

## BRIEF COMMUNICATION

## Activities of carbonic anhydrase and ribulose-1,5-bisphosphate carboxylase, and dry mass accumulation in *Brassica juncea* following defoliation

N.A. KHAN

Department of Botany, Aligarh Muslim University, Aligarh-202002, India

### Abstract

The effect of 30 % defoliation of shaded leaves in lower layers of plant was studied on activities of carbonic anhydrase (CA) and ribulose-1,5-bisphosphate carboxylase (RuBPC), leaf dry mass per unit leaf area, and plant dry mass of mustard (*Brassica juncea*). Removal of 30 % of leaves resulted in increased CA and RuBPC activities of leaves, and leaf and plant dry masses.

*Additional key words:* dry mass accumulation; leaf dry mass; mustard.

Mustard is an important crop of the tropical region of the world, characterised by large number of leaves in the lower layers on the plant (Weiss 1983). Effective solar irradiation of these leaves is reduced. That leads to a low photosynthetic rate and unproductive leaves as the crop matures. Earlier work showed that removal of 30 % of such leaves increased plant biomass and seed production maximally, and other levels of defoliation were less effective (Khan *et al.* 2002b). However, an effect of defoliation on photosynthetic enzymes has not been studied yet. An increase in photosynthetic biomass formation due to defoliation could be achieved only if it increased the amount and activity of photosynthetic enzymes. CA and RuBPC are important enzymes in photosynthesis and thus in biomass accumulation. CA accelerates the reactions of  $\text{HCO}_3^-$  dehydration, increases the  $\text{CO}_2$  concentration in the place of carboxylation, and thus contributes to the effective work of RuBPC in the cell (Sültemeyer *et al.* 1993). Net photosynthetic rate is related to the activity of CA (Hayat *et al.* 2001). The reported research was, therefore, undertaken to study the effect of 30 % defoliation of lower leaves on CA and RuBPC activities and dry mass accumulation in mustard.

Mustard (*Brassica juncea* L. Czern & Coss. cv. Alankar) seeds were sown in 23-cm diameter earthen pots filled with acid washed sand. The plants were grown with Hoagland's nutrient solution (Hewitt 1966), and two

plants per pot were maintained. The pots were kept in a greenhouse of the Botany Department, Aligarh Muslim University, Aligarh, India under natural day length. At 40 d after sowing (pre-flowering stage), leaf number was 14 and 30 % of these leaves on lower layers were removed by excising the leaf blades. Control plants were not defoliated. Each treatment was replicated five times. At 60 d after sowing (post-flowering stage), activities of CA and RuBPC, leaf dry mass per unit leaf area, and plant dry mass were determined. Leaf area was measured with a leaf area meter (*LA 211, Systronics, India*) and dry mass was taken after drying the samples in an oven at 80 °C for 24 h.

For measurement of enzyme activities, leaves from apex to base on the plant axis were collected, and homogenised as a composite sample in 0.05 M Tris-HCl (pH 8.5), containing 1 mM dithiothreitol, 5 mM  $\text{MgCl}_2$ , 1 mM EDTA, and 1 % polyvinylpyrrolidone. Homogenate was passed through *Whatman 42* filter paper and centrifuged first at 1 000×g for 10 min and then at 5 000×g for 30 min. The supernatant was used as a source for determining the activities of CA and RuBPC. The CA activity was determined by an electrometric method (Rickli *et al.* 1964), and RuBPC by a spectrophotometric method (Lawlor *et al.* 1989). The values were statistically analysed.

Received 2 January 2003, accepted 13 January 2003.

Fax: +91-0571-2702016, e-mail: naf9@lycos.com

Abbreviations: CA, carbonic anhydrase; RuBPC, ribulose-1,5-bisphosphate carboxylase.

Table 1. Activities of carbonic anhydrase (CA) and ribulose-1,5-bisphosphate carboxylase (RuBPC), leaf dry mass per unit leaf area, and plant dry mass of mustard following defoliation of leaves in lower layers.

