BIOLOGIA PLANTARUM 48 (2): 301-304, 2004

BRIEF COMMUNICATION

Changes in the leaf polypeptide patterns of barley plants exposed to soil flooding

R.Y. YORDANOVA, K.G. KOLEV, Zh.G. STOINOVA and L.P. POPOVA*

Acad. M. Popov Institute of Plant Physiology, Bulgarian Academy of Sciences, Acad. G. Bonchev str., Bl. 21, BG-1113 Sofia, Bulgaria

Abstract

Exposure of barley plants (*Hordeum vulgare* L.) to soil flooding for 72 - 120 h led to decrease in the content of the both subunits of ribulose-1,5-bisphosphate carboxylase/oxygenase. The effect was more pronounced on the small subunit. Further, the changes in protein pattern were observed, mainly proteins with molecular masses 30 - 85 kD were down-regulated.

Additional key words: *Hordeum vulgare*, large subunit, leaf proteins, ribulose-1,5-bisphosphate carboxylase-oxygenase, small subunit.

Plants subjected to soil flooding undergo numerous metabolic and physiological changes. When tissues are hypoxic or anoxic the oxygen-dependent pathways, especially the energy-generating system, are suppressed. In this case the ability of plants to overcome hypoxia is related to stress-induced changes in the level of individual proteins (for review see Subbaiah and Sachs 2003). Anaerobic treatment of maize seedlings primary roots caused selective synthesis of at least 20 proteins, most of which were found to be enzymes of glycolysis or sugar-phosphate metabolism and inhibition of the expression of most aerobic, soluble proteins Sachs *et al*. (1980).

Physiological changes in roots inevitably affect leaf metabolism. The reduced Rubisco activity is among the early signals contributing to the reduction of leaf photosynthetic potentials in flooded plants (Vu and Yelenosky 1992). In recent investigations we demonstrated that soil flooding led to a noticeable decrease in the rates of CO_2 assimilation, transpiration, and stomatal conductance. A drop in the activity of ribulose-1,5-bisphosphate carboxylase and of the photorespiratory enzymes phosphoglycolate phosphatase and glycolate oxidase was also observed, while the activity of phosphoenolpyruvate carboxylase increased in flooded plants (Yordanova and Popova 2001). An explanation of these changes could be related to stomatal closure and reduced supply of CO_2. Another possibility is an alteration in leaf proteins, including the Rubisco protein.

In the present work we studied the effect of soil flooding on the content of Rubisco protein and its subunits. An attempt has been made to identify specific polypeptides in soluble leaf fractions where photosynthetic proteins were located.

Barley plants (*Hordeum vulgare* L. cv. Alfa) were grown for two weeks in soil in a growth chamber. The environmental conditions were: irradiance 160 μmol m^{-2} s^{-1} PAR, 12-h photoperiod, day/night temperature 24/20 °C, and relative humidity of 60 %. When the plants were at the 2- to 3-leaf stage, half of the plants (3 - 4 pots with 35 - 40 plants, each) were flooded in the early morning by placing the pots inside larger glass containers filled with tap water to 25 mm above the level of the soil surface. Control plants remained well watered (60 % soil moisture) during the period of the experiment. Samples were taken 72, 96 and 120 h after the start of the treatment. Each measurement was done independently and experiments were repeated at least three times.

Received 22 April 2003, *accepted* 21 January 2004.

Abbreviations: DTT - dithiothreitol; EDTA - ethylendiamine tetraacetic acid; LSU - large subunit; 1-D - one-dimensional; Mr - relative molecular mass; PAGE - polyacrylamide gel electrophoresis; PMSF - phenylmethyl sulfonyl fluoride; Rubisco - ribulose-1,5-bisphosphate carboxylase/oxygenase; SDS - sodium dodecylsulfate; SSU - small subunit;

* Author for correspondence; fax: (+359) 2 739 952; e-mail: lpopova@obzor.bio21.bas.bg

Fresh first-leaf tissue (500 mg) was homogenised in a mortar and pestle in 2.0 cm^3 extraction buffer which contained 330 mM sorbitol, 50 mM HEPES-NaOH, 2 mM KNO_3, 2 mM EDTA, 1 mM $MnCl_2$, 1 mM $MgCl_2$, 0.5 mM K_2HPO_4, 20 mM NaCl, 2 mM Na-isoascorbate, 1 mM PMSF, and 1 mM DTT (pH 7.6). The homogenate was centrifuged at 15 000 *g* for 15 min, and the supernatant was used directly for electrophoretic separation. Protein content of the supernatant was determined according to Bradford (1976).

