PHOTOSYNTHETICA 42 (3): 477-480, 2004

BRIEF COMMUNICATION

Activity of 1-aminocyclopropane carboxylic acid synthase in two mustard (*Brassica juncea* L.) cultivars differing in photosynthetic capacity

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Abstract

The pattern of activity of 1-aminocyclopropane carboxylic acid synthase (ACS) was similar to photosynthetic and growth traits observed at 30, 45, and 60 d after sowing in mustard (*Brassica juncea* L.) cultivars Varuna and RH 30 differing in photosynthetic capacity. Higher activity of ACS and therefore ethylene release in Varuna than RH 30 increased stomatal conductance, intercellular CO_2 concentration, carboxylation rate (carbonic anhydrase and intrinsic water use efficiency), and thus net photosynthetic rate (P_N) and leaf and plant dry masses (DM) at all sampling times. Moreover, Varuna also had larger leaf area which contributed to higher P_N and DM. A positive correlation between ACS activity and P_N and leaf area was found in both the cultivars. Thus ACS activity may affect P_N through ethylene-induced changes on foliar gas exchange and leaf growth.

Additional key words: ACC oxidase; carbonic anhydrase; dry mass; intercellular CO_2 concentration; leaf area; net photosynthetic rate; plant hormones; stomatal conductance.

Contradictory claims have been made on the effect of ethylene on photosynthesis with the use of ethylene-releasing compounds. An increase in the net photosynthetic rate, P_N (Bühler *et al.* 1978, Grewal and Kolar 1990, Subrahmanyam and Rathore 1992, Grewal *et al*. 1993, Pua and Chi 1993, Khan *et al*. 2000) or decrease (Kays and Pallas 1980, Rajala and Peltonen-Sainio 2001) has been reported with the use of ethephon. Since the rate-limiting step in the ethylene biosynthetic pathway is catalyzed by 1-aminocyclopropane carboxylic acid synthase (ACS), its activity can be used as an indicator of the status of regulation of ethylene-induced changes in photosynthesis and dry mass (DM) accumulation. In two cultivars of mustard (*Brassica juncea* L. Czern. & Coss.) differing in photosynthetic capacity relationship between ACS activity and P_N was tested.

Plants of mustard (*Brassica juncea* L. Czern. & Coss.) cultivars Varuna and RH 30 were raised from seeds sown in 10 m^2 field plots in complete randomized design with five replications. The cultivars were obtained from Indian Agricultural Research Institute, New Delhi. Cvs. Varuna and RH 30 are characterized by high and low P_N and DM, respectively. At seedling establishment a plant population of 12 plants m^{-2} was maintained and recommended plant cultivation cultural practice was. A uniform recommended soil application of 18 g N, 3 g P, and 3 g K per m^{-2} was given at the time of sowing so as the nutrients are non-limiting. Determinations were carried out at 30, 45, and 60 d after sowing (DAS).

Leaves used for photosynthesis measurements were cut into two halves. Half of the leaf was used for the assay of ACS activity and ethylene biosynthesis and the other half for carbonic anhydrase (CA) activity. ACS activity was measured according to Avni *et al*. (1994) and Woeste *et al*. (1999). Leaf tissue was ground in 100 mM N-2 hydroxyethylpiperazine–N-2 ethanesulfonic acid buffer (pH 8.0) containing 4 mM dithiothreitol, 2.5 mM pyridoxal phosphate, and 25 % polyvinylpolypyrrolidone.

Received 16 March 2004, *accepted* 13 April 2004.
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Abbreviations: ACC – 1-aminocyclopropane carboxylic acid; ACO – 1-aminocyclopropane carboxylic acid oxidase; ACS – 1-aminocyclopropane carboxylic acid synthase; AdoMet – S-adenosyl methionine; CA – carbonic anhydrase; C_i – intercellular CO_2 concentration; DAS – days after sowing; DM – dry mass; g_s – stomatal conductance; P_N – net photosynthetic rate; PAR – photosynthetically active radiation; WUE – intrinsic water use efficiency.
Acknowledgements: Financial assistance by the University Grants Commission, New Delhi for the work is gratefully acknowledged.

