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Gas exchange response of barley and pea cultivars to altitude variation in Himalaya

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Abstract

Leaf stomatal density (SD), net photosynthetic rates (P_N), and stomatal conductance (g_s) of *Hordeum vulgare* and *Pisum sativum* cultivars in Himalaya increased with altitude. Higher P_N and leaf temperature under low CO_2 partial pressure at high altitudes could evoke a higher g_s and SD to allow sufficient influx of CO_2 as well as more efficient leaf cooling through transpiration.

Additional key words: acclimation; *Hordeum*; photosynthesis; *Pisum*; pore length; species differences; stomatal conductance; stomatal density; stomatal index.

Introduction

Photosynthetic activity and stomatal conductance (g_s) are positively related, and high g_s associated with high net photosynthetic rate (P_N) are useful traits to maximize growth during short period of water availability in otherwise dry conditions (Hetherington and Woodward 2003). Schlüter *et al.* (2003) underline that an increase of stomatal density (SD) increases photosynthetic performance in a mutant of *Arabidopsis thaliana*. Stomatal sensitivity to CO_2 acclimation has great ecological significance in enabling plants to occupy habitats with fluctuating environment (Šantrůček and Sage 1996, Morrison 1998). Stomatal acclimation to sub-ambient CO_2 concentration in wheat plants increases P_N in response to SD and g_s (Ulman *et al.* 2000).

The partial pressure of CO_2 drops with increasing altitude. Photosynthetic efficiency at elevated altitude is related with g_s and/or SD (Woodward 1986, Hovenden and Brodribb 2000, Woodward *et al.* 2002, Kumar *et al.* 2005, Vats and Kumar 2006). The g_s and SD are affected by changes in CO_2 partial pressure at high altitude (Woodward and Bazzaz 1988, Woodward *et al.* 2002). The objective of this research was to compare P_N, g_s, and SD of barley and pea at high and low altitude in Himalaya.

Materials and methods

Site description, environmental parameters, and field trials: Barley (*Hordeum vulgare* L.) and pea (*Pisum sativum* L.) are grown as winter and summer crops, respectively, at high and low altitude in Himalaya (Viswanath 2002). Seeds of high altitude cultivars (cvs.) of barley and pea (barley Kaza and pea Kaza) were collected from Spiti valley of Himachal Pradesh (4 200 m a.s.l.), whereas those of the low altitude cvs. barley

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Abbreviations: C_i – intercellular CO_2 concentration; cv. – cultivar; EC – epidermal cells; g_s – stomatal conductance; PL – pore length; P_N – net photosynthetic rate; PPFD – photosynthetic photon flux density; SD – stomatal density; SI – stomatal index; Tr – trichomes; WUE_i – intrinsic water use efficiency.

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Dolma and pea Lincoln) from the State Agricultural University, Palampur (1 300 m). All the four cvs. were sown at Kibber (4 200 m, 32°20′N, 78°00′E, 61.1 kPa) and Palampur (1 300 m, 32°06′N, 76°33′E, 86.8 kPa) under natural field conditions in the months of October and May, respectively. The different sowing months are the conventional sowing periods for cereal crops. Daily maximum irradiance for growth during the day ranged 9−12 h at Palampur (PPFD 1 500−2 000 μmol m^{-2} s^{-1}) and 12−14 h at Kibber (PPFD 2 300−2 700 μmol m^{-2} s^{-1}). The mean daily air temperature recorded at Palampur and Kibber was 19.2±2.2 and 18.6±2.5 °C, respectively. Plants were irrigated and fortified with NPK following standard agronomic practices for the two crops (Viswanath 2002).

Plants attained maximum height 10−12 weeks after sowing at the two locations. Gas exchange and morphological analysis were carried out on leaves collected 12 weeks after sowing, using the flag leaf for barley and the two opposite leaves adjacent to stem at 3rd node position for pea.

