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Expression of Early Light-Induced Protein in field-grown pea plants is regulated by low temperature and developmental stage of the plant

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Introduction

Plants in the nature frequently perceive environmental extreme conditions such as light stress. Even relatively low light intensities can induce light stress syndrome when connected with low temperature. Exposure of plants to light stress impairs the photosynthetic apparatus and influences other important physiological processes mainly due to photooxidative damage of various cell components (reviewed in Aro et al., 1993). In order to maintain their normal functions under light stress conditions plants developed multiple repair and protection strategies. The induction of light stress proteins, the Elips (Early Light-Induced Proteins) can be considered to be a part of such protective responses.

Elips are distant relatives of Cab (chlorophyll *a/b*-binding) proteins but they are expressed only transiently during the early stages of greening, under light stress conditions and during a long-term acclimation of plants to increasing light intensities (reviewed in Adamska, 1997). All higher plant Elips investigated so far are nuclear-encoded proteins, posttranslationally imported into the chloroplast and inserted into non-appressed regions of the thylakoid membrane. A recent purification of Elips from light-stressed pea leaves revealed that these proteins bind chlorophyll *a* and lutein, however, the quantity of pigments bound to Elip, as well as their binding characteristics, differed significantly from those reported for Cab proteins (Adamska et al., 1999). Firstly, an extremely high carotenoid content was calculated for purified Elips and secondly, a very weak excitonic coupling between chlorophyll molecules was measured. Although no define function was found for this group of proteins these data strongly suggest that chlorophylls bound to Elips are not involved in energy transfer but fulfil completely different functions. We believe that these proteins might play a protective role within the thylakoids under light stress conditions either by transient binding of free chlorophyll molecules and preventing the formation of free radicals and/or by acting as sinks for excitation energy.

In this work the expression of Elips in a field-grown pea plants was investigated. We demonstrated that low temperature during the night in the field resulted in the presence of Elips also in the absence of light stress. However, this effect of low temperature on Elip

expression was restricted only to the vegetative phase of the plant growth and was absent in flowering plants or plants being in the phase of seed formation.

Materials and methods

Growth of plants and stress conditions. To investigate Elip expression under field conditions the experimental pea varieties (*Pisum sativum* var. x695 or x5) were grown in 1999 under agricultural conditions in a field of Svenska Findus AB near Bjuv, Sweden. Leaves from the latest developed node of 38 days old plants were collected at the afternoon at 3 p.m. during a cloudy day (200-500 $\mu mol\, m^{-2}\, s^{-1}$) that was proceeded by at least three sunny days (1,000-2,000 $\mu mol\, m^{-2}\, s^{-1}$). As a control, leaves were collected before sunrise at 4 a.m. The temperature during the growth period varied between 6 and 29°C.

To investigate Elip expression under controlled conditions pea plants were grown in a growth chamber at a photon flux density of 100 $\mu mol\, m^{-2}\, s^{-1}$ at 20°C during the daytime and at 18°C or 10°C during the night. In order to simulate the field conditions, light stress treatment (1000 $\mu mol\, m^{-2}\, s^{-1}$ for 6 hours) was applied three days before collection of the leaves. On the day of the sample collection the light intensity was lowered to 700 $\mu mol\, m^{-2}\, s^{-1}$.

Isolation and assay of proteins. Leaves frozen in liquid nitrogen were homogenized in medium containing 300 mM sorbitol, 20 mM Hepes-NaOH pH 7.4, 5 mM $MgCl_2$, 2.5 mM EDTA and 10 mM KCl, supplemented with a protease inhibitor cocktail according to the advice of the manufacturer´s (Sigma). The homogenate was solubilized in a sample buffer and polypeptides separated by SDS-PAGE (Laemmli 1970) on 14% polyacrylamide gel. After electrophoresis proteins were transferred to a polyvinylidene diflouride PLUS transfer membrane (Micron Separations Inc.) and the membrane used for immunodetection of Elip using polyclonal antibodies and chemiluminescence method. Obtained signals were quantified by laser-densitometry scanning.

Results and discussion

Elip expression was assayed in pea leaves collected from field-grown plants, which encountered light stress at least 3 days before sample collection and compared with the expression of these proteins in leaves of plants grown in a growth-chamber under controlled temperature, nutrition and water-supply conditions, where a similar light stress treatment as that in the field were applied. Since it was previously shown (Adamska et al, 1991) that the expression of Elip proteins undergoes a diurnal oscillation, where the maximal level of these proteins was measured during the day and no Elips were detected during the night, we collected the samples at 4 a.m. before sunrise (corresponding to the dark phase in the growth-chamber) and at 3 p.m. in the afternoon (corresponding to the light phase in the growth chamber). The results revealed (Fig. 1A) that there are significant differences in Elip expression under both growth conditions tested. The most pronounced difference was the presence of Elip protein in the morning-sample collected from the field-grown pea and its absence in the sample collected from the chamber-grown plants. Secondly, a much higher level of Elip expression was measured under controlled growth condition than in the field. The latter effect can be explained by a better preadaptation of field-grown plants to high irradiances than plants from the growth-chamber, which offers a better protection against light stress-induced damage.

