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BRIEF COMMUNICATION

Influence of drought, high temperature, and carbamide cytokinin 4-PU-30 on photosynthetic activity of plants. 2. Chloroplast ultrastructure of primary bean leaves

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Abstract

Ultrastructural changes in chloroplasts of primary leaves of 15-d-old bean plants (*Phaseolus vulgaris* L. cv. Cheren Starozagorski) in response to a single stress (increasing water deficit, WD) as well as to combined stress (WD *plus* high temperature, WD+HT) were investigated under the possible protective or reparatory effects of the carbamide cytokinin 4-PU-30 [N-(2-chloro-4-pyridyl)-N-phenylurea] applied before or after the stress. Essential structural changes in chloroplast ultrastructure occurred mainly in plants that had experienced WD+HT: the thylakoids were swollen, the envelope was destroyed, and the spatial orientation of inner membrane system was not typical. Changed starch accumulation was also observed. 4-PU-30 protected chloroplast ultrastructure under WD+HT.

Additional key words: membrane system; *Phaseolus vulgaris*; starch; stress; thylakoid.

High temperature (HT) and water deficit (WD) are typical stresses that induce a spectrum of structural and functional deviations of photosynthetic apparatus. The mechanisms of injury vary and depend on the intensity and duration of stress. The combined action of WD and HT complicates plant response. The effect of WD imposed separately and in combination with HT on some essential functional

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Abbreviations: HT - high temperature; 4-PU-30 - N-(2-chloro-4-pyridyl)-N-phenylurea; WD - water deficit.
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parameters of photosynthetic apparatus of young bean plants has been intensively studied in our laboratory. We found that the combined stress (WD+HT) strongly suppresses the rate of photosynthesis, almost completely inhibits oxygen evolution (Yordanov *et al.* 1997), changes the chlorophyll fluorescence induction kinetics (Goltsev *et al.* 1994, Yordanov *et al.* 1997), and changes the fatty acid composition of lipids (Ivanova *et al.* 1998). These variations may be connected with changes in structural characteristics of plastid apparatus. We also studied the changes in ultrastructural organization of chloroplasts during the acclimation of bean plants to HT and proved its high resistance (Yordanov *et al.* 1986a). Generally, water stress induces swelling of chloroplast thylakoids in wheat (Freeman and Duysen 1975), *Festuca vaginata* and *Sedum sexangulare* (Maróti *et al.* 1984).

The functional deviations in the photosynthetic apparatus caused by the combined WD+HT stress induce a question which ultrastructural changes may occur. Some physiologically active substances (plant growth regulators) can either partly eliminate the induced deviations or enhance the processes of reparation (Yordanov *et al.* 1997). The carbamide cytokinin 4-PU-30 [*i.e.*, N-(2-chloro-4-pyridyl)-N-phenylurea] is a synthetic compound with biological properties similar to those of the cytokinins of adenine type (Mok *et al.* 1987, Sudo 1994). In this study we tried to establish whether 4-PU-30 can protect the ultrastructural organization of chloroplasts and also help in reparation processes at the structural level during WD applied separately or in combination with HT.

Young bean plants (*Phaseolus vulgaris* L.) cv. Cheren starozagorski were grown on sand with Knop's nutrient solution in a climatic chamber (temperature 23-25 °C, irradiance 120 μmol m^{-2} s^{-1}, 12 h photoperiod, relative air humidity about 60 %, and sand moisture 80 % of total capacity). After emergence of the first compound leaf the following variants were formed: control plants (C); plants that endured increasing WD by withholding irrigation (WD); plants that endured increasing WD and HT (WD+HT); plants pretreated with 1 μM 4-PU-30 and then exposed to increasing WD (4-PU-30+WD); plants pretreated with 1 μM 4-PU-30 and then exposed to WD+HT (4-PU-30+WD+HT); plants that endured 3 d of WD and then were treated with 4-PU-30 (WD+4-PU-30); plants that endured 3 d of combined stress and then were treated with 4-PU-30 (WD+HT+4-PU-30). All the HT treatments took 5 h at 42 °C during the first two days, whereas on the third day the treatment at 45 °C lasted only 2 h. Immediately after the stresses all treated plants (except the C plants) were rewatered and kept to recover for 24 h at 25 °C. All measurements were done on the primary leaves of bean plants.