Defoliation [%]	CA [mmol m <sup>-2</sup> (leaf) s <sup>-1</sup> ]	RuBPC [μmol m <sup>-2</sup> (leaf) s <sup>-1</sup> ]	Leaf dry mass [mg cm <sup>-2</sup> ]	Plant dry mass [g plant <sup>-1</sup> ]
0	10.6	50	16.0	9.4
30	18.2	120	24.6	12.6
LSD ( <i>p</i> < 0.05)	2.2	20	2.4	1.4

Removal of 30 % of leaves increased CA and RuBPC activities, and leaf and plant dry masses. The removal of unproductive lower leaves helped in diverting plant resources in the formation of new leaves. In defoliated plants, more new leaves emerged. These leaves contributed to the increased activities of CA and RuBPC of composite leaf sample (Table 1). The co-localisation of CA and RuBPC in stroma helped rapid dehydration of  $\text{HCO}_3^-$  for RuBPC by CA resulting in an increase of RuBPC activity and high leaf and plant dry masses

(Table 1). The leaves in lower layers on mustard (Khan *et al.* 2002a) and soybean (Wells 1991) have been found less photosynthetically efficient. Chhabra and Krishnamoorthy (1995) and Singh and Singh (1996) have also reported that surplus leaves in mustard do not contribute to biomass accumulation but prevent translocation of assimilates to the reproductive sink. My results suggested that an increase in photoassimilation efficiency and plant dry mass of defoliated plants was caused by increased activities of CA and RuBPC.

## References

Chhabra, M.L., Krishnamoorthy, H.N.: Investigations on the effect of partial defoliation on  $^{14}\text{C}$ -transport in Indian mustard. – *Cruciferae Newsletter* **17**: 62, 1995.

Hayat, S., Ahmad, A., Mobin, M., Fariduddin, Q., Azam, Z.M.: Carbonic anhydrase, photosynthesis, and seed yield of mustard plants treated with phytohormones. – *Photosynthetica* **39**: 111-114, 2001.

Hewitt, E.J.: Sand and Water Culture Methods used in the Study of Plant Nutrition. – Commonwealth Agricultural Bureaux 1966.

Khan, N.A., Khan, M., Ansari, H.R., Samiullah: Auxin and defoliation effects on photosynthesis and ethylene evolution in mustard. – *Sci. Hort.* **96**: 43-51, 2002a.

Khan, N.A., Khan, M., Samiullah: Changes in ethylene level associated with defoliation and its relationship with seed yield in rapeseed-mustard. – *Brassica* **4**: 12-17, 2002b.

Lawlor, D.W., Kontturi, M., Young, A.T.: Photosynthesis by flag leaves of wheat in relation to protein, ribulose bisphosphate carboxylase activity and nitrogen supply. – *J. exp. Bot.* **40**: 43-52, 1989.

Rickli, E.E., Ghazanfar, S.A.S., Gibbons, B.H., Edsall, J.T.: Carbonic anhydrase from human erythrocytes. Preparation and properties of two enzymes. – *J. biol. Chem.* **239**: 1065-1078, 1964.

Singh, S.P., Singh, G.S.: Effect of defoliation on growth, yield and yield contributing characteristics in mustard (*Brassica juncea* L. Czern & Coss.). – *Indian J. Plant Physiol.* **1**: 123-124, 1996.

Sültemeyer, D., Schmidt, C., Fock, H.P.: Carbonic anhydrases in higher plants and aquatic microorganisms. – *Physiol. Plant.* **88**: 179-190, 1993.

Weiss, E.A.: Oilseed Crops. – Longman, New York 1983.

Wells, R.: Response of soybean growth to plant density, relationship among canopy photosynthesis, leaf area and light interception. – *Crop Sci.* **31**: 775-781, 1991.