Role of fructose-1,6-bisphosphatase, fructose phosphotransferase, and phosphofructokinase in saccharide metabolism of four C_3 grassland species under elevated CO_2

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Abstract

We studied the effects of 15-months of elevated (700 μmol mol^{-1}) CO_2 concentration (EC) on the CO_2 assimilation rate, saccharide content, and the activity of key enzymes in the regulation of saccharide metabolism (glycolysis and gluconeogenesis) of four C_3 perennial temperate grassland species, the dicots *Filipendula vulgaris* and *Salvia nemorosa* and the monocots *Festuca rupicola* and *Dactylis glomerata*. The acclimation of photosynthesis to EC was downward in *F. rupicola* and *D. glomerata* whereas it was upward in *F. vulgaris* and *S. nemorosa***.** At EC, *F. rupicola* and *F. vulgaris* leaves accumulated starch while soluble sugar contents were higher in *F. vulgaris* and *D. glomerata*. EC decreased pyrophosphate-D-fructose-6-phosphate l-phosphotransferase (PFP, EC 2.7.1.90) activity assayed with Fru-2,6-P_2 in *F. vulgaris* and *D. glomerata* and increased it in *F. rupicola* and *S. nemorosa*. Growth in EC decreased phosphofructokinase (PFK, EC 2.7.1.11) activity in all four species, the decrease being smallest in *S. nemorosa* and greatest in *F. rupicola*. With Fru-2,6-P_2 in the assay medium, EC increased the PFP/PFK ratio, except in *F. vulgaris*. Cytosolic fructose-1,6-bisphosphatase (Fru-1,6-P_2ase, EC 3.1.3.11) was inhibited by EC, the effect being greatest in *F. vulgaris* and smallest in *F. rupicola*. Glucose-6-phosphate dehydrogenase (G6PDH EC 1.1.1.49) activity was decreased by growth EC in the four species. Activity ratios of Fru-1,6-P_2ase to PFP and PFK suggest that EC may shift sugar metabolism towards glycolysis in the dicots.

Additional key words: acclimation; CO_2 assimilation; fructose-1,6-bisphosphatase; gluconeogenesis; glucose-6-phosphate dehydrogenase; glycolysis; oxidative pentose phosphate pathway; phosphofructokinase; pyrophosphate D-fructose-6-phosphate l-phosphotransferase; starch.

Introduction

Photosynthetic acclimation of C_3 plants to elevated atmospheric CO_2 concentration (EC) is often attributed to soluble saccharide accumulation. Upon changes from ambient to elevated CO_2, contents of non-structural saccharides in stems and leaf sheaths increased significantly. More than 70 % of this increase is due to sucrose accumulation, indicating that excess assimilate is being rapidly exported to vegetative sinks (Gesch *et al.* 2002). Plants grown under EC for a long term produce more saccharides than those grown under ambient CO_2,

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Abbreviations: AC – ambient CO_2 concentration; EC – elevated CO_2 concentration; Fru-6-P – fructose-6-phosphate; Fru-1,6-P_2 – fructose-1,6-bisphosphate; Fru-1,6-P_2ase – fructose-1,6-bisphosphatase; Fru-2,6-P_2 – fructose-2,6-bisphosphate; G6PDH – glucose-6-phosphate dehydrogenase; OPPP– oxidative pentose phosphate pathway; P_N – net photosynthetic rate; PFP – pyrophosphate-D-fructose-6-phosphate l-phosphotransferase; PFK – phosphofructokinase; PPFD – photosynthetic photon flux density; PPi – pyrophosphate; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase.
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AC (Körner and Miglietta 1994). The amount of saccharides produced in high CO_2 is strongly dependent on the photosynthetic and overall physiological acclimation of the plant (Jarvis 1993). An upward regulation of photosynthesis results in an increase of net photosynthetic rate, P_N (Arp and Drake 1991, Chen *et al*. 2000). The increased P_N is usually accompanied by accumulation of soluble sugars in the upward acclimated plants (Tuba *et al*. 1996). In the case of downward acclimation, accumulation of starch is often observed (*e.g*. Peet *et al*. 1986, Stitt 1993, Tuba *et al.* 1996). Starch accumulating in chloroplasts reduces P_N through negative feedback (Azcón-Bieto 1983, Lea *et al.* 2001). Plants in high CO_2 optimize the carbon source/sink ratio, and through this the carbon acquisition, with the aid of down regulation of photosynthesis, since they would be unable to utilize the excessive amount of saccharides beside unchanged levels of N and P (Jarvis 1993, Ceulemans and Mousseau 1994, Bassirirad *et al*. 1996, Bowes *et al.* 1996). The primary factor in the downward acclimation of photosynthesis is the decrease of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) capacity (Sage *et al*. 1989). The acclimation and its consequences will affect the cellular structure, stomatal regulation, water use, allocation pattern, and chemical composition (Newton 1991, Jarvis 1993).

