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# Effect of benzylaminopurine on rehydration of bean plants after water stress

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## Abstract

The possibility of improving the recovery of plant photosynthesis after water stress by cytokinin-induced stimulation of stomatal opening or delay of leaf senescence was tested. The 6-benzylaminopurine (BAP) in concentrations 1 and 10 μM was applied to the substrate (sand + nutrient solution) or sprayed on primary leaves of 14-d-old *Phaseolus vulgaris* L. plants sufficiently supplied with water or water-stressed for 4 d. The later ones having relative water content decreased to 69 % were fully rehydrated during the following three days. Parameters of photosynthesis and water relations were measured in primary leaves of 7-, 10-, 14-, and 17-d-old plants. Application of 1 μM BAP slightly delayed leaf senescence: in 17-d-old control plants, net photosynthetic rate ($P_N$) and chlorophyll (Chl) content, and when sprayed on leaves also some of Chl *a* fluorescence kinetic parameters of BAP-treated leaves were slightly higher than those of untreated leaves. Both types of application of 1 μM BAP slightly improved recovery of plants during rehydration after water stress in terms of increased $g_{ad}$, $g_{ab}$ and $P_N$, *i.e.*, parameters which were markedly decreased by mild water stress. However, contents of Chl *a*, Chl *b* and carotenoids and parameters of Chl *a* fluorescence kinetic were not markedly affected by mild water stress and after rehydration were not stimulated by 1 μM BAP. 10 μM BAP had mostly negative effects on the parameters measured.

*Additional key words*: chlorophyll content, chlorophyll fluorescence, net photosynthetic rate, *Phaseolus vulgaris*, stomatal conductance.

## Introduction

One of the most serious environmental stresses is drought. Different mechanisms were developed during plant evolution to avoid severe, dangerous water deficit, and to tolerate mild water deficit. For the plant drought resistance, not only successful survival during stress but also the recovery after stress is crucial. While a huge amount of literature deal with changes in plant morphology, anatomy, and physiology during drought, less attention has been paid to events during plant rehydration.

In many cases, water deficit firstly reduces growth and leaf area development and duration. Stomatal closure which decreases the water efflux decreases also the $CO_2$ influx, and usually limits photosynthesis under mild water stress. Under high irradiance, restricted $CO_2$ influx supports photoinhibition. The severe water stress directly affects photosynthetic capacity of mesophyll causing a decrease in carboxylation, electron transport chain

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*Abbreviations*: ABA - abscisic acid; BAP - 6-benzylaminopurine; Chl - chlorophyll; CK - cytokinin(s); $F_m$ - maximum fluorescence; $F_v$ - variable fluorescence; $g_{ab}$ - abaxial stomatal conductance; $g_{ad}$ - adaxial stomatal conductance; $g_s$ - total stomatal conductance ($g_s = g_{ad} + g_{ab}$); $P_N$ - net photosynthetic rate; $q_N$ - non-photochemical quenching; $q_p$ - photochemical quenching; RWC - relative water content; Φ - quantum yield of photosystem 2.

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** Corresponding author; fax: (+420) 2 24310113; e-mail: pospisilova@ueb.cas.cz activities, and/or ultrastructural changes in chloroplasts. Water stress affects many other metabolic pathways, mineral uptake, membrane structure, *etc.* Therefore, it is not surprising that also phytohormone content is changed. It is very important because plant hormones are main signals in root-to-shoot communication and *vice versa* (for review see, *e.g.*, Schulze 1986, Davies and Zhang 1991, Tardieu and Davies 1993, Davies 1995, Naqvi 1995). In consequence, the change of hormonal balance might play the key role in the sequence of events induced by stress (Itai 1999). Despite of this, the mechanism of induction of a hormonal shift by an environmental change is unknown.

Cytokinins (CK) antagonize many physiological processes induced by water stress, mainly those mediated by abscisic acid (ABA). Well known is the reversal of ABA-induced stomatal closure (for review see, *e.g.*, Incoll and Jewer 1987, Incoll *et al.* 1990). Thus the reduction in CK concentration in xylem sap and delivery rate (Bano *et al.* 1993, Shashidhar *et al.* 1996) as soil dries might amplify shoot responses to an increasing concentration of ABA (Davies and Zhang 1991). In plantlets growing *in vitro* the addition of benzylaminopurine (BAP) stimulates opening of stomata and transpiration (Diettrich *et al.* 1992, Pospíšilová *et al.* 1993). The mechanism of CK action on guard cells might involve direct induction of membrane hyperpolarization by stimulation of electrogenic $H^+$-pump, stimulation of adenylate cyclase activity which could lead to an increase in intracellular adenosine 3′,5′-cyclic monophosphate content, stimulation of guanylate cyclase activity, or interactions with calcium-calmodulin system and with ABA regulation of ion channel permeabilities (Incoll *et al.* 1990, Morsucci *et al.* 1991, Pharmawati *et al.* 1998). CK may partially ameliorate negative effects of water stress, *e.g.*, by stimulation of osmotic adjustment in *Cicer* by BAP (Yadav *et al.* 1997) or in *Vigna* by kinetin (Agarwal and Gupta 1995). Recently, Cowan *et al.* (1999) proposed a model illustrating metabolic interrelations between CK and ABA.