After thorough homogenization, the preparation was centrifuged at 12 000×*g* for 15 min on *CPR 24* centrifuge (*Remi,* New Delhi, India). One cm^3 of the supernatant was placed in a 30 cm^3 tube and 0.1 cm^3 of 5 mM S-adenosyl methionine (AdoMet) was added. This was incubated for 1 h at 22 °C. The 1-aminocyclopropane carboxylic acid (ACC) formed was determined by its conversion to ethylene by addition of 0.1 cm^3 of 20 mM $HgCl_2$, followed by 0.1 cm^3 of 1 : 1 mixture of saturated NaOH : NaOCl. The tubes were capped immediately after addition of NaOH/NaOCl and incubated on ice for 10 min. Ethylene evolution was monitored on a gas chromatograph. For control set, AdoMet was not added. ACC oxidase (ACO) activity was measured as the ability of leaves to convert exogenous ACC to ethylene. 50 mg leaves were cut to small pieces and incubated with 0.5 cm^3 of 5 mM ACC in 30 cm^3 tubes. The tubes were capped and kept in light for 1 h under the similar conditions used for plant growth and ethylene evolution was determined.

A 5 cm^3 of gas phase was removed with a syringe and ethylene was measured on a gas chromatograph *GLC 5700* (*Nucon,* New Delhi, India) equipped with 1.8 m *Porapack N* (80/100 mesh) column, a flame ionization detector, and an integrator. Nitrogen gas was used as a carrier. The flow rates of nitrogen, hydrogen, and oxygen were 0.5, 0.5, and 5 $cm^3\ s^{-1}$ respectively. The oven temperature was 100 °C and the detector was at 150 °C. Ethylene identification was based on the retention time and quantified comparison with the peaks from standard ethylene concentrations.

Carbonic anhydrase (CA) activity in the leaf was measured by adopting the method of Dwivedi and Randhava (1974). Leaves were cut into small pieces in 10 cm^3 of 0.2 M cysteine at 4 °C. The solution adhering to the leaf surface was removed and immediately transferred to a tube containing 4 cm^3 phosphate buffer (pH 6.8). A 4 cm^3 of 0.2 M sodium bicarbonate in 0.002 M sodium hydroxide and 0.2 cm^3 of 0.002 % bromothymol blue was added to the tube. The tubes were kept at 4 °C for 20 min after shaking. Liberated CO_2 during the catalytic action of enzyme on sodium bicarbonate was estimated by titrating the reaction mixture against 0.05 M hydrochloric acid.

Table 1. Activities of 1-aminocyclopropane carboxylic acid synthase (ACS) [ng(ACC) kg^{-1}(leaf FM) s^{-1}], 1-aminocyclopropane carboxylic acid oxidase (ACO) [ng(C_2H_4) kg^{-1}(leaf FM) s^{-1}], ethylene evolution [ng kg^{-1}(leaf FM) s^{-1}], net photosynthetic rate, P_N [μmol(CO_2) $m^{-2}\ s^{-1}$], stomatal conductance, g_s [mmol $m^{-2}\ s^{-1}$], intercellular CO_2 concentration, C_i [μmol mol^{-1}], carbonic anhydrase activity, CA [mmol m^{-2}(leaf) s^{-1}], water use efficiency, WUE [μmol mol^{-1}], leaf area [cm^2 per plant], leaf dry mass (DM) [g m^{-2}], and plant DM [g per plant] in two cultivars of mustard (*Brassica juncea* L.). Means ± SE. *Values statistically different at $p<0.05$.