Thus saccharides must play a significant role in the physiological acclimation to EC (Stitt 1993). This was also indicated by our previous study (Nádas *et al*. 1997). Such role of the saccharides cannot be explained without a precise knowledge of regulation of saccharide metabolism. Therefore it is surprising that information on this regulation in plants under EC is difficult to find or rather sparse (Stitt 1991, 1993, van Oosten *et al*. 1992, Vu *et al.* 2001). The role of enzymes of sugar synthesis and breakdown, namely the key enzymes of glycolysis and gluconeogenesis are very important in saccharide regulation. The PFP/PFK enzyme system plays a crucial role in the regulation of glycolysis and gluconeogenesis in plants (Lea *et al.* 2001, Widodo *et al.* 2003).

The Fru-6-P → Fru-1,6-P_2 transformation has a strategic role in regulation of glycolysis. This transformation in different tissues is catalyzed by two enzymes: an ATP dependent phosphofructokinase (PFK) found both in the cytoplasm and chloroplasts catalyzing a one-way process (Fru-6-P → Fru-1,6-P_2) and the PPi dependent phosphofructotransferase (PFP) which is a cytoplasmic enzyme (Uyeda and Furuya 1982, Carnal and Black 1983, Cséke *et al*. 1984, Stitt 1985) catalyzing the above reaction reversibly. PFP is often more active than PFK (ap Rees 1988) and, unlike this, is activated by the important regulator metabolite, Fru-2,6-P_2 (Stitt 1990). At the same time, the Fru-2,6-P_2 inhibits the cytoplasmic Fru-1,6-P_2ase and so reduces gluconeogenesis (Cheng *et al.* 1998, Vu *et al*. 2001, Widodo *et al*. 2003). Diverse roles have been proposed for PFP, including a role in glycolysis, gluconeogenesis, or general adaptability to stress (Paul *et al.* 1995), although dramatic decreases in PFP protein had little effect on overall fluxes or growth of potato tubers (Hajirezaei *et al*. 1994).

Glucose-6-phosphate dehydrogenases (G6PDHs, EC 1.1.1.49) catalyze the oxidation of glucose-6-phosphate (G6P) to 6-phosphogluconolactone concomitant with reduction of NADP to NADPH. The product 6-phosphogluconolactone is then converted to 6-phosphogluconate by 6-phosphogluconolactonase (EC 3.1.1.31) and finally decarboxylated by 6-phosphogluconate dehydrogenase (G6PDH, EC 1.1.1.44) yielding another mole of NADPH and ribulose-5-phosphate. The first enzyme, G6PDH, controls the flux through this non-reversible limb of the oxidative pentose phosphate pathway (OPPP) (Hauschild and Schaewen 2003). Reducing power (NADPH) generated by the OPPP sustains reductive biosynthesis (*e.g*. fatty acids, isoprenoids, and aromatic amino acids) in the dark and nitrogen assimilation in heterotrophic tissues. In addition, OPPP intermediates are continuously withdrawn to fuel other metabolic pathways. For example, erythrose-4-phosphate is produced by Calvin cycle in the light and by the OPPP in the dark (Hauschild and Schaewen 2003).