Gas exchange was measured on the flag leaf of the plant using an open gas-exchange system *LI-6400* (*Li-COR*, Lincoln, NE, USA). The instrument was equipped with a transducer to measure atmospheric pressure in the range 0−115 kPa, with an accuracy of ±0.1 % of full scale. Infrared gas analyzers were zeroed for CO_2 and H_2O using a CO_2 scrubber and desiccant, respectively, before calibration of the span for CO_2 and H_2O using a calibrated gas (505 cm^3 m^{-3}; *Li-COR*) and a portable dew point generator (*LI-610, Li-COR*). All observations were made under ambient irradiance on a clear day between 09−11 h. Incoming gas was partially passed through a desiccant column to maintain a relative humidity of about 30 % inside the leaf chamber of the portable photosynthesis system (average % relative humidity at low and high altitude locations was 32.8±1.5 and 29.5±2.8, respectively). The P_N, g_s for water vapour, and intrinsic water use efficiency (WUE$_i$, P_N/g_s) were recorded in five different plants representing five replications at each location.

The leaf internal CO_2 concentration (C_i) was obtained using a *6400-01* CO_2 injector. All the measurements were made at 2 000 μmol m^{-2} s^{-1}, using a blue-red radiation emitting diodes (*6400-02* LED) capable of providing irradiances of 0−3 000 μmol m^{-2} s^{-1} inside the leaf chamber. Leaf chamber temperature during measurements was maintained at 25 °C through the integrated Peltier coolers. A time interval of 90 s was given for leaf to equilibrate to the new conditions in each measurement. For P_N *versus* C_i curves, 3 sets of measurements were made on different plants from each cv.

Morphological studies: Nail enamel imprints were obtained at the middle portion of the adaxial and abaxial surfaces of uppermost fully expanded leaves at both the low and high altitude. Two leaves from five plants per cv. of barley and pea were used. The mounted imprints on the microscope slides were studied for SD [number of stomata per mm^2], stomatal pore length (PL), epidermal cells density (EC) [epidermal cells per mm^2], and trichomes (Tr) [number per unit area]. SD and stomatal index, SI [SI = SD/(SD + EC) × 100] were recorded at 40× magnification under the *Nikon Diaphot* inverted microscope. PL was defined as the distance between the two junction points of guard cells, using an eye piece micrometer.

Statistical analysis: Morphological and gas exchange parameters were computed by one-way analysis of variance (ANOVA) separately for each test crop, using general linear model procedures in *SPSS* statistical package (*SPSS*, Chicago, USA). The source of variation was low or high altitude location. The differences for all comparable morphological and gas exchange parameters were calculated at the significance level of $p<0.05$. Significant results were compared using Gabriel *post hoc* comparison.

Results

Leaf morphological parameters showed the same trend with altitude (Table 1), with the exception in PL in barley, and they were statistically significant ($p<0.05$). SD and SI were significantly ($p<0.05$) higher in all the cvs. of barley and pea at high than at low altitude. There were significant ($p<0.05$) difference in SI on both the adaxial and abaxial leaf surface in barley and pea. SD was on an average higher on adaxial than abaxial leaf surface. SD correlated with g_s in all the four cvs. Tr was higher on both abaxial and adaxial leaf surfaces of barley Kaza, and it did not differ significantly ($p>0.05$) from barley Dolma at the two altitudes. PL did not change significantly ($p>0.05$) with altitude.

The high altitude cvs. of both barley and pea showed significantly ($p<0.05$) higher g_s and P_N compared to those of low altitude at different C_i. On an average, the mean P_N in barley Dolma and pea Lincoln was higher by 17.4 and 21.6 % at high altitude but decreased by 21.4 and 16.6 % in barley Kaza and pea Kaza, respectively (Fig. 1). Similarly, g_s increased by 47 and 55 % in barley Dolma and pea Lincoln at high altitude but decreased by 28 and 24 % in barley Kaza and pea Kaza, respectively (Fig. 1). Regression analysis between g_s and P_N revealed a significant negative correlation at both high and low altitudes irrespective of the test cvs. Correlation between g_s and P_N was significantly higher at high altitude.

Table 1. Difference in leaf morphological parameters in low and high altitude cultivars of *Hordeum vulgare* (barley Dolma and barley Kaza) and *Pisum sativum* (pea Lincoln and pea Kaza) grown at 4 200 and 1 300 m a.s.l.. Epidermal cell density (EC) [mm^{-2}], stomatal density (SD) [number of stomata per mm^2], stomatal index (SI), trichome density (Tr) [number of trichomes per mm^2] (absent in pea), pore length (PL) [μm] on abaxial (Ab) and adaxial (Ad) leaf surfaces. Means ($n = 10$). *Different letters* for each parameter at high and low altitude denote significant difference ($p<0.05$). Values in parenthesis indicate % increase (+) or decrease (−) in low and high altitude cultivars at high and low altitudes, respectively.

