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BRIEF COMMUNICATION

Effect of Red Light on Ribulose 1,5-Bisphosphate Carboxylase Activity in Pea Leaves

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Abstract. The ribulose 1,5-bisphosphate activity and its relative content in pea (*Pisum sativum* L., cv. Bordi) seedlings grown either under white or red light were investigated. Plants grown under red light had a lower ribulose 1,5-bisphosphate carboxylase (RuBPCO) activity as compared to plants grown under white light, if expressed on a fresh mass. These activities were very similar under both lights, as calculated on protein basis, although the relative content of RuBPCO was higher in the red one. The activity of RuBPCO under red light corresponds to the lower rate of net photosynthesis. The results are discussed in respect to possible presence of RuBPCO inhibitor in pea plants growth under red light.

Red light conditions during plant growth usually reduce their rate of CO_2 assimilation (Hernischfeger *et al.* 1974, Romanowska *et al.* 1984, Voskresenskaya *et al.* 1984, Leong *et al.* 1985, Drozdova *et al.* 1986). This effect of red light has been recently explained as a result of incomplete development of the light harvesting system (Buschmann *et al.* 1978, Leong *et al.* 1986, Eskins and McCarthy 1987).

The content of soluble proteins in leaves also decreased under red light conditions (Berger and Bergmann 1966, Wild and Holzapfel 1980). According to Voskresenskaya *et al.* (1984) and Frosch *et al.*(1985) carboxylase activity of RuBPCO in plants grown under red light was lowered. However Frosch *et al.* (1985) noted that the activity of RuBPCO did not depend on the red radiation during growth. We investigated the RuBPCO activity and relative content of this enzyme in pea plants grown either under red or white light.

Seeds of *Pisum sativum* L. cv. Bordi were soaked for 20 h in water. After six days of germination in darkness the seedlings were transferred on Knop's nutrien solution. Pea seedlings were grown in growth chamber. The light source consisted of 4 lamps LH 11/1G 1000 W with running tap water filter 15 cm

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below to reduce infra red radiation. Additionaly, for the red light a 5 mm red plexiglass filter was used. The white light irradiance (PAR: 400–700 nm) was 340 Wm^{-2} and red light 170 Wm^{-2} (band width 600–700nm, 660 nm max). The temperature during the 16 h of illumination period was 25 °C, while in the darkness 20 °C. Experiments were carried out on 21 d old seedlings. Measurements of CO_2 gas exchange in saturating white light were described previously (Romanowska *et al.* 1984).

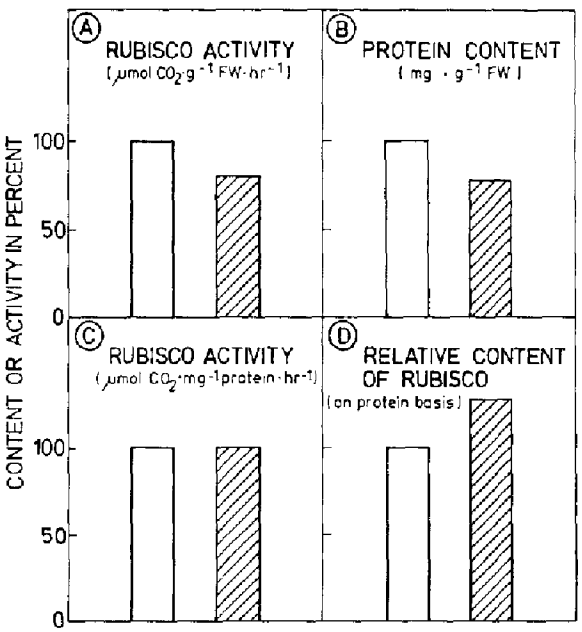

Fig. 1. The activity of RuBPCO (A – per fresh mass; C – per protein), protein content (B) and relative content of RuBPCO (D) in leaves of pea seedlings grown under "white" (emply columns) or red (dasted columns) "light".

Determination of Carboxylase Activity of RuBPCO: 180–280 mg leaf material was ground in a chilled mortar with acid washed quartz sand and 5 ml of isolation medium (150 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 10 mM EDTA, 2 % (w/v) Polyclar AT). The suspension was centrifuged 2 min at 24000 × g and the supernatant immediately used for experiments. For preincubation 8 min. in 30 °C 50 μl of the extract were pipetted to reaction tubes yielding incubation concentrations of 250 mM Tris—HCl, pH 8.3, 12.5 mM $MgCl_2$, 12.5 mM MET (mercaptoethanol), 12.5 mM $NaH^{14}CO_3$ (16×10^9 Bq mol^{-1}). The reaction was started by addition of 50 μl of RuBP (ribulose 1,5-bisphosphate), resulting in an assay medium containing 200 mM Tris-HCl pH 8.3, 10 mM $MgCl_2$, 10 mM $NaH^{14}CO_3$ and 0.7 mM RuBP. Incorporation of ^{14}C was stopped after 1 min. with 250 μl of 5 N acetic acid. Volumes of 250 μl were them transferred to

scintillation vials and evaporated to dryness. The residue was dissolved in 0.5 ml of distilled water, 10 ml of scintillation solution were added and the amount of ^{14}C was measured using a scintillation counter.