Effects of 15-months' exposure to elevated (700 μmol mol^{-1}) CO_2 on four perennial C_3 temperate grassland species, the dicotyledons *Filipendula vulgaris* and *Salvia nemorosa* and the monocotyledons *Festuca rupicola* and *Dactylis glomerata*, were examined. Our main purpose was to determine the effects of high CO_2 exposure on P_N, starch and soluble sugar contents, activities of PFP, PFK, and Fru-1,6-P_2ase, and how this may affect saccharide metabolism.

Materials and methods

Plants and CO_2 exposure: We studied a xeric temperate loess steppe (Salvio-Festucetum rupicolae pannonicum) situated at the border of the Hungarian Great Plain (Albertirsa, Monor-Irsa hills, 48 km south east of Budapest) at 160 m altitude a.s.l. The parent rock is sandy loess and loess with thick humus-and nutrient-rich A layer (humus layer: 100 cm, humus content: 6.1 %, and available nitrogen: 14.2 g m^{-2}). The original grassland is made up of more than 90 species. The open air CO_2 fumigation experiment was carried out in the Botanical Garden of the Agricultural University at Gödöllő (28 km east of Budapest). The climate of the two locations did not differ: temperate continental with hot dry summers; mean annual precipitation 500 mm or less; annual mean temperature of 11 °C; and large annual amplitude of temperature changes (22 °C).

The CO_2 exposure was carried out in the Global Climate Change and Plants Long-Term Experimental Ecological Research Station (Gödöllő) for 15 months using open top chambers (Tuba *et al*. 1996). Monoliths

(50×50×30 cm) were extracted from the grassland and transplanted into the open top chambers described by Tuba *et al.* (1996). The soil in the chambers was removed and it was replaced by soil from the profiles the monoliths had been collected from. Four weeks after the transplantation of the monoliths the grass was cut. Following a two-month adaptation period, the monoliths were exposed gradually, over a 4-week period, to 700 μmol mol^{-1} CO_2. The air CO_2 concentrations in the EC (700 μmol mol^{-1}) and AC (350 μmol mol^{-1}) chambers were maintained as described by Tuba *et al*. (1996).

The measurements were made on *Festuca rupicola* Heuff., *Dactylis glomerata* L., *Filipendula vulgaris* Mönch., and *Salvia nemorosa* L. after 15 months' exposure to 700 and 350 μmol mol^{-1} CO_2. *F. rupicola,* the dominant species of the grassland, has sclerophyllous erect leaves of waxy surface, while the others are frequent characteristic species of the grassland with different leaf characteristics: *D. glomerata* has flat blades; *F. vulgaris* has soft, large incised leaves; and *S. nemorosa* has broad, entirely waxy, abaxially hairy rosette and stem-leaves. All species are perennial and have C_3 photosynthesis.

Measurements: Photon-saturated P_N was calculated according to Caemmerer and Farquhar (1981) and measured on single leaves using an IRGA system (type *LCA4*, *ADC Co*., Hoddesdon, UK), operated in differential mode at a photosynthetic photon flux density (PPFD) of 1 200 μmol m^{-2} s^{-1} as described by Tuba *et al*. (1996).

Saccharides: The soluble sugar content of the leaves was measured by a colorimetric method using the phenol-sulfuric acid reaction according to Dubois *et al*. (1956). The starch content was determined according to McCready *et al*. (1950), after solubilisation of starch with perchloric acid *in vitro*, using the sugar anthrone-sulfuric acid reaction.