		Barley Kaza		Barley Dolma		Pea Kaza		Pea Lincoln	
		4 200 m	1 300 m	4 200 m	1 300 m	4 200 m	1 300 m	4 200 m	1 300 m
SD	Ab	80.3^a	50.0^b (−37.7)	88.8^a (+19.5)	74.4^b	129.1^a	88.2^b (-31.7)	207.3^a (+33.5)	155.3^a
	Ad	102.0^a	70.4^b (−30.9)	100.7^a (+18.6)	84.9^b	12.6^a	74.4^b (−33.9)	177.0^a (+35.2)	130.9^b
EC	Ab	186.9^b	132.9^a (−28.9)	154.6^a (+2.2)	151.3^a	335.1^a	290.8^b (−13.2)	419.2^a (+33.5)	313.9^b
	Ad	206.6^b	155.9^b (−24.5)	163.8^a (+2.4)	159.9^a	313.6^a.	275.7^b (−12.1)	394.8^a (+31.6)	300.0^b
SI	Ab	30.1^a	27.3^c (−9.4)	36.5^a (+10.8)	32.9^b	27.8^a	23.4^b (−16.1)	41.4^a (+24.8)	33.1^b
	Ad	33.1^a	31.1^b (−6.0)	37.9^a (+9.3)	34.7^b	26.4^a	21.3^b (−19.5)	38.7^a (+27.2)	30.4^b
Tr	Ab	37.50^b	18.42^b (−50.9)	31.58^a (+4.3)	30.27^b	–	–	–	–
	Ad	40.14^a	28.90^b (−27.9)	36.20^a (+5.8)	34.20^b	–	–	–	–
PL	Ab	27.8^a	27.5^a (−0.9)	28.8^a (−1.3)	29.1^a	15.3^a	16.1^b (+5.3)	13.9^b (+11.0)	12.5^b
	Ad	27.9^a	26.9^a (−3.6)	28.1^a (+0.5)	28.0^a	14.3^a	14.4^a (+0.9)	12.3^b (+2.1)	12.0^b