For electron microscopy the samples were taken from the middle part of lamina, fixed immediately after the treatment in 3 % glutaraldehyde in phosphate buffer (pH 7.4) for 12 h at 4 °C, and postfixed in 2 % $KMnO_4$ for 4 h at room temperature. After dehydration the material was embedded in *Durcupan* (*Fluka*, Swizerland) and cut with *Tesla* (Prague, Czech Republic) ultramicrotome. Observations were carried out with *JEOL 1200 EX* (Japan) electron microscope.

Chloroplasts in mesophyll cells of the primary leaves of 15-d-old bean plants were well-differentiated. They had a well-developed inner membrane system (grana and

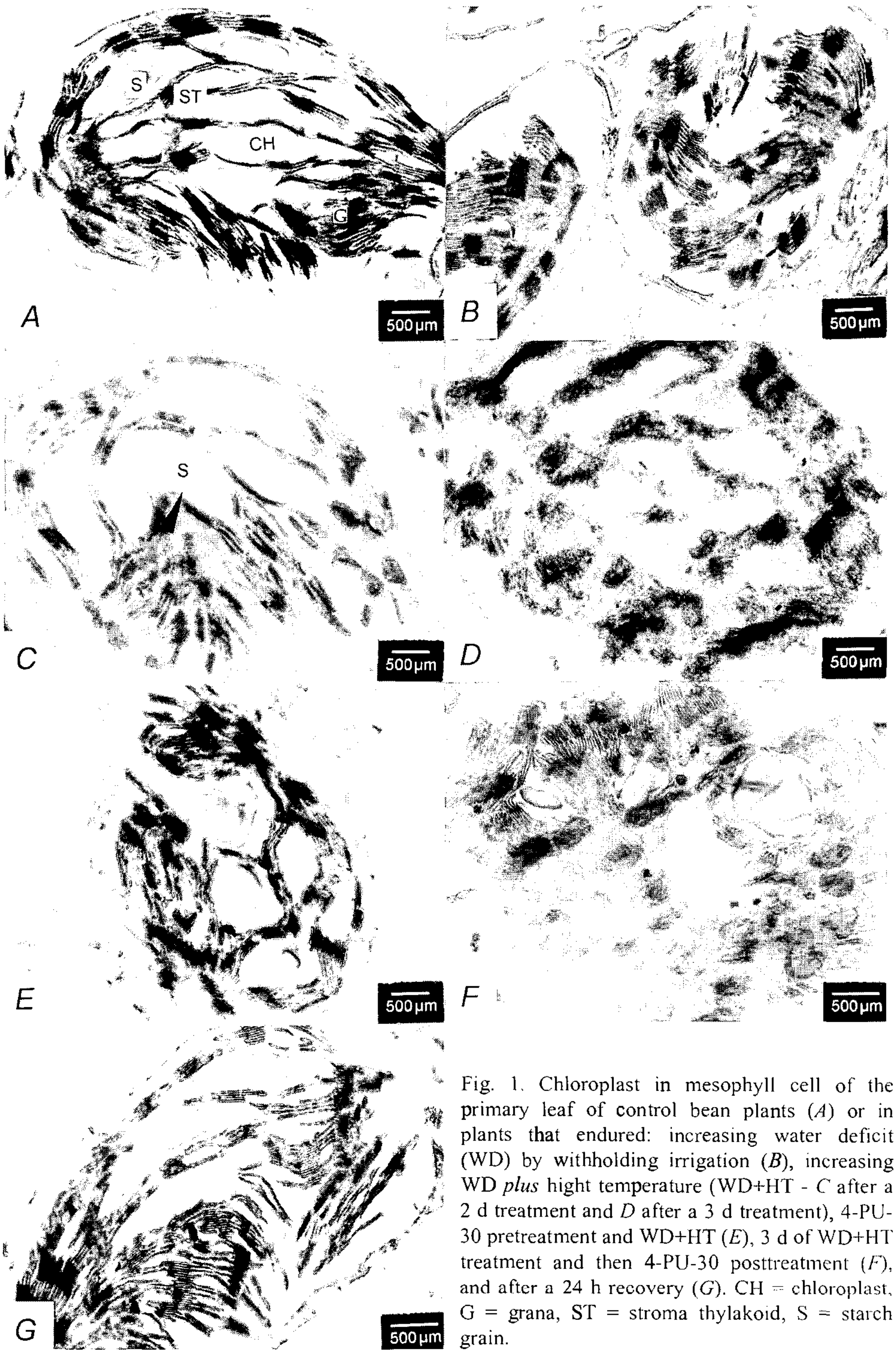

Fig. 1. Chloroplast in mesophyll cell of the primary leaf of control bean plants (*A*) or in plants that endured: increasing water deficit (WD) by withholding irrigation (*B*), increasing WD *plus* hight temperature (WD+HT - *C* after a 2 d treatment and *D* after a 3 d treatment), 4-PU-30 pretreatment and WD+HT (*E*), 3 d of WD+HT treatment and then 4-PU-30 posttreatment (*F*), and after a 24 h recovery (*G*). CH = chloroplast, G = grana, ST = stroma thylakoid, S = starch grain.

stroma thylakoids) (Fig. 1*A*). Grana were of different heights (from 3 to 5 thylakoids) and were linked by a few stroma thylakoids. In the stroma relatively small starch grains with well-shaped zone of saccharides and a capsule containing enzymes were observed. The chloroplasts of control plants preserved their structural characteristics during the experiment. The structural organization of chloroplasts of bean plants that had experienced increasing WD was rather similar to that of control ones. However, fragmentation of stromal thylakoids and destruction of part of them were observed in the internal membrane system caused by WD (Fig. 1*B*). The chloroplast stroma was free of starch grains. This chloroplast architecture was characteristic also for the plants treated with 4-PU-30. The chloroplast response was very typical for a stressed photosynthetic apparatus but obviously it was not controlled at the structural level by 4-PU-30 under WD.

Considerable changes in chloroplast ultrastructure were found in plants under WD+HT. After a 48 h combined stress (Fig. 1*C*), the thylakoid destruction was considerably stronger compared with that observed after WD. On the sites of starch grains gaps were observed (Fig. 1*C*, *arrow*). Obviously the combined stress caused very fast degradation of starch as a result of which the starch grain sites were preserved without changing the chloroplast structure. A previous study on the acclimation of young bean plants to extremely high temperatures showed that chloroplast ultrastructure is a more resistant parameter than some photosynthetic functions (Yordanov *et al.