Enzymes: The samples were frozen in liquid N_2 and homogenized with 50 mM TRIS-HCI buffer, pH 8.0 containing 5.0 mM $MgCl_2$, 1 mM EDTA, 10 % (M/m) glycerine, and 0.1 % (M/m) 2-mercaptoethanol (Cséke *et al*. 1982, Wong *et al*. 1988). The homogenate was centrifuged at 20 000×*g* for 20 min. The enzymes were partially purified by Fast Protein Liquid Chromatography with *DEAE-52* columns. All procedures were performed below +4 ºC.

For measuring PFK and PFP activities in the glycolytic direction, the reaction mixture contained in 500 mm^3 final volume the following [mM]: TRIS-HCl buffer (pH 7.5) 50; $MgCl_2$ 5; EDTA 1; NADH 0.1; Fru-6-P 2; ATP (for PFK) 0.2; PPi (for PFP) 0.2; Fru-2,6-P_2 (activator) none or 1 μM; coupling enzymes [unit]: aldolase 0.5; α-glycerophosphate dehydrogenase 0.5; triosephosphate isomerase 5.0. Fru-1,6-P_2ase activity was assayed in 500 mm^3 total volume of reaction mixture as follows [mM]: TRIS-HCl buffer (pH 7.5) 50; $MgCl_2$ 5; EDTA 1, Fru-1,6-P_2 0.4; NADP 0.5; Fru-2,6-P_2 none or 1 μM; and the coupling enzymes [unit]: glucose-6-phosphate dehydrogenase 1, phosphohexose isomerase 1 (Cséke *et al*. 1982, Wong *et al.* 1988). Protein was determined by the method of Bradford (1976). Enzyme activities were determined by computer using *Enzfitt* softwares. Activation of enzymes was estimated as the relative change in activity with and without F-2,6-P_2.

Results

At 350 μmol mol^{-1} CO_2 concentration, P_N of plants grown at EC was lower in *F. rupicola* and higher in the other three species than in plants grown under AC (Table 1). However, when P_N was measured at 700 μmol mol^{-1} CO_2 concentration, the plants grown at EC had a higher rate in *F. rupicola, F. vulgaris*, and *S. nemorosa*, but a slightly lower one in *D. glomerata* than in plants grown at AC.

Table 1. Net photosynthetic rate, P_N [μmol m^{-2} s^{-1}] and starch and soluble sugar contents [g kg^{-1}(d.m.)] in the leaves of *F. rupicola, F. vulgaris, D. glomerata,* and *S. nemorosa* grown at ambient, AC (350 μmol mol^{-1}) and elevated, EC (700 μmol mol^{-1}) air CO_2 concentration were measured all at AC and EC. P_N was measured at 1 200 μmol(photon) m^{-2} s^{-1} irradiance and 23±0.5 ºC leaf temperature after 15 months of exposure. Means±standard deviation. *significant difference ($p \leq 0.05$) between means at both growth CO_2 concentrations.

Species	Growth CO_2	Measurement CO_2 [μmol mol^{-1}] 350	700	Starch	Soluble sugars
F. rupicola	AC	8.90±0.37	10.80±0.19	156.30±1.80	161.20±2.67
	EC	6.51±0.61	12.60±0.39	196.50±3.40*	111.70±3.91*
F. vulgaris	AC	8.55±0.38	11.27±0.71	90.69±9.01	76.29±5.34
	EC	10.80±0.55	15.89±0.33	114.83±7.53*	96.56±7.35*
D. glomerata	AC	5.35±0.43	12.89±0.46	56.55±8.06	49.15±2.06
	EC	7.32±0.58	8.04±0.60	55.40±3.64	99.74±12.81
S. nemorosa	AC	8.75±0.16	15.26±0.24	92.19±1.30	58.01±1.30
	EC	11.14±0.30	17.43±0.29	92.60±5.21	56.58±2.81

The leaf starch content of *F. rupicola* and *F. vulgaris* was higher at EC than AC (Table 1). There was no significant difference in the starch content of *D. glomerata* and *S. nemorosa* grown at the two CO_2 concentrations (Table 1). EC increased soluble sugar content in *F. vulgaris* and, non-significantly, in *D. glomerata*, decreased it in *F. rupicola*, and left it almost unchanged in *S. nemorosa* (Table 1).

