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Effect of cytokinins on the photosynthetic apparatus in water-stressed and rehydrated bean plants

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Abstract

Effects of the cytokinins 6-benzylaminopurine (BAP) and N-2-chloro-4 pyridyl-N′-phenylurea (4-PU-30) on the photochemical activity, oxygen flash yields, and thermoluminescence in bean plants under a water stress were studied. The cytokinins increased the photochemical (Hill reaction) activity and thermoluminescence "B"-band in control as well as in stressed and rehydrated plants, while the oxygen flash yields were affected only in the stressed and rehydrated plants.

Additional key words: oxygen flash yields; photochemical activity; photosynthesis; photosystem 2; thermoluminescence.

Introduction

A low leaf water potential affects photosynthesis in at least three ways: by closing the stomata, by inhibition of the dark fixation, and by inhibition of the electron transport. Depending on the stress severity one among these ways is prevailing (*e.g.*, Kicheva *et al.* 1994). Water stress influences also the hormonal balance in plants, *e.g.*, decreases the cytokinin production (Seeley 1990). We suppose that during rehydration, an addition of exogenous cytokinins may promote the plant recovery. Cytokinins of the purine type, *e.g.*, 6-benzylaminopurine (BAP), and of phenylurea type, *e.g.*, N-2-chloro-4-pyridyl-N′-phenylurea (4-PU-30), stimulate the cell division and differentiation, RNA-polymerase activity, protein synthesis, *etc.* Positive effects of the phenylurea cytokinins on the plant productivity (Nickell 1986, Georgiev *et al.* 1992, Iliev *et al.* 1994, Stefanov *et al.* 1994) were demonstrated for several plant species.

The purpose of this work was to study effects of two cytokinins, BAP and

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Abbreviations: BAP - 6-benzylaminopurine; FWC - full water holding capacity; PS - photosystem; 4-PU-30, N-2-chloro-4 pyridyl-N′-phenylurea.
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4-PU-30, on the recovery of photosynthetic apparatus in bean plants after a water stress.

Materials and methods

Bean plants (*Phaseolus vulgaris* L.) cv. Cheren Starozagorski were grown in pots with 1 kg soil under controlled conditions: temperature 25±2 °C, photon flux density 400 μmol m^{-2} s^{-1} at 12/12 h light/dark cycle, and relative air humidity 50 %. Water stress was imposed 35 d after sowing. For 10 d, the soil moisture of stressed plants was kept at 30 % of the full water holding capacity (FWC). At the same time the soil moisture of pots with control plants was 60 % FWC. On the 10th d the stressed plants were separated into two groups. In the first group the soil moisture in pots was recovered to 60 %, whereas in the second one it was left low (30 %) and the plants were sprayed with water solution of either 10^{-4} M 4-PU-30 or 10^{-4} M BAP. All measurements were taken on the second trifoliate leaf two weeks after the treatment with phytohormones.

Fresh chloroplasts (thylakoids) isolated according to Camm and Green (1980) were suspended in a medium containing 0.4 M sorbitol, 10 mM NaCl, 5 mM $MgCl_2$, and 10 mM Tricine-NaOH (pH 7.5) at a chlorophyll concentration of 300 g m^{-3}. The photochemical activity (Hill reaction) was measured with a *Rank* oxygen electrode at 20 °C. The reaction mixture contained 0.4 M sorbitol, 10 mM NaCl, 5 mM $MgCl_2$, 70 μM 2,6-dichloro-*p*-benzoquinone, 10 mM Tricine-NaOH (pH 7.5), and thylakoids equivalent to 20 g(Chl) m^{-3}. An irradiance of about 200 W m^{-2} was effected with "white light" from a 150 W halogen lamp.

The oxygen flash yields were recorded at room temperature with a rate oxygen electrode of the Joliot type (Joliot and Joliot 1968) and a universal polarograph *OH-105* (*Radelkis*, Hungary). The chlorophyll concentration was about 300 g m^{-3}. Using a 0.08 cm^3 sample volume instead of 0.004 cm^3 in the original Joliot electrode increased the reproducibility of the oxygen flash yields. The oxygen flash yields were examined with a series of short saturating flashes (4 J, $t_{1/2}$ = 8 μs) at 0.5 s dark intervals. The value of dark distribution of the oxygen evolving S_i states (S_0 and S_1), and the values of misses (α) and double hits (β) were calculated using a programme for minimalisation of deviations between experimentally obtained oxygen flash yields and the yields calculated according to the model of Kok *et al.* (1970).

Thermoluminescence measurements were done using a set-up analogous to that described by Ichikawa *et al.* (1975). The samples were fastened to a heatable stage that was inserted into a Dewar flask containing liquid N_2. The heating rate was approximately 1 °C s^{-1}. The leaves had been kept in the dark for 2 h before measurements. Leaf pieces of equal size were used in order to obtain reproducible results. The output signal from the radiometer, which was proportional to the frequency of the thermoluminescence photons, and the output signal from a bridge amplifier proportional to the temperature of the holder (sample), were connected to two different channels of the analogue digital converter in an *IBM* compatible computer. A special programme composite in C and machine languages read and

transferred data to the memory of computer every 2 ms, and allowed their simultaneous graphical presentation on the computer display. The mathematical treatment of the about 50 000 pairs of numbers, temperature, and thermoluminescence, was done using the programme package *Origin 3.0*.