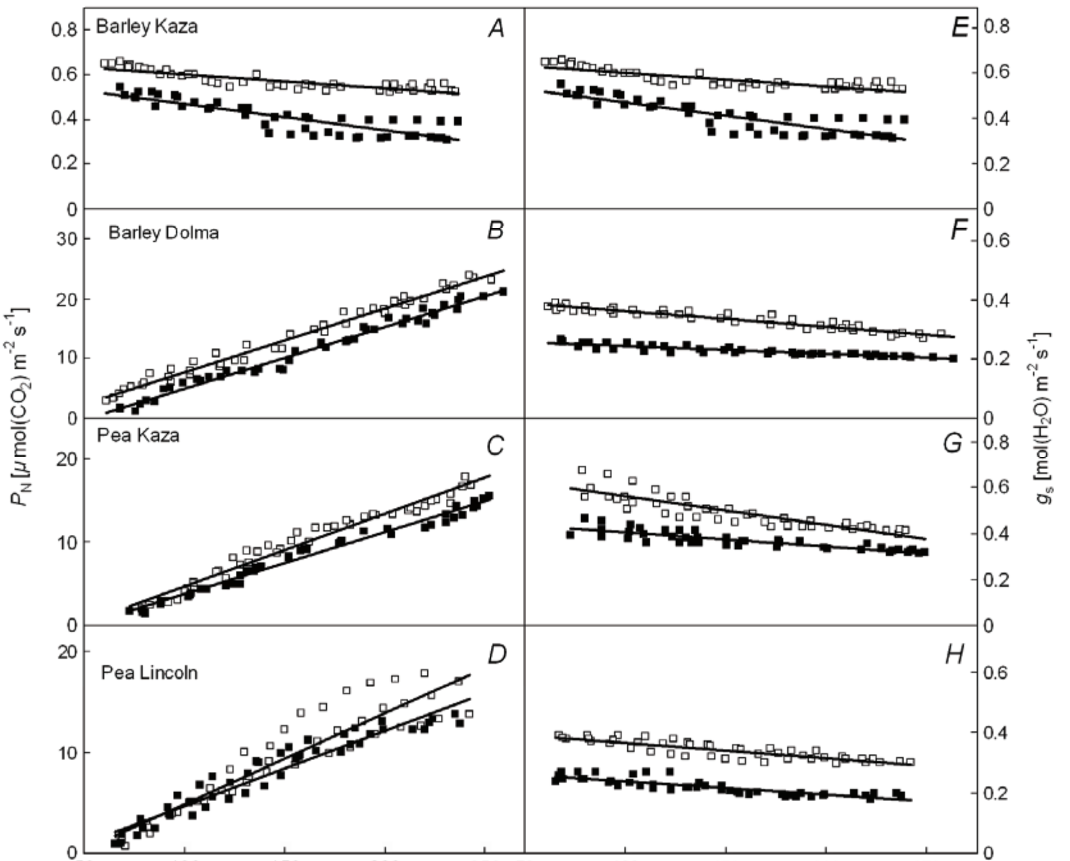

Fig. 1. Correlations between leaf internal CO_2 concentration (C_i) and (*A−D*) net photosynthetic rate (P_N) or (B) stomatal conductance (g_s) at high (□) and low (■) altitude. (*A−D*): Barley Kaza ($y = −4.1349 + 0.0593\ x + 0.0003\ x^2$; $r^2 = 0.920$), barley Dolma ($y = −3.0339 + 0.0915\ x + 3.8181\ x^2$; $r^2 = 0.926$), pea Kaza ($y = −9.3017 + 1.707\ x + 0.1572\ x^2$; $r^2 = 0.945$), and pea Lincoln ($y = −9.2682 + 0.1696\ x + (−0.0002)\ x^2$; $r^2 = 0.896$). (*E−H*): Barley Kaza ($y = 0.6289 + 1.0000\ x + (−0.0551\ x^2)$; $r^2 = 0.668$), barley Dolma ($y = 0.3712 + 1.0000\ x + (−0.0357\ x^2)$; $r^2 = 0.804$), pea Kaza ($y = 0.6072 + (−0.0118)\ x + 1.0000\ x^2$); $r^2 = 0.522$), and pea Lincoln ($y = 0.4596 + (−0.1204)\ x + 1.0000\ x^2$); $r^2 = 0.822$). Based on 90 measurements.

WUE$_i$ was lower in barley Dolma and pea Lincoln (20 and 24 %) at higher elevation, but higher in barley Kaza (24%) and pea Kaza (8%) at low altitude (Fig. 2). The differences were significant (p<0.05) except for pea Kaza.

Discussion

The observed change in SI in the low and high altitude cvs. of barley and pea was in accordance to the results of Bonnier (1895), Körner *et al.* (1989), and Woodward *et al.* (2002). SI is a good measure of response to change in SD (Royer 2001). Since SI also changed along with SD with altitude in leaves of barley and pea, the response was not due by the change in dimension of epidermal cells. The percent change in SD in barley Kaza and pea Lincoln was highest and indicated a cv. response.

Royer (2001) using experimental and fossil data showed an inverse relationship between SI and CO$_2$ mole fraction. McElwain (2004) related increase in SI in leaves of California black oak to the drop of CO$_2$ partial pressure with elevation. The decreasing SD might be responsible for decreased g_s at elevated CO$_2$ (Woodward 1987, Beerling and Woodward 1993). SI and g_s exhibited a similar response in barley and pea with altitude, increasing (by 47 and 55 %, respectively) at low altitude; however, it was much higher than g_s decrease (28 and 24 %) in their high altitude cvs. grown at low elevation, indicating that high altitude determined a greater g_s up-regulation. The low altitude cvs. showed increment in g_s as well as in P_N at high altitude, whereas the high altitude cvs. exhibited decline in the two parameters al low altitudes; this indicated g_s to be a significant parameter to influence P_N.

WUE$_i$ in both barley and pea decreased with elevation despite enhanced g_s and P_N. Higher g_s and P_N, but lower WUE$_i$ at saturation irradiance was reported in early succession species in traditional agro-ecosystem in the Venezuelan High Andes (Llambí *et al.* 2003). Higher P_N values were linked with fast growth in these species, whereas enhanced WUE$_i$ in the late succession flora was associated with dominance during increasing drought stress. High g_s might be advantageous to achieve higher P_N under non-limiting moisture.