One-dimensional SDS-PAGE was performed according to Laemmli (1970). An aliquot of the leaf extract was mixed with sample buffer (3:1, v/v). Sample buffer contained 125 mM Tris-HCl, 4 % (m/v) sodium dodecyl sulphate (SDS), 20 % (v/v) glycerol, 200 mM dithiothreitol (DTT), 0.02 % (m/v) bromophenol blue, pH 6.8. The samples were heated for 1 min in boiling water and 20 µg of proteins loaded per lane. Separation was conducted in 12 % polyacrylamide gel at constant current 30 mA per gel using mini-dual vertical gel electrophoresis apparatus (*Sigma-Aldrich*, Germany). After electro-phoresis gels were fixed for 30 min in methanol:acetic acid:H_2O (40:10:50, v/v/v) and stained overnight with standard Coomassie stain. The stain contained 40 % (v/v) methanol, 7 % (v/v) acetic acid and 0.025 % (m/v) Coomassie Brilliant Blue R250. Stained gels were transferred to destaining solution of methanol:acetic acid (20:10, v/v), and then kept in distilled water.

The dried gels were scanned at 560 nm using *Shimadzu* (Japan) *CS 930 TLC*. Molecular masses were estimated from standard plot using lyzozym (14.4 kD), trypsinogen (20.1 kD), CA (30 kD), ovalbumin (43 kD), albumin (67 kD), and phosphorylase A (94 kD), (*Amersham Pharmacia Biotech*, Uppsala, Sweden).

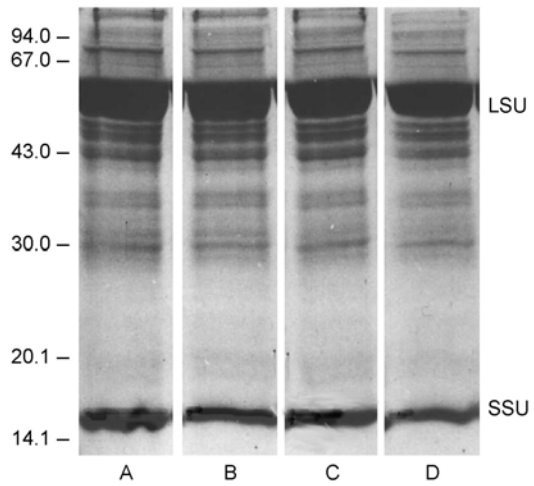

Fig. 1. Polypeptide profiles of soluble protein of barley leaves after 12 % 1D-PAGE: *A* - control, *B* - 72 h after soil flooding, *C* - 96 h after soil flooding, *D* - 120 h after soil flooding. Positions of Mr standards in kD are indicated at the left. Data are representative of three independent experiments.

About 19 or 20 polypeptide bands with molecular masses (Mr) from 98 to 14.4 kD were resolved in the soluble fractions (supernatants after 15 000 *g*) (Figs. 1, 2). The major polypeptide bands in the control have Mr of 85, 55, 47, 43, 37 - 35, 30, and 15 kD. As compared to the control, soil flooded variants show the following changes. The increasing time of treatment leads to a reduction in the content of 55 and 15 kD polypeptides corresponding to the LSU and SSU of Rubisco, respectively. The position of both subunits of Rubisco were previously identified by running purified barley Rubisco as an additional marker (data not shown).