	Varuna			RH 30		
	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS
ACS	46.4 ± 0.44	61.8 ± 0.30	87.8 ± 0.30	26.6 ± 0.31*	41.0 ± 0.21*	64.6 ± 0.37*
ACO	41.8 ± 0.36	49.5 ± 0.35	97.7 ± 0.42	28.6 ± 0.45*	31.9 ± 0.45*	38.4 ± 0.50*
Ethylene	2.50 ± 0.15	3.40 ± 0.13	4.30 ± 0.13	1.40 ± 0.07*	2.40 ± 0.10*	3.40 ± 0.09*
P_N	14.30 ± 0.24	20.60 ± 0.78	24.50 ± 0.67	10.50 ± 0.23*	14.00 ± 0.31*	19.40 ± 0.33*
g_s	348.0 ± 6.3	450.6 ± 5.9	475.0 ± 7.4	323.8 ± 5.6*	379.2 ± 3.6*	452.0 ± 4.4*
C_i	240.0 ± 3.7	269.4 ± 4.4	300.0 ± 7.9	208.5 ± 7.9*	250.2 ± 3.5*	271.0 ± 5.7*
CA	1.60 ± 0.20	2.10 ± 0.15	3.00 ± 0.24	0.90 ± 0.17*	1.40 ± 0.16*	2.20 ± 0.14*
WUE	5.20 ± 0.23	6.30 ± 0.23	6.90 ± 0.05	3.50 ± 0.13*	4.60 ± 0.17*	4.80 ± 0.23*
Leaf area	122.2 ± 4.4	161.6 ± 6.2	214.2 ± 8.5	82.7 ± 6.2*	109.4 ± 7.2*	162.6 ± 5.1*
Leaf DM	234.0 ± 4.1	254.0 ± 3.9	433.0 ± 4.03	191.0 ± 2.8*	236.0 ± 4.3*	354.0 ± 4.5*
Plant DM	4.40 ± 0.41	7.80 ± 0.51	12.90 ± 0.48	2.60 ± 0.33*	4.90 ± 0.48*	8.60 ± 0.45*

P_N, stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were measured using infrared gas analyzer *Licor 6200* (Lincoln, NE) on fully expanded uppermost leaves on four plants from each replicate in the two cultivars. The atmospheric conditions during the measurements between 11:00–13:00 were: photosynthetically active radiation (PAR) of about 900 μmol $m^{-2}\ s^{-1}$, relative humidity of 60 %, and temperature of 22 °C. Intrinsic water-use efficiency (WUE) was calculated by dividing P_N with g_s (Dudley 1996). Leaf area on the plant axis was determined with a leaf area meter *LA 211* (*Systronics*, New Delhi, India). Above-ground plant DM was determined after drying in an oven at 80 °C till constant mass.

Data were analysed statistically and standard error of the mean value was calculated. Analysis of variance was performed to identify the significant differences at $p<0.05$ between the cultivars.

At 30, 45, and 60 d after sowing (DAS) the ACS activity was higher in Varuna, and maximal activity was recorded at 60 DAS (Table 1). Varuna showed higher capacity of ethylene biosynthesis than RH 30. ACS activity was 74.4, 50.7, and 37.7 % higher in Varuna than in RH 30 at 30, 45, and 60 DAS, respectively. ACC produced with the activity of ACS was converted to ethylene by ACO. Ethylene evolution was 78.6, 41.7, and 26.4 % higher in Varuna than in RH 30 at 30, 45, and 60 DAS, respectively (Table 1).

At all sampling times, P_N in Varuna was higher than

in RH 30 by 71.6, 47.1, and 29.9 % at 30, 45, and 60 DAS, respectively (Table 1). Varuna also exerted higher g_s, C_i, CA activity, and WUE than RH 30. Leaf area, leaf dry mass (DM), and plant DM followed the pattern of P_N in the cultivars, being higher in Varuna than in RH 30 at all sampling times (Table 1).

The ACS activity was correlated to P_N and leaf area in both the cultivars (Fig. 1). A strong positive correlation of ACS activity with P_N (r^2 = 0.983) and leaf area (r^2 = 0.976) was observed in Varuna (Fig. 1*A, C*). Also in RH 30 a positive relationship was found. A correlation of ACS activity with P_N (r^2 = 0.991) and with leaf area (r^2 = 0.976) was noted (Fig. 1*B, D*).

