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PHOTOINHIBITION EFFECTS ON PEA PLANTLETS

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Abstract: In our experiment we showed that the high light intensity had photoinhibitory effect on pea plants. On pea plantlet exposed to 600 μmol m^{-2} s^{-1} different periods of time, the content of chlorophyll a is slightly increased after 30' of exposure, and after 60' from exposure is strongly increased (50,93% from control). In case of chlorophyll *b* it could be observed a strong rise of content after 30' of exposure (50,23% from control) and 90,29% from control after 60' of exposure. The carotenoids have a protecting effect, the content of carotenoids was 44,66% from control,after 60' of exposure. The content of glucose in leaves and roots of exposed plantlets is increased in comparison with control, glucose is stored in leaves as starch and parte of it is translocated to roots. The stomatal conductance measured immediately after 30' of exposure, is strongly increased, more than 100% from control, and increasing 5 times after 60' of exposure and 7 times after 120' of exposure. After 4 days, the value of stomatal conductance after 120' of exposure are decreased (30% from control) because of perturbation of stomatal apparatus activity or the water supply. At high light intensity as 900 and 1200 μmol m^{-2} s^{-1} the content of chlorophyll *a* and chlorophyll *b* is decreased. The parameters of fluorescene show a strong inhibition of photosynthetic activity under high light intensity, the F_v/F_m ratio is the most sensitive marker of negative effects of inhibitory light on photosynthetic apparatus.

Introduction

Light is the ultimate source for photosynthesis. However, if the absorbed light energy that is transferred to the reaction centers exceeds the consumption, this may cause damage. Initially, mechanism such as nonphotochemical thermal dissipation of excess energy (Demmig-Adams and Adams 1992, Horton et al., 1994, Darkó et al., 1996) and short- and long-term dynamic regulation of the antenna size (Aro et al., 1993, Barzda et al., 1996, 1998, Garab et al., 1998) serve to protect the photosynthetic machinery. At later stages, when the protective capacities are exceeded, photoinhibition take place. This damage results in a net decrease in photosynthetic efficiency (Aro et al., 1993) and has been estimated to cause losses of carbon assimilation. A combination of high light with other stress factors such as chilling or heat, drought, or low carbon dioxide supply greatly increases the inhibition process (Demmig-Adams, 1992, Aro et al., 1993, Tjus et al., 1998, Fodorpataki and Papp, 2002). The photosystem two (PS II) has long been considered the primary target for photoinhibition (Andersson and Styring, 1991, Barber and Andersson 1992, Prasil et al., 1992, Aro et al., 1993) because photosystem one (PS I) is more stable that PS II during strong light treatments and because its inactivation has rarely been observed in vivo (Havaux and Eyletters, 1991). It has been showed that oxygen is required for light-induced inactivation of PS I to take place (Tjus and Andersson, 1993), which suggest that damage is caused by active oxygen species. PS II is unique among various types of photosynthetic systems in that it produces a very high redox potential so as to oxidize water. As a consequence it is unable to protect itself completely against singlet oxygen production generated by chlorophyll triplets. Mass spectrometry has shown that this leads to successive light induced oxidations of the D1, and to a lesser extent, the D2 proteins which constitute the PS II reaction centre (Barber, 1998).

The accommodation and protection mechanisms of photosynthetic structures are more complicated in case of other factors that act simultaneously (Lichtenthaler, 1996, Olson, 1997, Pugnaise and Valladares, 1999). This make difficult to distinguish the effect of different factors

that generally act together in natural environment. For all of these reasons, the investigation of the effects of several factors on photosynthetic efficiency is required to do in experimental conditions.

In our paper we show the influence of the inhibitory light on photosynthetic activity of pea plantlets by mean of several biochemical and fluorescence parameters.

