impact of 4-Cl-IAA on the receptor of signal transduction pathway was also suggested as another reason for its higher auxin-like activities (Reinecke *et al.* 1998).

The leaves of plants treated with IAA, 4-Cl-IAA, 7-Cl-IAA, and 4,7-Cl_2-IAA photosynthesised at a faster rate (Fig. 1*B*) for an extended period because of delayed senescence (Menon and Srivastava 1984) than the control. The availability of larger quantities of metabolites, therefore, facilitated an increase in dry mass and pod bearing capacity of the plants, consequently leading to an increase in seed yield at harvest (Table 1).

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