PHOTOSYNTHETICA 42 (2): 303-305, 2004

BRIEF COMMUNICATION

Differences in the activation state of ribulose-1,5-bisphosphate carboxylase/oxygenase in barley, pea, and wheat at two altitudes

Narinder KUMAR, Sanjay KUMAR*, and Paramvir Singh AHUJA

Biotechnology Division, Institute of Himalayan Bioresource Technology, Palampur-176 061 (HP), India

Abstract

Activation state of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) is an important parameter determining the rate of net photosynthesis (P_N) *in situ* for which no information is available with reference to altitude. We analyzed activation state along with P_N in three plant species and their cultivars grown at low (LA, 1 300 m) and high (HA, 4 200 m) altitudes. No significant change in P_N and the initial activity of RuBPCO was obtained with reference to altitude. However, activation state of RuBPCO was reduced significantly in the HA plants as compared to the LA ones. Hence low partial pressure of CO_2 prevailing at HA might be responsible for the lower activation state of RuBPCO.

Additional key words: CO_2 partial pressure; cultivar differences; *Hordeum*; net photosynthetic rate; *Pisum*; *Triticum*.

High altitude (HA) is the natural site of lower partial pressure of CO_2 (p_{CO2}). The prevailing lower p_{CO2} is expected to limit carbon fixation. However, net photosynthetic rate (P_N) plotted against the intercellular partial pressure of CO_2 (C_i) presented a higher slope value, indicating higher efficiency of carbon uptake (ECU) in the plants growing at HA (Körner and Diemer 1987). This is further supported by isotope discrimination (^{13}C) analysis, which shows a lower discrimination by the plants at HA, meaning thereby higher carboxylation efficiency (Körner *et al*. 1988). Higher ECU at HA was attributed to features such as thicker palisade layer and greater leaf nitrogen content per unit area (Körner and Diemer 1987). Also, a higher total ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO; EC 4.1.1.39) activity was observed in *Selinum vaginatum* at HA as compared to those grown at lower altitude, LA (Pandey *et al*. 1984). However, there is no information available on the activation state of RuBPCO with reference to altitude. Activation state is defined as the ratio of initial to final activity of RuBPCO (Tissue *et al*. 1995) and is an important parameter determining P_N *in situ*. In the present communication, we analyzed activation state of RuBPCO in plant species grown at two altitudes exhibiting 30 % difference in partial pressure.

Barley (*Hordeum vulgare* L. cv. HBL-113 and Local Kaza), pea (*Pisum sativum* L. cv. Azad and Local Kaza), and wheat (*Triticum aestivum* L. cv. VL-616) were grown at LA (Palampur of 1 300 m; 32°06'32"N; 76°33'43"E) and HA (Kibber of 4 200 m; 32°20'11"N; 78°00'52"E). The plants were sown in the first week of November at LA and in the first week of May at HA. These are the conventional sowing months at the two altitudes. Mean monthly day temperatures during the month of data recording at Kibber and Palampur were 18.6±2.5 and 19.2±2.2 °C, respectively.

Plants for analyses were collected starting 85 d after sowing. Flag leaves of barley and wheat and two opposite leaves adjacent to the stem at 3rd node in pea were used for RuBPCO analyses. Leaves were harvested during 09:00–10:00 under full sunlight and stored in liquid nitrogen. RuBPCO analyses were done at Palampur. Leaf samples were ground using a pre-chilled mortar and pestle in chilled extraction buffer containing 50 mM Tris-Cl buffer, pH 7.5, 1 mM $MgCl_2$, 5.0 mM dithiothreitol (DTT), 1 mM PMSF, insoluble 2 % polyvinylpyrrolidone,

Received 18 November 2003, *accepted* 23 April 2004.
*Corresponding author; fax: +91 1894 230433, e-mail: sanjayplp@rediffmail.com
Abbreviations: C_i – intercellular partial pressure of CO_2; ECU – efficiency of carbon uptake; HA – high altitude; LA – low altitude; p_{CO2} – partial pressure of CO_2; P_N – net photosynthetic rate; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase.
Acknowledgements: This research was supported by the Department of Biotechnology (DBT), Government of India vide grant number BT/PR/502/AGR/08/39/966-VI. NK acknowledges DBT for financial assistance. The manuscript represents IHBT communication number 0347.

Table 1. Initial and final activity of RuBPCO [μmol(CO_2) m^{-2} s^{-1}] in the plants grown at low (LA) and high (HA) altitudes. Means ± SE (n = 4). Values indicated by different letters in the superscript, to be compared with corresponding row values, show significant difference at p<0.01 whereas those marked with the same letters exhibit non-significant difference. Values in parentheses are % of HA values.