Material and Methods

Two series of experiments have been achieved. In the first one, the accommodation capacity of photosynthetic apparatus to high intensity of light has been studied. The experiments were performed with pea plantlets obtained by seed germination on filter paper embedded with distillated water, at 25 ºC, under dark. After 5 days, plantlets were transferred under light at 100 μmol m^{-2} s^{-1}. Plantlets were preserved in these conditions until leaves appeared. This light intensity is enough for plants growth and to obtain fhotoinhibition effect at low light intensity in experimental conditions. After 3 weeks, plantlets were exposed to 600 μmol m^{-2} s^{-1} light intensity (to induce photoinhibition) during 30', 60' and 120'. After exposure, evolution of stomatal conductance has been studied by AP4ΔT porometer. Leaves were harvested and several analyses were performed as: determination of assimilatory pigments and carotenoids content (according to Arnon, 1945), glucose content (according to Nelson, 1944; Somogyi, 1952). 3 repetitions were achieved for every analyses, and values were statistically processed.

An other experiment was performed to establish the effects of different light intensities on photosynthetic activity of pea plantlets. 3 weeks old plantlets were exposed at 100, 300, 600, 900 and 1200 μmol m^{-2} s^{-1} at 16/8 photoperiod regime, during 7 days. After exposure, the photosynthes was studied by fluorescence parameters and leaves were harvested and the content of assimilatory pigments were determined (Arnon, 1945). The photosynthesis efficiency was studied with Hansatech device, the sensor was applied on 15 min dark adapted leaves. The following fluorescence parameters have been studied (according to Strasser et al., 1995): a) initial fluorescence (F_0) that appears in the first ms from actinic light action on photosynthetic apparatus under dark. The intensity of F_0 fluorescence is directly proportional with the level of light harvesting complexes organization. This parameter offers information about thylacoid membrane integrity and the capacity of energy transduction between pigment molecules; b) maximal fluorescence (F_m), that is due to reduction of quinonic receptors of PS II by expelled electrons from reaction center P_{680} during primary photochemical reaction of electric charge separation as a result of light absorbtion. This parameter gives information about functional state of acceptor region of PS II and about capacity of electron transport to b_6/f cytocroms complex; c) variable fluorescence (F_v) that shows the quantitative relation between light harvesting and light energy conversion; d) potential efficiency of photosynthesis (F_v/F_m), that shows the level of transformation of light energy in chemical energy that is used in ATP synthesis by phosphorylation and $NADP^+$ reduction. 5 repetitions were achieved for every analyses, and values were statistically processed by *t* test.

Results and Discussions

The results regarding biosynthesis and biodegradation of assimilatory pigments and carotenoids under inhibitory light intensity are shown in Fig. 1. After 30' of exposure to 600 μmol m^{-2} s^{-1} light intensity, the content of chlorophyll *a* is slightly increased (4,68% from control), but after 60' of exposure, the content of chlorophyll *a* is strongly increased (50,93% from control). After 120' of exposure it could be observed just a small rise of value because of low capacity of plants to synthetise new molecules of assimilatory pigment under long exposure to inhibitory light. In case of chlorophyll *b* it could be observed a strong rise of content after 30' of exposure (50,23% from control), 90,29% from control after 60' of exposure but after 120' of exposure the content of chlorophyll *b* was only 74,48% from control, by the same reasons as we

already explained. In case of carotenoids, the results show that after 30' of exposure the synthesis of these pigments is inhibited (15,40% from control). This inhibitory mechanism acts immediatly because after 60' of exposure the mechanism of accomodation of photosynthetic apparatus induces the biosynthesis of new molecules of carotenoids that have a protecting effect. After 60' of exposure the content of carotenoids was 44,66% from control. Despite of this, after 120' of exposure, the biosynthesis of carotenoids is decresed, the content was only 1,55% from control, because of long action of inhibitory light. The role of carotenoids in photosynthetic systems is to quench the chlorophyll triplet state (^{3}Chl). In so doing they prevent the formation of singlet oxygen $^1O_2$ The carotenoid protection mechanism is vital for aerobic photosynthetic organisms since $^1O_2$ is a very reactive and toxic species (Barber, 1998).