At high altitude the partial pressure of CO$_2$ was relatively low under which ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) could be the most prominent limitation for CO$_2$ assimilation, and its activity in plants exhibiting photosynthetic acclimation should be enhanced relative to non-limiting processes such as electron transport capacity (Sage and Coleman 2001). High RuBPCO activity was reported in cvs. of barley and wheat grown at high elevation (Kumar *et al.* 2006). A greater CO$_2$ influx, which could be achieved through enhanced g_s might allow a higher carboxylase activity.

g_s could also influence plant photosynthetic function by altering leaf surface temperature (Lu and Zeiger 1994, Sellers *et al.* 1996). g_s was directly related to crop yield in irrigated Pima cotton and bread wheat grown at high temperature (Lu *et al.* 1998). At high altitude, the higher leaf temperature during the day, associated with a lower partial pressure of CO$_2$, could allow a greater g_s and SD. High g_s and SD could lead to efficient leaf cooling through transpiration for optimal photosynthesis, and are important traits to enhance photosynthetic performance at high altitude.

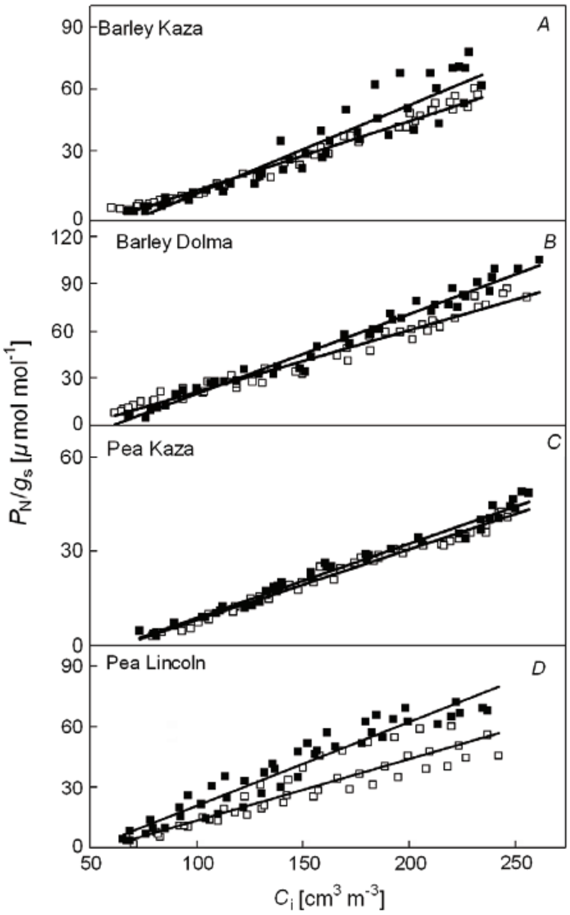

Fig. 2. Correlations between the intrinsic water use efficiency (WUE$_i$; P_N/g_s) and leaf internal CO$_2$ concentration (C_i) in barley Kaza (y = −13.0394 + 0.1628 x + 0.0007 x^2; r^2= 0.922), barley Dolma (y = −7.5779 + 0.2068 x + 0.0008 x^2; r^2= 0.959), pea Kaza (y = −15.9046 + 0.2479 x + (−1.1483) x^2; r^2= 0.979), and pea Lincoln (y = −36.3266 + 0.6328 x + (−0.0009) x^2; r^2= 0.807) at high (□) and low (■) altitude. Based on 90 measurements.

References

Beerling, D.J., Woodward, F.I.: Ecophysiological responses of plants to global environmental change since the Last Glacial Maximum. – New Phytol. **125**: 641-648, 1993.

Bonnier, G.: Recherches expérimentales sur l'adapatation des plantes au climat alpin. – Ann. Sci. nat. Bot. **20**: 217-360, 1895.

Hetherington, A.M., Woodward, F.I.: The role of stomata in sensing and driving environmental change. – Nature **424**: 901-908, 2003.