* 1986a). Evidently WD and HT stresses applied independently within certain limits could not cause strong destruction of chloroplasts; however, their combination might lead to a multiplication of the stress effect. In addition, a strong destruction of chloroplast inner membrane system was observed upon 72 h combined stress (Fig. 1*D*). This destruction causes a decrease of the photosynthetic rate (Yordanov *et al.* 1997). Therefore, only in bean plants that had endured mild HT stress (42 °C) a visible acclimation process at the structural level may occur in the chloroplasts and result in an enhanced termotolerance of the photosynthetic apparatus. The combined stress lead to substantial disturbances in chloroplast architecture. In plants pretreated with 4-PU-30, chloroplast structure was similar to that observed in the control plants (Fig. 1*E*). Hence 4-PU-30 could protect the photosynthetic apparatus against injury under the studied stresses. On the other hand, in plants treated with 4-PU-30 after the WD+HT was applied, the plastid apparatus changed differently: the chloroplasts had entirely destroyed envelope, their thylakoids were swollen, and their inner membrane system had untypical spatial orientation (Fig. 1*F*). A similar bean chloroplast destruction was observed after treatment with chloramphenicol (Yordanov *et al.* 1986b) and cadmium (Barceló *et al.* 1988). Such structural changes were very weakly expressed under water stress (Freeman and Duysen 1975) as well as during drought (Maróti *et al.* 1984). Therefore, the stress response of the chloroplast was not specific and the extent of destruction assumed its irreversible character. Under our experimental conditions 4-PU-30 did not favour the reparation of chloroplast structure when applied after HT. In HT variants only in plants treated with 4-PU-30 before the stress was applied the chloroplast structure was similar to that of control plants; this confirmed the protective effect of the cytokinin (Fig. 1*G*).

WD did not influence significantly the structure of chloroplasts, however, its combination with HT caused strong destruction of chloroplasts and sharply decreased the reparation ability of plants. Pretreatment of plants with the carbamide cytokinin 4-PU-30 had a certain protective effect on the structural organisation of chloroplasts.

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