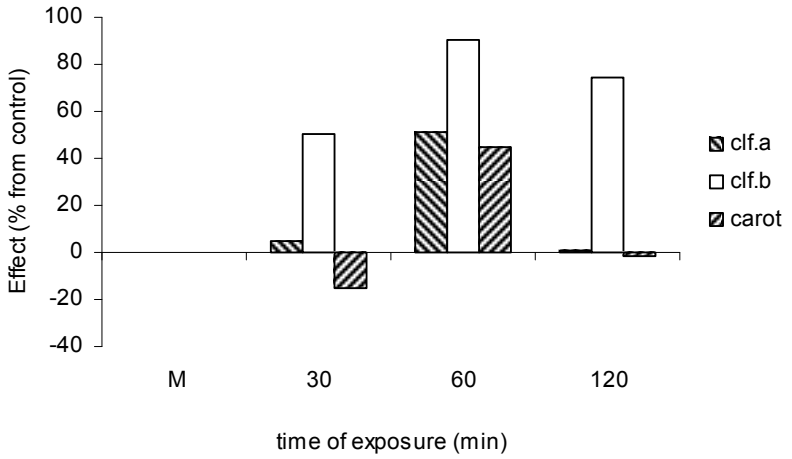

Fig. 1: The content of chlorophyll *a, b* and carotenoids in pea leaves exposed at 600 µmol m^{-2} s^{-1} light intensity

The effect of inhibitory light action on pea plantlets could be seen on the glucose content that is stored as starch in leaves of higher plants. We determined the glucose content after 30', 60' and 120' of exposure, in leaves and roots as well, and the results are shown in Fig. 2. After 30' of exposure the glucose content is increased in leaves, but in roots is decreased. After 60' of exposure the content of glucose is increased in leaves and roots as well, which means that a small content of glucose is translocated from leaves to roots. After 120' of exposure the glucose content is decreased because of low rate of photosynthesis.

The photosynthesis is directly proportional with stomatal activity that could be estimated by the intensity of transpiration. The values regarding stomatal conductance on plantlets leaves exposed 30', 60' and 120' to 600 µmol m^{-2} s^{-1} light intensity are shown in Fig. 3. The measurements have been done immediately after exposure and after 4 days. Immediately after 30' of exposure, the stomatal conductance is strongly increased, more than 100% from control, and increasing 5 times after 60' of exposure and 7 times after 120' of exposure. After 4 days, the value of stomatal conductance after 120' of exposure are decreased (30% from control) because of perturbation of stomatal apparatus activity on the water supply of foliar tissues, due to some perturbations at the root level.

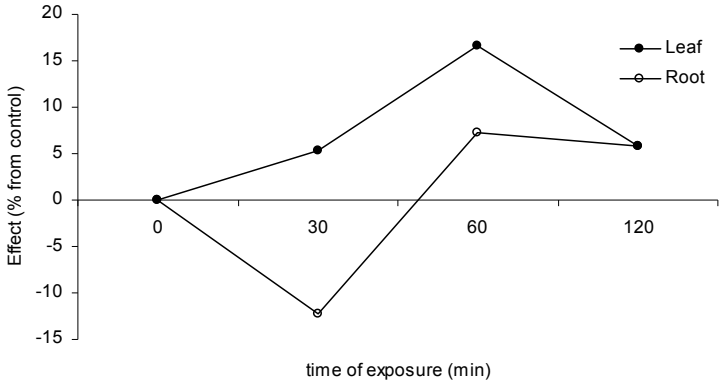

Fig. 2: The content of glucose in pea leaves exposed at 600 µmol m^{-2} s^{-1} light intensity

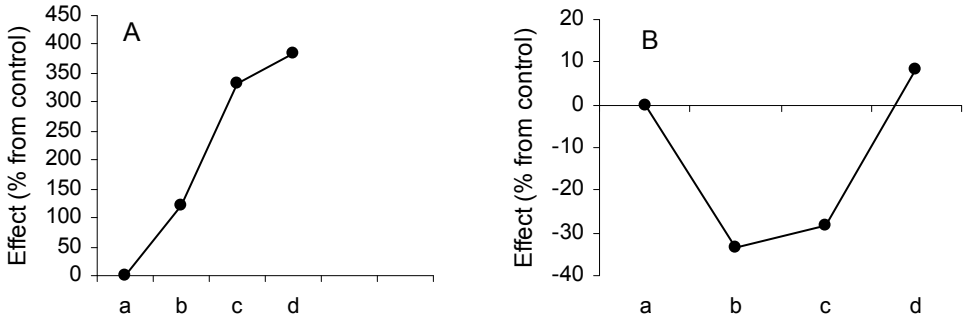

Fig. 3: Stomatal conductance evolution in pea leaves exposed at 600 µmol m^{-2} s^{-1} light intensities (A-immediately after exposure, B-after 4 days of exposure, a-control, b-30' of exposure, c-60' of exposure d-120' of exposure)