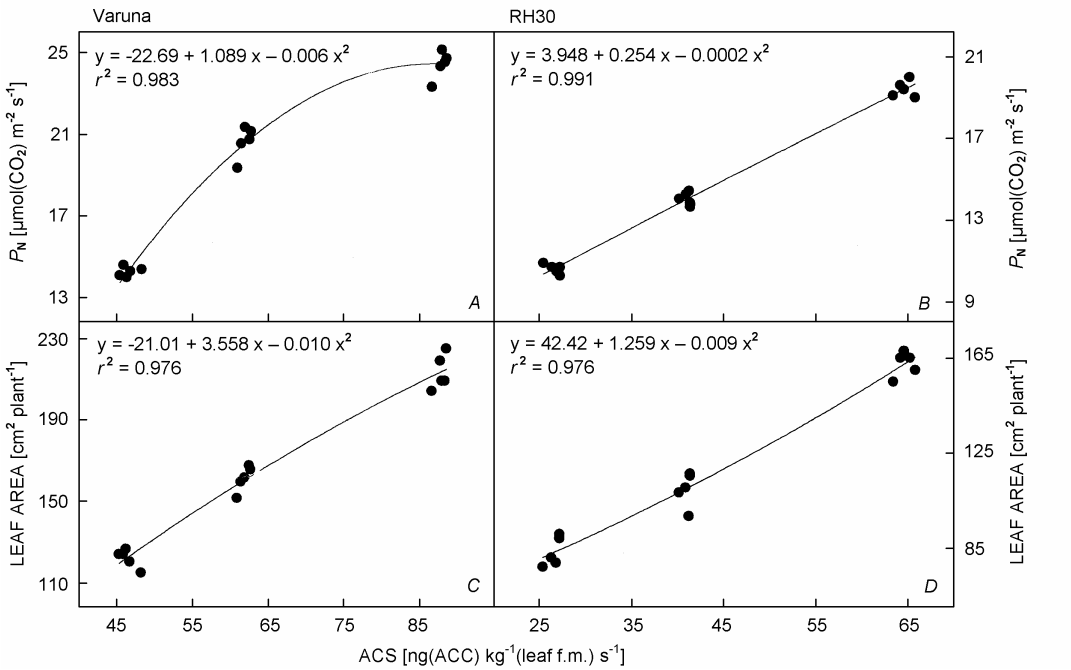

Fig. 1. Relationship of 1-aminocyclopropane carboxylic acid synthase (ACS) with net photosynthetic rate, P_N (*A, B*) and leaf area (*C, D*) of cv. Varuna (*A, C*) and cv. RH 30 (*B, D*) of *Brassica juncea* L.

The high P_N of Varuna leaves was manifestation of ethylene-induced variation in g_s. This caused greater influx of CO_2 which increased C_i and CO_2 fixation. The higher CA activity and WUE in Varuna than RH 30 reflected the increased P_N. A correlation between ethylene-enhanced g_s and ethylene-enhanced P_N was found by Taylor and Gunderson (1986). High concentration of CO_2 (>1 %) acts as antagonist of ethylene, but atmospheric CO_2 concentration is needed for the conversion of ACC oxidase to ethylene (Mattoo and White 1991). According to these authors, CO_2 could promote or inhibit ethylene evolution depending on its concentration in the tissue. Thus there is an interrelation between ethylene and CO_2 metabolism, and ethylene evolution controls the growth of plants. Bassi and Spencer (1982) showed increase in ACO activity with an increase in CO_2 concentration. Dhawan *et al*. (1981), Kao and Yang (1982), and Grodzinski *et al*. (1982) also showed that inhibition of ethylene evolution resulting from a decrease in C_i and regulated photosynthesis. Similarly, Dong *et al*. (1992) showed completely abolished ACO activity in the absence of CO_2. ACO binds to Lys residue (Fernandez-Maculet *et al*. 1993) and results in carbamate formation (Veverides and Dilley 1994). With the increase in ACO activity ACC was converted to ethylene at enhanced rate and ACS activity increased the autocatalytic regulation of ethylene.

Due to larger leaf area, Varuna was expected to have higher photon interception and thus photosynthesis than RH 30. The role of ethylene in regulating leaf growth of plants (Abeles *et al*. 1992, Hussain *et al*. 1999, Khan *et al*. 2000), ethylene-induced leaf emergence in cereal seedlings (Ivenish and Kreicbergs 1992), and leaf expansion (Kieber *et al*. 1993, Rodrigues-Pousada *et al*. 1993, Khan *et al*. 2000, 2002) have also been reported.

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