PFP activity was higher in the monocots *F. rupicola* and *D. glomerata* than in the dicots *F. vulgaris* and *S. nemorosa* (Table 2). Growth under EC decreased the Fru-2,6-P_2-free activity of PFP in *F. vulgaris, S. nemorosa*, and *D. glomerata*, while it increased it in *F. rupicola*. In the presence of Fru-2,6-P_2, EC decreased this activity in *F. vulgaris* and *D. glomerata*, while increased it in *F. rupicola* and *S. nemorosa*.

Table 2. Activity [mkat kg^{-1}(protein)] of PFP, PFK, G-6-PDH, and Fru-1,6-P_2ase in the leaves of *F. rupicola, F. vulgaris, D. glomerata*, and *S. nemorosa* grown at ambient, AC (350 μmol mol^{-1}) and elevated, EC (700 μmol mol^{-1}) CO_2 concentration, after 15 months' exposure. PFP and Fru-1,6-P_2ase were assayed without (–) and with (+) F2,6-P_2.

Species	Growth CO_2	PFP –F2,6-P_2	PFP +F2,6-P_2	PFK	G-6-PDH	Fru-1,6-P_2ase –F2,6-P_2	Fru-1,6-P_2ase +F2,6-P_2
F. rupicola	AC	21.12	28.23	83.15	31.48	241.35	199.46
	EC	41.51	47.46	43.22	16.29	209.21	159.67
	% change	96.5	68.1	–48.0	–48.3	–13.3	–19.9
F. vulgaris	AC	4.17	8.03	1.83	2.15	26.48	17.63
	EC	1.78	3.52	1.15	0.93	12.76	5.73
	% change	–33.3	–56.2	–37.2	–56.7	–51.8	–67.5
D. glomerata	AC	15.26	19.43	18.35	37.71	185.19	143.35
	EC	10.97	16.86	12.39	14.43	116.27	102.47
	% change	–28.1	–13.2	–32.5	–61.7	–37.2	–28.5
S. nemorosa	AC	8.66	9.10	14.45	3.16	18.57	12.45
	EC	6.32	11.37	12.28	2.23	12.85	6.29
	% change	–27.0	24.9	–15.0	–29.4	–30.8	–49.5

PFK activity was highest in *F. rupicola* and lowest in *F. vulgaris* (Table 2). Growth in EC decreased PFK activity in all four species; the decrease was greatest in *F. rupicola* and smallest in *S. nemorosa*. Consequently, in the Fru-2,6-P_2-free assay EC caused a large increase in the PFP/PFK ratio in *F. rupicola* (284 %), a relatively small (14 %) decrease of this ratio in *S. nemorosa*, and no major change (+6 %) in the other two species. With Fru-2,6-P_2 in the assay medium, EC increased the PFP/PFK ratio, noticeably in *F. rupicola* (223 %), and to a lesser extent in *S. nemorosa* (47 %) and *D. glomerata* (28%), but decreased this ratio in *F. vulgaris* (–30 %).

Among species, Fru-1,6-P_2ase activity was higher in the monocots than the dicots (Table 2). Growth at EC decreased Fru-1,6-P_2ase activity, the effect being greatest in *F. vulgaris* and smallest in *F. rupicola*. The F-1,6-P_2ase/(PFP+PFK) ratio (assay with no Fru-2,6-P_2) was hardly changed in *F. rupicola*, but decreased in the other species. When the ratio was computed with the F-2,6-P_2-affected activities, a large (31–50 %) relative decrease was observed in the dicots, and no or a small decrease in the monocots.

The activity of G-6-PDH, as that of most of the enzymes analyzed in this work, was higher in monocots than dicots. There was a decrease in this activity under EC in all four species, which was relatively smaller in *D. glomerata*.