Regarding the content of assimilatory pigments content in pea leaves exposed at different light intensities, the results are shown in Fig. 4. The content of chlorophyll *a* and chlorophyll *b* shows moderate decreases at 300 and 600 µmol m^{-2} s^{-1} light intensity and strong decreases at 900 and 1200 µmol m^{-2} s^{-1} light intensity. The moderate decreases at 300 and 600 µmol m^{-2} s^{-1} light intensity are due to accomodation of photosynthetic apparatus to increased light intensity, that is shown by decresing of internal areas of light harvesting from thylakoid complexes. The strong decreases of chlorophyll content at 900 and 1200 µmol m^{-2} s^{-1} light intensity are due to photooxidative degradation of chlorophyll molecules by singlet oxygen, that is accumulated in photochemical systems when the excess of light energy could be not used for biosynthesis of organic compounds.

As all higher plants, pea has two types of chlorophylls (*a* and *b*) that mutual complete their absorbtion capacity of blue and red light. These two types of chlorophyll are not uniform distributed in thylakoids. The chlorophyll *a* is preponderant in fixed light harvesting complexes arround reaction centers, while peripheral light harvesting systems associted with PS II especially are rich in chlorophyll *b*. Because of this distribution, the content of these two types of chlorophylls are not affected in the same way by photoinhibition. In our experiments we show that the content of chlorophyll *b* is strongly decreased in high intensity of light (900 and 1200 µmol m^{-2} s^{-1}) in comparison with chlorophyll *a*. It has been shown that the content of cholophyll

a and *b* decreased in moderate light intensity (600 µmol m^{-2} s^{-1}). In high light intensity, the content of chlorophyll *b* is stronger decreased that the content of chlorophyll *a* and the chlorophyll *a/b* ratio is increased.

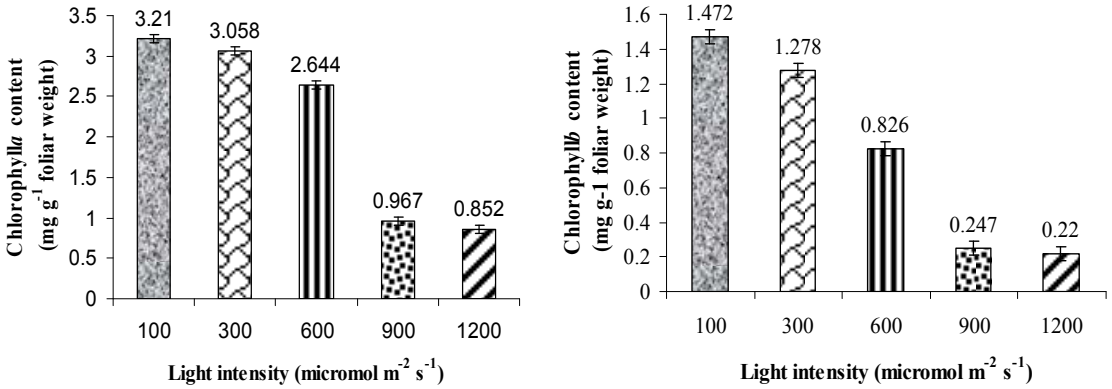

Fig. 4: The content of chlorophyll *a* and *b* in pea leaves exposed at different light intensities (n=5, p<0,01)

The induced chlorophyll fluorescence in pea leaves of plantlets grown under different light intensities gives information about the functional state of photosynthetic apparatus that makes the conversion of light energy in chemical energy used in different biosynthesis processes. The values of F_0 fluorescence show that the system of harvesting pigments has lower extension in dependence of light intensity (Fig. 5). Under 1200 µmol m^{-2} s^{-1} light intensity the level of organization of light harvesting complex of PS II is stronger effected that under 900 µmol m^{-2} s^{-1} light intensity, while the content of chlorophylls is similar.