Plant	RuBPCO				Activation state	
	Initial		Final			
	LA	HA	LA	HA	LA	HA
Barley HBL-113	24.62^{a}±1.74	24.28^{a}±1.58	26.19^{a}±2.08	30.84^{b}±2.08	94.0 (119.4)	78.7
Barley Local Kaza	39.95^{a}±3.69	35.45^{a}±1.24	49.85^{a}±4.76	62.34^{b}±5.54	80.1 (140.9)	56.8
Pea Azad	55.67^{a}±4.53	61.61^{a}±3.62	74.15^{a}±5.71	114.30^{b}±6.42	75.0 (139.1)	53.8
Pea Local Kaza	26.57^{a}±2.88	24.47^{a}±2.46	28.63^{a}±1.91	38.33^{b}±2.26	92.8 (145.4)	63.8
Wheat VL-616	34.50^{a}±2.38	30.69^{a}±2.08	41.18^{a}±1.43	49.41^{b}±5.26	83.7 (134.8)	62.1

10 % glycerol, and 0.1 % *Triton X-100*. The extract was centrifuged at 12 000×*g* for 10 min at 4 °C and the supernatant was used for determination of RuBPCO activity in medium containing 50 mM Tris-Cl (pH 8.0), 10 mM $MgCl_2$, 5 mM DTT, 1.3 mM ribulose-1,5-bisphosphate (*Sigma*), and 20 mM $NaH^{14}CO_3$ (specific activity of 1×10^6 DPM μmol^{-1}) (Kumar and Kumar 2001). $NaH^{14}CO_3$ (specific activity 1.92×10^9 Bq $mmol^{-1}$) was obtained from the Board of Radiation and Isotope Technology, Mumbai, India. After 1 min at 25 °C, the reaction was terminated by the addition of trichloroacetic acid (8 % final concentration) to remove the unused $NaH^{14}CO_3$. Hot air was passed through all the samples to remove the unbound $^{14}CO_2$. Samples in scintillation cocktail (*Sisco Research Laboratories*, India) were counted using a *Beckman* scintillation counter *LS 6000TA*. To estimate the total activity, RuBPCO was activated using 20 mM $NaHCO_3$ and 10 mM $MgCl_2$ in 50 mM Tris-Cl (pH 8.0) for 10 min. Activation state of RuBPCO was calculated by dividing initial activity with the total activity. P_N at the two locations were measured on-site using an infrared gas analyzer *Li-6400* (*LI-Cor*, Lincoln, USA) following manufacturer's instructions. All the measurements were conducted at 2 000 μmol m^{-2} s^{-1} maintained through a blue-red LED source. Temperature of the leaf-chamber was maintained at 25 °C through a Peltier cooling system. Values were analyzed using Complete Randomized Block Design and differences between the means were tested against critical difference p<0.01.

High altitude (Kibber; 61.1 kPa) in the present study represented a location of 30 % decrease in partial pressure compared to low altitude (Palampur; 86.8 kPa). A measurement of initial activity of RuBPCO showed no significant difference at the two altitudes (Table 1). However, total activity increased by 17 to 54 % in plants under study (Table 1). No significant difference was observed in P_N at the two altitudes (Table 2). This was in accordance with the earlier deduction that initial rather than total activity is related to P_N (Yelle *et al*. 1989). Higher total activity of RuBPCO at high altitude could be due to increase in RuBPCO content *per se* (Yelle *et al*. 1989). Work with Texas prairie grasses showed that RuBPCO content increased at low CO_2 concentrations relative to those grown at current levels (Sage and Coleman 2001). Further, Körner and Diemer (1987) found an increased nitrogen content with increase in altitude. Higher N and RuBPCO contents are positively correlated with each other (Warren *et al*. 2000).

Table 2. Net photosynthetic rate, P_N [μmol(CO_2) m^{-2} s^{-1}] in plants grown at the two altitudes. Means ± SE (n = 4). Values indicated by different letters in the superscript, to be compared with corresponding row values, show significant difference at p<0.01 whereas those marked with the same letters exhibit non-significant difference.

Plant	P_N	
	LA	HA
Barley HBL-113	17.80^{a}±2.45	18.00^{a}±3.17
Barley Local Kaza	13.70^{a}±1.79	15.40^{a}±2.36
Pea Azad	26.40^{a}±3.47	28.30^{a}±4.57
Pea Local Kaza	15.10^{a}±2.87	14.20^{a}±3.07
Wheat VL-616	15.80^{a}±1.67	17.60^{a}±2.47

Plants at LA showed activation state ranging between 75–94 % whereas at HA the activation state ranged at 53–78 % (Table 1). Depending upon the plant type, activation state of RuBPCO in HA plants was lower by 19–45 % as compared to LA plants. Since p_{CO2} is important in activating RuBPCO (Sage and Coleman 2001), lower p_{CO2} at HA could be an important factor affecting activation state of RuBPCO. Tissue *et al*. (1995) found a lower activation state of RuBPCO at low pCO_2. Enhanced CO_2 increased activation state of RuBPCO (Rey *et al*. 1990).

To our knowledge, this is the first report exhibiting different activation state of RuBPCO in response to altitude variation. It will be important to analyse metabolites and other factors, which possibly modulate RuBPCO activase and hence affect the activation state of RuBPCO at various altitudes.

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