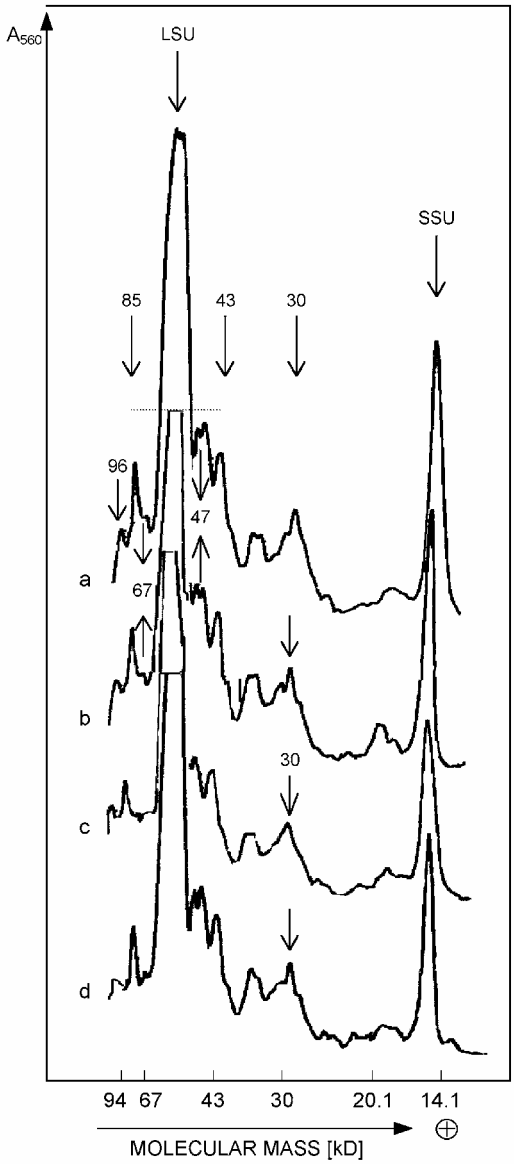

Fig. 2. Gel scans of soluble protein of barley leaves after 1D-PAGE: *a* - control, *b* - 72 h after soil flooding, *c* - 96 h after soil flooding, *d* - 120 h after soil flooding. The major protein differences are indicated with *vertical arrows*. The *horizontal arrow* denotes the direction of band migration. Data are representative of three independent experiments.

Coomassie blue-stained gels (Fig. 1) were scanned at 560 nm (Fig. 2) to quantify separated polypeptides (Table 1). The percentage content of LSU declined from 40.5 % at the control down to 35.5 % at 120 h flooded plants. The average percentage of decrease in LSU level was 13 % for the whole period of soil flooding. For SSU this decrease was much more pronounced: from 12.4 % at the control it decreased to 8.6 % at 120 h flooded plants (over 40 % inhibition). Further, 85, 47,43, and 30 kD bands were less intensive in flooded plants. At the same time one polypeptide with molecular mass 67 kD well expressed in the control became slightly discernible in treated plants.

Changes in proteins (from inhibition of synthesis of a great number of proteins to the induction of synthesis of new sets of proteins) can result from a variety of environmental stresses, such as heat shock (Sebehat *et al*. 1998), anaerobiosis (Sachs *et al*. 1980), water stress (Kicheva *et al*. 1993), cold acclimation (Gray *et al*. 1997), and salt stress (Maslenkova *et al*. 1992). Changes in the composition of proteins are important in the mechanism of adaptation to stress. Among these proteins, Rubisco is considered to be involved in the process of plant growth, development and productivity (Weidner and Fehling 1985).

Table 1. Major differences in polypeptides between the control and plants exposed to soil flooding for 72, 96 or 120 h. The content of each individual polypeptide was expressed as percentage (%) of the total scanning area (100 %) of all polypeptides. The values correspond to those represented graphically in Fig. 2 (∗ - 32 - 30 kD, ? - not detectable).