Results and discussion

The Hill reaction activity in isolated thylakoids increased up to 150 % after treatments with BAP (10^{-4} M) and 4-PU-30 (10^{-4} M) (Table 1). The Hill activity in the water-stressed plants was 50 %, and after the treatment with 4-PU-30 and BAP it increased up to 75.6 and 71.0 %, respectively. The photochemical activity in the rehydrated plants increased to 60.6 %, and the positive effect of the phytohormones was also observed. The results confirm the hypothesis about the protective role of cytokinins.

Table 1. Photochemical activity of chloroplasts [mmol(O_2) kg^{-1}(Chl) s^{-1}], isolated from BAP and 4-PU-30 treated control, water stressed and rehydrated bean plants, and the values of kinetics parameters according to the model of Kok *et al.* (1970). Means ± SE of 5 independent experiments.

Variant	Photochemical activity		S_0+S_1		Misses (α)	Double hits (β)
		[%]	[relative]	[%]		
Control	48.9±1.2	100.0	199.3	100.0	0.254	0.046
Control + 4-PU-30	73.1±2.5	149.5	219.8	110.3	0.276	0.049
Control + BAP	69.6±2.1	142.3	199.9	100.3	0.265	0.046
Stressed	23.2±1.1	47.4	108.3	54.3	0.280	0.056
Stressed + 4-PU-30	36.9±1.8	75.5	183.1	91.9	0.247	0.048
Stressed + BAP	34.7±1.2	71.0	150.8	75.7	0.270	0.038
Rehydrated	29.6±1.1	60.5	172.5	86.6	0.273	0.050
Rehydrated + 4-PU-30	43.6±1.5	89.2	193.4	97.0	0.269	0.043
Rehydrated + BAP	41.3±2.3	84.5	169.9	85.2	0.278	0.042

The oxygen flash yields (Table 1) also pointed in this direction, but the effect of the cytokinins was not so strong as in the case of the photochemical activity (Table 1). The effect of phytohormones in control and rehydrated plants on the number of oxygen evolving centres (S_0 and S_1) was not significant while in stressed plants a pronounced increase was seen. There were no remarkable differences in the values of the misses (α) and the double hits (β).

As the thermoluminescence curves were obtained after the excitement with 1 flash of the dark (2 h) adapted leaves, the amplitude of the "B" band reflected the number of the oxygen-evolving centres in S_2 state according to the model of Kok *et al.* (1970), *i.e.*, the recombination of the $S_2Q_B^-$ pairs (Demeter and Vass 1984). Both cytokinins induced a positive effect (Fig. 1). The amplitude of the "B" band increased especially in the 4-PU-30 treated plants.

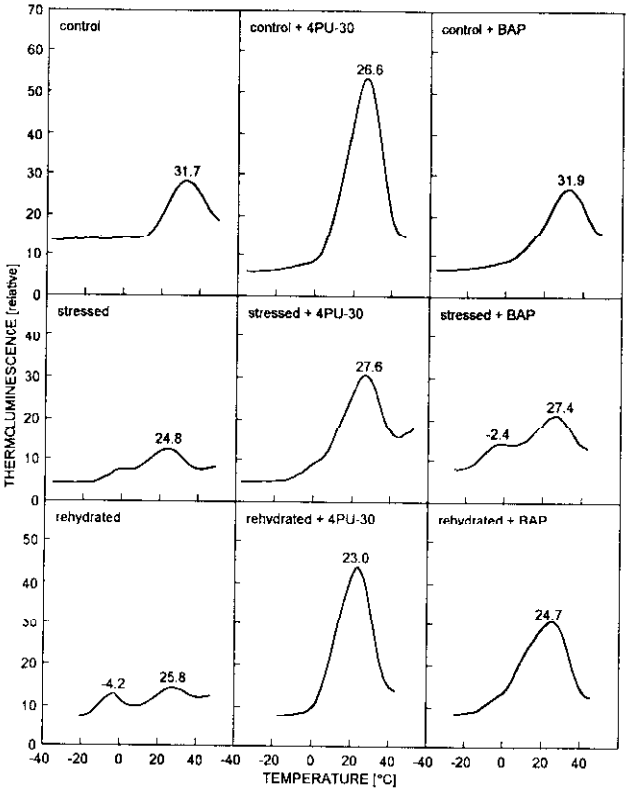

Fig. 1. The effect of 4-PU-30 (10^{-4} M) and BAP (10^{-4} M) on the thermoluminescence curves of control, water stressed, and rehydrated plants.

The 4-PU-30 and BAP increased the photochemical activity at all types of treatment (Table 1). The cytokinins also affected the number of the oxygen-evolving centres ($S_0 + S_1$) in the stressed plants (Table 1). On the other hand, the oxygen flash yields and the kinetics parameters calculated according to the model of Kok showed no significant effect in both the control and rehydrated plants. The water stress significantly decreased the photochemical activity and the number of the oxygen-evolving centres. According to Maslenkova *et al.* (1993), the so-called PS2α centres, situated in grana regions, operate by non-cooperative Kok's mechanism for O_2 production and are especially sensitive to different stress factors. The promoting effect of the BAP and 4-PU-30 on the photochemical activity and on the number of the oxygen-evolving centres is thus probably a consequence of certain changes in the chloroplast structure and oxygen-evolving enzyme system of the photosynthetic apparatus. The finding of Wilhelmová and Kutík (1995) that BAP increased grana stacking is in agreement with our results. As the samples had equal chlorophyll concentrations, the higher number of oxygen-evolving centres in the cytokinin treated plants may reflect either less antennae chlorophyll molecules in their photosynthetic units or their functioning with a higher rate (shorter turnover time). The significant increase in the amplitude of the thermoluminescence "B" band, reflecting the number

of the centres in the S_2 state, pointed to the first possibility. Undoubtedly, this statement needs further investigations.

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