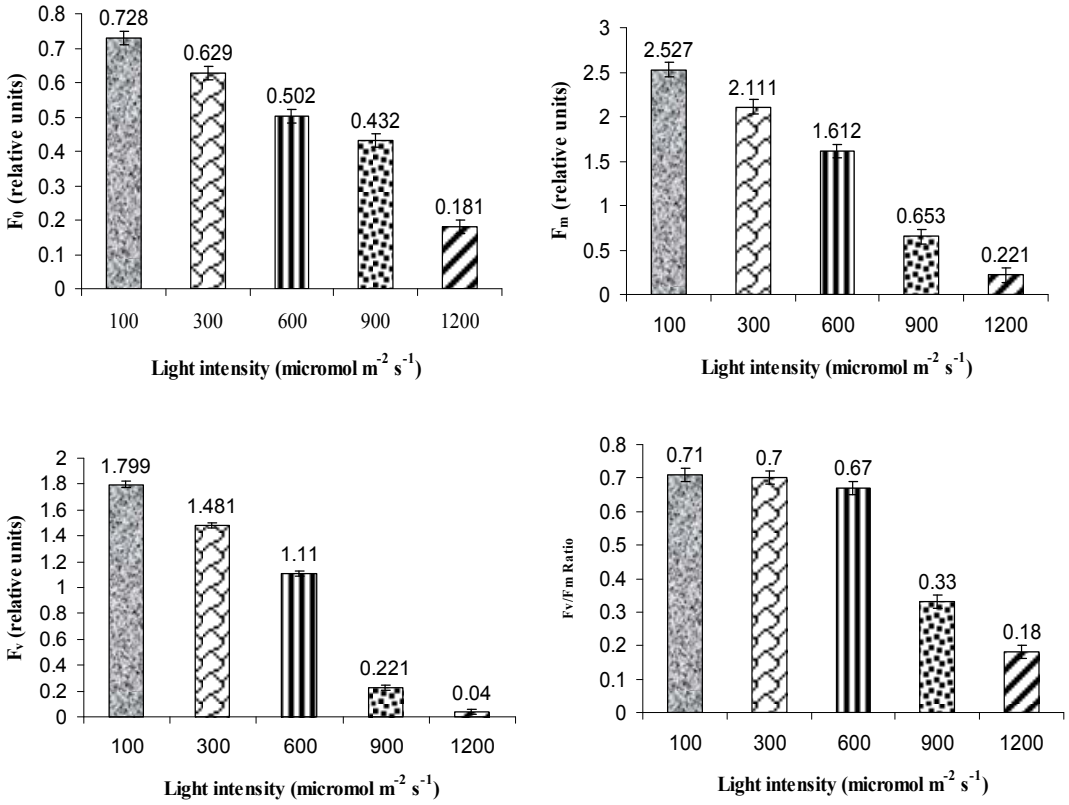

Fig. 5: Influence of different light intensities on fluorescence parameters as F_0, F_m, F_v and F_v/F_m ratio (n=5, p<0,01)

The similar results have been obtained regarding F_m fluorescence (Fig. 5) but the values of this parameter decreases stronger that the values of F_0 parameter even under 900 μmol m^{-2} s^{-1} light intensity.

Because the different light intensities affect in different ways the F_0 and F_m parameters, the values of F_v fluorescence show significant decreasing in inhibitory light (Fig. 5).

The F_v/F_m ratio, as a marker of potential efficiency of photosynthesis, is maintained at similar values around 0.7 under 100-600 μmol m^{-2} s^{-1} light intensity, but is strongly decreased in high light intensity. Based on our results it could be seen that the F_v/F_m ratio is the most sensitive marker of negative effects of inhibitory light on photosynthetic apparatus (Fig. 5).

Conclusions

The content of chlorophyll *a* is slightly increased after 30' of exposure, and after 60' from exposure is strongly increased (50,93% from control).

In case of chlorophyll *b* it could be observed a strong rise of content after 30' of exposure (50,23% from control), 90,29% from control after 60' of exposure but after 120' of exposure the content of chlorophyll *b* was only 74,48%.