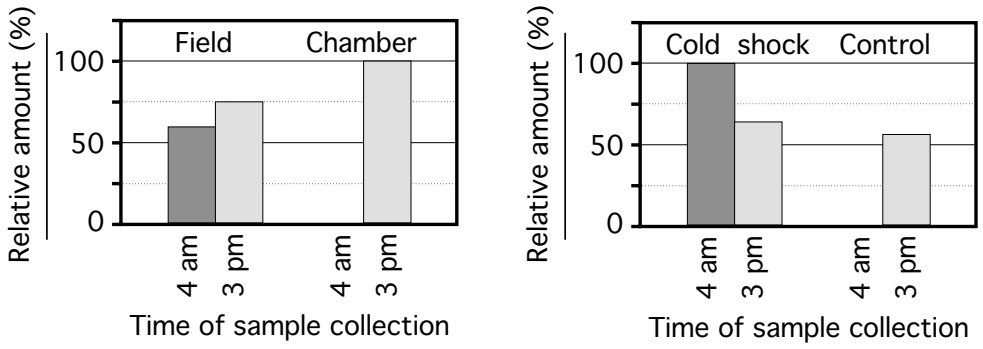

Figure 1. The effect of low temperature on Elip expression in the field and under controlled growth conditions. (A) Quantification of the Elip level in leaves collected from the field- and chamber-grown pea plants. (B) Quantification of the Elip level in leaves collected from chamber-grown pea plants, where the temperature during the night was lowered either to 10°C (cold treatment) or to 18°C (control). The samples were collected twice at 4 a.m. during the night/dark phase or at 3 p.m. in the afternoon during the day/light phase.

It was shown previously (Adamska and Kloppstech, 1994) that the combination of light stress with low temperature increased expression of Elip mRNA in pea leaves. Furthermore, exposure of non-hardened barley plants to prolonged light stress and cold treatment applied in a growth-chamber under light-dark light conditions resulted in a constantly high Elip level due to the lack of its degradation during the night (Montané et al., 1997). Thus, the high level of Elip in the morning in the field-grown pea plant might result from the effect of low temperature on Elip expression.

Data obtained from the climate station revealed that night temperature in the field was approximately 10°C lower than in that was set in the growth-chamber and was at average close to 13°C. To prove whether a lowering of night temperature in the growth-chamber will increase Elip level during the dark phase pea plants were exposed to two different temperatures, 10°C (cold-treatment) and 18°C (control) during the night and the Elip content was assayed by immunoblotting. The results showed (Fig 1B) that similarly to field-grown plants (compare with Fig. 1A) a high level of Elip was detected in the morning in leaves from plants exposed to cold treatment during the night and no Elip was assayed in control plants exposed to 18°C. No significant differences in Elip expression were measured in samples collected at the afternoon from plants grown at both temperature regimes (Fig. 1B). Thus, we can conclude that the high level of Elip expression during the night in the field is related to low temperature.

In addition it is expected that the specific light stress responses might occur during various phases of the plant life cycle, such as vegetative stadium, flowering phase, reproductive stadium and finally the senescence. Therefore, we investigated changes in Elip expression during various phases of the plant cell cycle in the field-grown pea plants. The results (Fig 2) demonstrated that the Elips were expressed to a high extent only during the vegetative phase of the pea life cycle. A small amount of Elip was detected in leaves collected from plants being in the early stages of flowering and no Elip was assayed in older plants, which entered a reproductive phase. In addition, the stabilizing effect of low temperature on Elip level in the night was detected only in the vegetative phase of plant growth. No Elip was detected in the morning sample collected at the time of flowering or fruit formation.

It was previously shown (Binyamin et al., 2001) that Elip mRNA in tobacco plant is up regulated during the leaf senescence. Our data revealed that in contrast to the Elip mRNA level that was significantly enhanced in senescing leaves exposed to light stress the Elip protein was not detectable by immunoblotting (not shown). This indicates a posttranscriptional regulation of Elip expression during senescence.

Based on presented data we conclude that Elips are expressed only in photosynthetically active tissues and such an expression pattern correlates with a light stress protective function proposed for these proteins (Adamska, 1997).

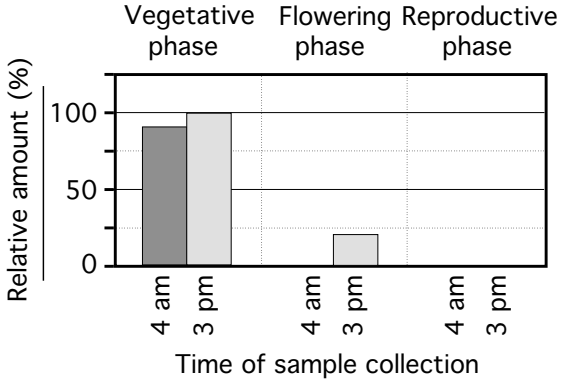

Figure 2. Expression of Elip during a life cycle of pea plants grown in the field**.** Elip expression was tested in leaves collected from plant being in vegetative, flowering or reproductive phases that experienced light stress for three consecutive days. The samples were collected twice at 4 a.m. during the night or at 3 p.m. in the afternoon and Elip amounts assayed by immunoblotting.

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