Determination of the Content of Soluble Proteins: The tests were carried out in accordance with the method of Bradford (1976) using commassie brilant blue R – 250 reagent (CBB – R 250).

Determination of Chlorophyll Content: Chlorophyll was extracted by 80 % (v/v) acetone and determined by the method of Arnon (1949).

Determination of RuBPCO Content: Determination of relative RuBPCO content was done according to the method of Makino *et al.* (1986). Protein samples (the same as for the determination of the carboxylase activity) were treated in 10 % glycerol (v/v), 1 % SDS (sodium dodecyl sulphate) (w/v) and 5 % MET at 100 °C for 1 min. After cooling these preparations containing 4–10 μg protein were loaded into slab gels ($12.5 \times 13.5 \times 0.1$ cm) containing a 15 % (w/v) resolving PAG (polyacrylamide gel) with a 3 % stacker using the buffer system of Laemmli (1970). Electrophoresis was done for 5 h at 10 to 15 mA. Gels were stained in 0.25 % (m/v) CBB – R in 45 % (w/v) methanol and 7 % (w/v) acetic acid at room temperature for 2 h and destained with successive changes of 20 % (v/v) methanol and 7 % (v/v) acetic acid until the background was colourless. The stained bands (represented by both the large and small RuBPCO subunits) were cut out of the gels with razor blade and eluted in 1 ml of formamide in a stoppered test tube at 50 °C for 5 h with shaking (250 strokes min^{-1}). The absorbance of the resultant solution was read at 595 nm. The extinction of the control extracts per mg protein was taken as 100 %.

All values result from at least eight experiments with four replications. The results were statistically evaluated.

The CO_2 assimilation rate of leaves of pea plants grown under red light conditions is reduced to 85 % compared to the control plants (data not presented). This is not due to a lower chlorophyll content as leaves of both plants contained the same amount of chlorophyll on fresh mass basis (1.1 mg chl g^{-1} fresh mass).

Carboxylase activity of RuBPCO was decreased to a similar degree as rate of photosynthesis in plants grown under red light, [from 273 to 233 μmol (CO_2) g^{-1} (fresh mass) h^{-1}]. As soluble protein content was lowered also in these plants (from 25.8 to 19.6 mg g^{-1} (fresh mass). Carboxylase activity expressed as μmol (CO_2) mg^{-1} (protein) h^{-1} yields about the same value as in control plants (38.7 and 38.3, respectively). The relative content of RuBPCO was significantly higher in plants grown under red light than in control plants [126.3 and 100 mg mg^{-1} (protein), respectively].

Our results referring to the carboxylase activity of RuBPCO in pea leaves are in accordance with those obtained by Servaites *et al.* (1986) and by Farineau

et al. (1988). The content of this enzyme as expressed on protein basis was higher in red light plants than in the controls. Therefore the decline in RuBPCO activity as well as net photosynthesis rate in plants grown under red light conditions, cannot be explained by a lower content of enzyme protein (Fig. 1). The activity measurements were carried out with fully active enzyme in plant extracts. This means that the difference in activity cannot be explained by another degree of activation but that the specific activity of the enzyme had changed. It suggests the occurence of the tight binding inhibitor of RuBPCO in red light plants. Servaites *et al.* (1986) showed that a day/night inhibitor carboxyarabinitol – 1 – phosphate (CA1P) in pea leaves was absent. Our preliminary experiments also confirmed this observation. It seems that the higher RuBPCO content in plants grown under red light may be tentatively explained as follows; (a) blue light might be necessary for the synthesis of some proteins which red light supressed: (b) red light might suppress release of an unknown RuBPCO inhibitor.

Further studies are necessary before a precise explanation of the effect of red light on regulation of RuBPCO activity.

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With an increasing demand for plant products, people have turned toward more and more intensive management practices to increase the productivity of land. One of the most effective way of increasing productivity is to use genetically improved material which can be quickly multiplicate by micropropagation techniques. During last ten years the interest in micropropagation also of woody plant species increased markedly and in connection with it also some books on this theme recently appeared in world literature. This one is the first in Slovak language and so it is welcomed by many researchers, practitioners as well as students in our country. In this book different ways of *in vitro* culture are evaluated. The attention is paid to the effects of the composition of nutrient media, the environmental factors and the type of primary explant. All these questions are demonstrated on the data from literature as well as on authors' own experiments on micropropagation of *Aesculus hippocastanum, Actinidia arguta, A. chinensis* and *Castanea sativa*. The synthesis of secondary metabolites is also considered. From the point of view of genetic fund preservation the tissue cultures are shown as one of possibility of preserving species. The economic comparison of new biotechnological methods with the traditional methods is also included.

The readable text is accompanied by many figures and tables. Abstracts in English and Russian are added.

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