Discussion

The gas exchange measured at AC shows that under growth EC there was a downward regulation of RuBPCO capacity in *F. rupicola* and increase in this capacity in the other three species, similarly to our former results (Tuba *et al*. 1996). In contrast, measurements at EC showed an upward acclimation of ribulose-1,5-bisphosphate regeneration or inorganic phosphate (P_i)-recycling capacities in all species, except *D. glomerata*. Obviously, in EC there was an increase in capacity of the whole regulatory control of photosynthesis in the dicots. The upward and downward acclimation of the investigated species is also reflected in their P_N/C_i response curves (Tuba *et al*. 1996). Species differences in response to growth at EC have been described, with reduction or removal of P_i-regeneration limitation of photosynthesis being associated with increased rates of starch or sucrose synthesis (Sage *et al*. 1989). In our experiment, however, there was no simple relationship between photosynthetic acclimation and changes in saccharide contents which could indicate increased rates of their synthesis. Thus, although starch accumulation is often associated with down-regulation of photosynthesis under EC (Peet *et al*. 1986, Stitt 1993,

Tuba *et al*. 1996), starch build-up was associated with decreased RuBPCO capacity in *F. rupicola* but not in *F. vulgaris*. Moreover, with a high nitrogen supply sugar contents increase at EC but no acclimation of photosynthesis or decreased transcripts for Calvin cycle enzymes is observed (Geiger *et al.* 1999). Similarly at high measurement CO_2, enhanced photosynthetic capacity in *S. nemorosa* plants grown at EC was not associated with higher saccharide content and photosynthesis was not enhanced along with soluble sugar accumulation in *D. glomerata*.

Responses of enzyme activities to growth in EC were observed when expressed on a protein basis and therefore are not a consequence of the decrease in N content frequently caused by EC (Nakano *et al*. 1997). Most of the enzymes of gluconeogenesis/glycolysis analyzed here displayed higher activities in monocotyledonous than dicotyledonous species, suggesting this is a specific difference between plant groups. *F. rupicola* was the only species in this study with increased PFP activity in response to EC, both in presence and absence of the activator Fru-2,6-P_2. With this metabolite, which may be expected to occur in the cytosol of irradiated leaves (Theodorou and Kruger 2001), PFP activity was also increased in *S. nemorosa*. This response contrasts with the decrease in activity caused by EC in the other two species. PFP activity increases in response to saccharide accumulation (Spilatro and Anderson 1988, Stitt *et al*. 1991, Krapp *et al*. 1993, Krapp and Stitt 1994). However, we found no relation between saccharide content and PFP activity responses to EC. Van Oosten *et al*. (1992) found no change in this activity after long-term CO_2 enrichment.

Although PFP has often been found as more active than PFK (ap Rees 1988), the plants in our experiment, with the exception of *F. vulgaris*, displayed lower PFP than PFK activities. Without Fru-1,6-P_2 in the assay medium, only in *F. rupicola* there was an increase in PFP/PFK ratio under EC, but in the assay with this metabolite, which is closer to *in vivo* conditions, CO_2 enrichment increased this ratio in all species but not in *F. vulgaris*. An increase in this ratio is usually observed under stress (*e.g*. shortage of nitrogen and phosphorus), when PFP takes over the role of the ATP dependent PFK in glycolysis (Stitt 1991). P starvation induces PFP activity and increases the PFP/PFK ratio (Theodorou and Plaxton 1994), and growth in EC may induce a shortage of N and P relative to the excessive saccharide loading (Jarvis 1993, Bassirirad *et al*. 1996) and hence create a stress situation. From an energetic point of view, the use of PPi in stress situations, such as a nutrient deficiency, is advantageous in comparison with the use of ATP. Our results show this response does not occur in all the species investigated. Reasons for this difference are not known.