Hovenden, M.J., Brodribb, T.: Altitude of origin influences stomatal conductance and therefore maximum assimilation rate in Southern Beech, *Nothofagus cunninghamii*. – Aust. J. Plant Physiol. **27**: 451-456, 2000.

Körner, C., Neumayer, M., Menendez-Riedl, S.P., Smeets-Scheel, A.: Functional morphology of mountain plants. – Flora **182**: 353-383, 1989.

Kumar, N., Kumar, S., Ahuja, P.S.: Photosynthetic characteristics of *Hordeum, Triticum, Rumex*, and *Trifolium* species at contrasting altitudes. – Photosynthetica **43**: 195-201, 2005.

Kumar, N., Kumar, S., Vats, S.K., Ahuja, P.S.: Effect of altitude on the primary products of photosynthesis and the associated enzymes in barley and wheat. – Photosynth. Res. **88**: 63-71, 2006.

Llambí, L.D., Fontaine, M., Rada, F., Saugier, B., Sarmiento, L.: Ecophysiology of dominant plant species during old-field succession in a high tropical Andean ecosystem. – Arct. antarct. alp. Res. **35**: 447-453, 2003.

Lu, Z.M., Percy, R.G., Qualset, C.O., Zeiger, E.: Stomatal conductance predicts yields in irrigated Pima cotton and bread wheat grown in high temperatures. – J. exp. Bot. **49**: 453-460, 1998.

Lu, Z.M., Zeiger, E.: Selection for higher yields and heat-resistance in Pima cotton has caused genetically-determined changes in stomatal conductances. – Physiol. Plant. **92**: 273-278, 1994.

McElwain, J.C.: Climate-independent paleoaltimetry using stomatal density in fossil leaves as a proxy for CO_2 partial pressure. – Geology **32**: 1017-1021, 2004.

Morrison, J.I.L.: Stomatal response to increased CO_2 concentration. – J. exp. Bot. **49**: 443-452, 1998.

Royer, D.L.: Stomatal density and stomatal index as indicators of paleoatmospheric CO_2 concentration. – Rev. Palaeobot. Palynol. **114**: 1-28, 2001.

Sage, R.F., Coleman, J.R.: Effects of low atmospheric CO_2 on plants: more than a thing of the past. – Trends Plant Sci. **6**: 18-24, 2001.

Šantrůček, J., Sage, R.F.: Acclimation of stomatal conductance to a CO_2-enriched atmosphere and elevated temperature in *Chenopodium album*. − Aust. J. Plant Physiol. **23**: 467-478, 1996.

Schlüter, U., Muschak, M., Berger, D., Altmann, T.: Photosynthetic performance of an *Arabidopsis* mutant with elevated stomatal density (*sdd1-1*) under different light regimes. – J. exp. Bot. **54**: 867-874, 2003.

Sellers, P.J., Bounoua, L., Collatz, G.J., Randall, D.A., Dazlich, D.A., Los, S.O., Berry, J.A., Fung, I., Tucker, C.J., Field, C.B., Jensen, T.G.: Comparison of radiative and physiological effects of doubled atmospheric CO_2 on climate. – Science **271**: 1402-1406, 1996.

Ulman, P., Čatský, J., Pospíšilová, J.: Photosynthetic traits in wheat grown under decreased and increased CO_2 concentration, and after transfer to natural CO_2 concentration. – Biol. Plant. **43**: 227-237, 2000.

Vats, S.K., Kumar, S.: Photosynthetic response of *Podophyllum hexandrum* Royle from different altitude in Himalayan ranges. – Photosynthetica **44**: 136-139, 2006.

Viswanath, C.S.: Handbook of Agriculture. – Indian Council of Agricultural Research, New Delhi 2002.

Woodward, F.I.: Ecophysiological studies on the shrub *Vaccinium myrtillus* L. taken from a wide altitudinal range. – Oecologia **70**: 580-586, 1986.

Woodward, F.I.: Stomatal numbers are sensitive to increase in CO_2 from pre-industrial levels. – Nature **327**: 617-618, 1987.

Woodward, F.I., Bazzaz, F.A.: The response of stomatal density to CO_2 partial-pressure. – J. exp. Bot. **39**: 1771-1781, 1988.

Woodward, F.I., Lake, J.A., Quick, W.P.: Stomatal development and CO_2, ecological consequences. – New Phytol. **153**: 477-484, 2002.