The sinthesys of carotenoids is initially inhibited (15,40% from control). This inhibitory mechanism acts immediatly because after 60' of exposure the mechanism of accomodation of photosynthetic apparatus induces the biosynthesis of new molecules of carotenoids that have a protecting effect and the content of carotenoids was 44,66% from control.

The content of glucose is leaves and roots of exposed plantlets is increased in comparison with control, glucose is stored in leaves as starch and parte of it is translocated to roots.

The stomata conductance measured immediately after 30' of exposure, is strongly increased, more than 100% from control, and increasing 5 times after 60' of exposure and 7 times after 120' of exposure. After 4 days, the values of stomata conductance after 120' of exposure are decreased (30% from control) because of perturbation of stomata apparatus activity on the water supply.

At high light intensity as 900 and 1200 μmol m^{-2} s^{-1} the content of chlorophyll *a* and chlorophyll *b* is decreased.

The parameters of fluorescene show a strong inhibition of photosynthetic activity under high light intensity, the F_v/F_m ratio is the most sensitive marker of negative effects of inhibitory light on photosynthetic apparatus.

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EFECTELE FOTOINHIBIȚIEI ASUPRA PLANTULELOR DE MAZĂRE

(Rezumat)

În urma studiilor noastre s-a constatat că lumina de intensitate crescută are efecte fotoinhibitorii asupra aparatului fotosintetic al plantulelor de mazăre, crescute în condiții de laborator la flux fotonic de 100 μmol m^{-2} s^{-1}. Aceste plantule au fost expuse diferite perioade de timp la un flux fotonic de 600 μmol m^{-2} s^{-1} şi s-au evidențiat efectele fotoinhibției cu ajutorul unor parametri biochimici. Astfel, în cazul plantulelor expuse 30', conținutul de clorofilă *a* din frunze şi stipele este uşor crescut, iar după 60' de expunere, este puternic crescut (50,93% față de martor). În cazul clorofilei *b* s-a observat o creştere accentuată a conținutului după 30' de expunere (50,23% față de martor) şi 90,29% față de martor după 60' de expunere. De asemenea, s-a evidențiat rolul protector al carotenoizilor, deoarece s-a constatat că după o scădere inițială a conținutului după o expunere timp de 30', se instalează mecanismul de acomodare a aparatului fotosintetic concretizat prin biosinteza de noi molecule de carotenoizi, iar conținutul în caroteni creşte, ajungând la 44,66% față de martor.

Conținutul de glucoză din frunze şi rădăcini este crescut la plantulele expuse fluxului fotonic inhibitor față de martor, o parte din glucoza sintetizată în frunze fiind translocată în rădăcini.

Activitatea fotosintetică este în relație directă cu nivelul schimburilor gazoase care au loc la nivelul stomatelor, care s-a evidențiat cu ajutorul conductanței stomatice. Măsurătorile realizate imediat după expunerea timp de 30' arată o creştere mai mare de 100% față de martor, iar după o expunere de 60' şi 120', valorile sunt crescute de 5, respectiv 7 ori. După 4 zile de la expunere, valorile conductanței stomatice după 120' de expunere sunt reduse cu 30% față de control, probabil datorită unei perturbări în funcționarea aparatului stomatic sau a unei dereglări în absorbția apei la nivelul rădăcinii, cu toate că plantulele au fost udate în fiecare zi, pe parcursul experimentului.

La valori mai mari ale intensității luminoase de 900 şi 1200 μmol m^{-2} s^{-1} conținutul de clorofilă *a* şi *b* este drastic redus. Parametrii fluorescenței arată o puternică inhibare a activității fotosintetice, raportul F_v/F_m fiind cel mai sensibil marker al efectelor negative ale fluxului fotonic inhibitor asupra aparatului fotosintetic. In the Arieşul Mare basin two associations have been identified on tree bark (Tab. 4). The association *Orthodicrano montani - Hypnetum filiformis* Wisn. 1930 settles on isolated spruce and beech - into lighter biotopes which are slightly drier. From the ecological point of view, the bryocoenoses of this association have a mesophilous, micro-mesothermal to microthermal, and acidophilous character.