CK affect photosynthetic parameters directly (*e.g.*, chlorophyll and photosynthetic protein synthesis and degradation, chloroplast composition and ultrastructure, electron transport, opening of stomata) or indirectly (mediated by changes in growth, sinks for photosynthates, morphology and anatomy) (for review see Synková *et al.* 1997). Further, CK delay leaf senescence (*e.g.*, Soejima *et al.* 1992, Čatský *et al.* 1996, Badenoch-Jones *et al.* 1996, Naqvi 1999), while water stress usually accelerates it. With leaf age, drought-induced inhibition of photosynthesis increases and ability to recover after rewatering decreases (*e.g.*, David *et al.* 1998). This fact accentuates the importance of delayed leaf senescence induced by CK.

Only few reports deal with the effect of CK on leaf photosynthesis during water stress (for review see Chernyad'ev 1997). Application of BAP alleviated negative effect of NaCl on chlorophyll content in detached wheat leaves, but not in intact seedlings (Mumtaz *et al.* 1997). BAP and synthetic CK 4-PU-30 increased the photochemical activity in control, water-stressed, and rehydrated bean plants (Metwally *et al.* 1997). In spring wheat, the CK-like compound kartolin-4 alleviated negative effect of water stress on ribulose-1,5-bisphosphate carboxylase content and activity (Chernyad'ev and Monakhova 1998).

The opposite to water stress effects of CK on plants and reduced CK content and activity in xylem sap and/or leaves of stressed plants led us to creation of a new hypothesis that recovery of plants after water stress might be accelerated by application of CK. The aim of this research was to prove this hypothesis and to determine whether the application of BAP can speed up recovery of photosynthetic rate of bean plants after water stress by promotion of stomatal opening and/or by retardation of leaf senescence.

## Materials and methods

French bean (*Phaseolus vulgaris* L. cv. Jantar) seedlings were grown in pots with coarse sand and Hewitt nutrient solution at 16-h photoperiod, irradiance (400 - 700 nm) of 350 μmol $m^{-2}$ $s^{-1}$, day/night temperature of 25/20 °C, and relative humidity of about 50 %. After 10 d, when the area of primary leaves was maximum, water stress was induced in half of plants by cessation of watering for 4 d. On the day 14 the stressed plants were re-irrigated. At the same time BAP in different concentrations was applied to control (sufficiently water-supplied) plants and to water-stressed plants either into the substrate or sprayed on leaves. BAP may be readily taken up by plants (Vogelmann *et al.* 1984), and it is not or only very slightly degraded by cytokinin oxidase (Galuszka *et al.* 1999). On days 7, 10, 14, and 17 the following parameters were measured.

Relative water content was measured gravimetrically in leaf discs (0.5 $cm^2$) saturated in holes of fully moistened polyurethane foam under dark (Čatský 1960). Diffusive conductances of abaxial and adaxial epidermes for water vapour were measured by a diffusion porometer *Delta-T* type *Mk3* (*Delta-T Devices*, Cambridge, UK) at a temperature of 25 °C, irradiance of 860 μmol(photon) $m^{-2}$ $s^{-1}$, and relative humidity of 50 - 60 %. Leaf net photosynthetic rate was determined as $CO_2$ uptake rate in a PC-assisted closed gas exchange system with an infra-red gas analyser *Infralyt IV* (*Junkalor,* Dessau, Germany) in a $CO_2$ concentration range from 20 to 2000 mg $m^{-3}$, leaf temperature of 20 °C, and near-saturating irradiance of 860 μmol $m^{-2}$ $s^{-1}$.

Chlorophyll *a* fluorescence characteristics of the adaxial surface of attached leaves were measured after a 15-min dark period with a *PAM Chlorophyll Fluorometer* (*Walz,* Effeltrich, Germany) at room temperature and ambient $CO_2$ concentration. $F_0$ was determined at a modulation frequency of 1.6 kHz. Actinic light (650 nm; 100 μmol $m^{-2}$ $s^{-1}$) was provided by *102L LED* lamp. Saturating pulses of "white light" (duration 700 ms, 2 500 μmol $m^{-2}$ $s^{-1}$) were applied in 100 s intervals. Data sampling, control, and calculation were served by the *DA 100 Data Acquisition System* (*Walz,* Effeltrich, Germany). The nomenclature of Van Kooten and Snel (1990) was used. The actual quantum yield of photosystem 2 photochemistry, Φ, was calculated as $(F_m'-F_s)/F_m'$ according to Genty *et al.* (1989).