Mr [kD]	Control	Flooding 72 h	96 h	120 h
96	2.6	4.9	4.8	4.1
85	5.0	1.9	1.6	1.5
67	2.0	?	?	?
55	40.5	36.8	33.9	35.5
49	3.7	3.7	3.6	3.6
47	5.9	4.9	4.3	4.8
43	8.4	6.2	7.7	7.1
37 - 35	5.1	5.6	4.5	5.1
32	3.8	3.2	7.7∗	3.5
30	6.2	5.8		5.1
29 - 27	0.5	0.3	1.0	1.3
24	0.5	0.5	0.7	0.6
18-16	2.2	3.0	2.0	2.2
15.1	12.4	9.8	9.4	8.6

The main result in this study is that soil flooding leads to decrease in the content of both subunits of Rubisco, the effect being more strongly expressed on the SSU. This result is consistent with our observation that soil flooding caused a substantial decrease in Rubisco activity: from 25 to 40 % following the 120-h flooding (Yordanova and Popova 2001). Vierling and Key (1985), estimating the protein synthesis, reported that high temperature stress caused inhibition on SSU of Rubisco. Similar mode of action has been reported for the effect of salt stress on the synthesis of Rubisco and its subunits (Maslenkova *et al*. 1992). This provides ground to suggest that under stress conditions, the synthesis of the chloroplast proteins is less sensitive than the synthesis of the cytoplasmic proteins. Although some stomatal limitation also occurred (Yordanova and Popova 2001), we suggest that one of the main reason for decreased carboxylating activity of Rubisco in flooded barley plants is related to the restriction of Rubisco protein synthesis or enhancement of its degradation.

Another observation is that soil flooding of barley plants leads to changes in the polypeptide profiles of leaf soluble proteins, mainly polypeptides with moderate molecular masses (85 - 30 kD) appeared to be down-regulated. Although the lack of proteome information concerning plants does not allow us to speculate about the nature of the affected polypeptides, there is no doubt that soil flooding mediated effects on protein expression deserve attention and further evaluation.

References

Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Gray, G.R., Chauvin, L.P., Sarhan, F., Huner, N.P.A.: Cold acclimation and freezing tolerance. A complex interaction of light and temperature. - Plant Physiol. **114**: 467-474, 1997.

Kicheva, M.I., Zhelev, N.Zh., Popova, L.P.: Effect of water stress on protein synthesis in two wheat cultivars differing in drought tolerance. - Compt. rend. Acad. bulg. Sci. **46**: 95-98, 1993.

Laemmli, U.K.: Cleavage of structural proteins during the assembly of the head of bacteriofage. - Nature **277**: 680-685, 1970.

Maslenkova, L.T., Miteva, T.S. Popova, L.P.: Changes in polypeptide patterns in barley seedlings exposed to jasmonic acid and salinity. - Plant Physiol. **98**: 700-707, 1992.

Sachs, M.M., Freeling, M., Okimoto, R.: The anaerobic proteins of maize. - Cell **20**: 761-767, 1980.

Sebehat, A., Weiss, D., Lurie, S.: Heat-shock proteins and cross-tolerance in plants. - Physiol. Plant. **103**: 437-441, 1998.

Subbaiah, C.C., Sachs, M.M.: Molecular and cellular adaptations of maize to flooding stress. - Ann. Bot. **90**: 119-127, 2003.

Vierling, E.J., Key, L.: Ribulose-1,5-bisphospahate carboxylase synthesis during heat shock. - Plant Physiol. **78**: 155-162, 1985.

Vu, J.C.V., Yelenosky, G.: Photosynthetic responses of rough lemon and sour orange to soil flooding, chiling and short-term temperature fluctuation during growth. - Environ. exp. Bot. **32**: 471-477, 1992.

Weidner, M., Fehling, E.: Heat modification of ribulose-1,5-bisphosphate carboxylase/oxygenase by temperature pretreatment of wheat (*Triticum aestivum* L.) seedlings. - Planta **166**: 117-127, 1985.

Yordanova, R.Y., Popova, L.P.: Photosynthetic response of barley plants to soil flooding. - Photosyntetica **39**: 515-520, 2001.