The activity of gluconeogenic enzyme Fru-1,6-P_2 was also decreased by EC, potentially imposing greater limitations to sucrose synthesis. The activity of sucrose phosphate synthase, another major enzyme of sucrose synthesis, may increase (Seneweera *et al*. 1995, Hussain *et al.* 1999) or decrease (Chen *et al*. 1995) in response to CO_2 enrichment. Compared to dicots, monocots exhibited higher activity of this enzyme and, with added Fru-2,6-P_2, lower decrease of activity under EC. This could favour the maintenance of relatively higher rates of gluconeogenesis in *F. rupicola* and *D. glomerata* at EC.

Comparison of effects of CO_2 on the gluconeogenic/glycolytic activity ratios of the enzymes involved in Fru-6-P↔Fru-1,6-P_2 conversion [F-1,6-P_2ase/(PFP+PFK)], especially in the presence of Fru-1,6-P_2, showed a decrease in the dicots, and little or no change in the monocots. This shift towards glycolysis, especially in the dicots, could limit sugar build-up and favour upward acclimation of photosynthesis in EC. These possible changes in fluxes may have occurred along with a net increase of sugar concentration at EC.

Although cytosolic G6PDH activity is up-regulated by sugar availability at the transcriptional level (Hauschild and Schaewen 2003), it decreases under EC (van Oosten *et al.* 1992), in agreement with the results of this study. The decrease under EC of G6PDH activity in all four species implies a reduction in the OPPP that can adversely affect the metabolic pathways of lipid, amino acid, and isoprenoid biosyntheses (Hauschild and Schaewen 2003).

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O'Leary, Z.: **The Essential Guide to Doing Research.** – Sage Publications, London – Thousand Oaks – New Delhi – Singapore 2004 (reprinted 2004, 2005, 2006, 2007). ISBN 978-0-7619-4198-9, 978-0-7619-4199-6 (paperback). 226 pp.

Holliday, A.: **Doing and Writing Qualitative Research. 2nd Ed.** – Sage Publications, London – Thousand Oaks – New Delhi 2007. ISBN 1 4129 X, 1 4130 X, 978 1 4129 1129 0, 978 1 4129 1130 0. 199 pp.

Now and then, Photosynthetica publishes reviews on textbooks dealing with scientific communication, it is how to lecture and write papers in science. The topic books are always useful for teachers and their students in photosynthesis or related fields of natural science. The two books I mention this time are a pure exception: they deal with research done mainly in social science that is usually based on interviewing people, making qualitative observations of people in their native environment, *etc*. The only border field accessible also to natural science students may be some questions connected with ecology, environment, and sustainable development of life where collection of views of individual inhabitants may be the basis of conclusions of scientific or political importance. A very significant field of research the preparation of which and realization is explained in detail in the reviewed books is critical ethnography.

In the first textbook, the author shows how to select the topic, how to work with literature, prepare and articulate appropriate questions, articulate a good question checklist, how to make sampling, explore populations, how to argue, filter observations, manage and analyse data, what is the ethics of such research, *etc*. The basic information on statistics, selection of proper methods, recognition what the reached information means in Chapter 12 (interesting Table 12.2) might certainly be used even by some natural science students. And the text ends with information how to prepare an article or thesis based on the results of the research (I like especially the sequence of preparing and reworking drafts till the production of a final one (pp. 210-213). Boxes bring important steps of doing research, selected examples, and references for further reading. Each chapter ends with a clear summary of most important facts. From the quotations presented in chapters I prefer these three: "Next week there can't be any crisis. My schedule is already full. – Henry Kissinger." "USA Today has come out with a new survey – apparently, three out of every four people make up 75% of the population. – David Letterman." "The pure and simple truth is rarely pure and never simple. – Oscar Wilde."

The second textbook shows research based on questioning people, observing situation in various countries and cultural surroundings, the description of which is based not only on interviews but also on photographs, films, radio and TV news, and other materials. Some examples are given, *e.g*. of shopkeeper research in an Iranian carpet shop (p. 142). A good idea is a list of topics for discussion (pp. 183-186) that enables repetition of all important facts.

Both books bring ample lists of references and carefully prepared indexes.

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