Chlorophyll and carotenoid contents were determined in 80 % acetone extracts of leaves with the spectrophotometer *Hitachi U-3300* (Tokyo, Japan) using the methods of Lichtenthaler (1987).

Experiments were repeated nine times, five times with BAP applied to substrate and four times with BAP sprayed on leaves. In each experimental set about 60 plants were used. Treated plants were always compared with control plants of the same age. Data obtained were statistically evaluated by *ANOVA* or Student's *t*-test.

## Results and discussion

During ontogeny of control bean seedlings the parameters measured in primary leaves followed a general course: increase to a maximum and then decline (for review see, *e.g.*, Čatský and Šesták 1997). Stomatal conductance ($g_s$), chlorophyll (Chl) content, and parameters of Chl *a* fluorescence kinetic increased to maximum on day 10 and then gradually decreased, while net photosynthetic rate ($P_N$) reached maximum on day 7 and then decreased to 27 % of its maximum on day 17. The earlier maximum of $P_N$ than of $g_s$ in primary bean leaves was observed also by Čatský *et al.* (1976). Application of 1 μM BAP to control plants slightly delayed leaf senescence. $P_N$, Chl content and when sprayed on leaves also parameters of Chl fluorescence [photochemical efficiency ($F_v/F_m$) and actual quantum yield of photosystem 2 photochemistry (Φ)] of treated leaves were slightly higher than those of untreated leaves on day 17 (Table 1). This effect of BAP may be connected with the observed increase in content of endogenous CK namely of isopentenyladenine and isopentenyladenosine after BAP application (unpublished results). Also Kuiper *et al.* (1990) observed increased content of endogenous CK in barley and wheat after addition of BAP into nutrient solution. Similarly Chernyad'ev (2000) observed increased Chl content in sugar beet leaves after application of BAP, and the difference between treated and control leaves increased with leaf ageing.

Table 1. Stomatal conductance, $g_s$ [cm $s^{-1}$], net photosynthetic rate, $P_N$ [$\mu g(CO_2)$ $m^{-2}$ $s^{-1}$], chlorophyll (Chl) *a+b* content [mg $m^{-2}$], and photosynthetic efficiency ($F_v/F_m$) during ontogeny of control bean plants. 1 μM BAP was sprayed on leaves on day 14 and possibility of delay of leaf senescence was checked on day 17. Means ± SE, *n* = 10 - 15.

| Age [d] | $g_s$ | $P_N$ | Chl *a+b* | $F_v/F_m$ |
|---|---|---|---|---|
| 7 | 0.97 ± 0.07 | 1110 ± 100 | 967.7 ± 35.9 | 0.79 ± 0.01 |
| 10 | 1.07 ± 0.11 | 720 ± 30 | 981.5 ± 32.9 | 0.84 ± 0.02 |
| 14 | 1.02 ± 0.07 | 430 ± 25 | 830.5 ± 45.1 | 0.80 ± 0.01 |
| 17 | 0.98 ± 0.08 | 290 ± 30 | 517.9 ± 44.0 | 0.77 ± 0.01 |
| 17 + BAP | 0.96 ± 0.09 | 340 ± 40 | 592.8 ± 41.7 | 0.84 ± 0.01 |

Table 2. Effect of water stress on relative water content, RWC [%], adaxial and abaxial stomatal conductances, $g_{ad}$ and $g_{ab}$ [cm $s^{-1}$], net photosynthetic rate $P_N$ [$\mu g(CO_2)$ $m^{-2}$ $s^{-1}$], contents of chlorophyll (Chl) *a*, Chl *b*, total carotenoids, Car [mg $m^{-2}$], photosynthetic efficiency ($F_v/F_m$), quantum yield of photosystem 2 (Φ), photochemical quenching ($q_p$), and non-photochemical quenching ($q_N$). Water stress was applied by cessation of watering for 4 d. Means ± SE, *n* = 15. Corresponding means significantly different (*P* = 0.05) are marked by different letters.

| Parameter | Control | Water stress |
|---|---|---|
| RWC | 88.5 ± $0.7^a$ | 68.8 ± $0.5^b$ |
| $g_{ad}$ | 0.35 ± $0.06^a$ | 0.08 ± $0.03^b$ |
| $g_{ab}$ | 0.67 ± $0.04^a$ | 0.09 ± $0.03^b$ |
| $P_N$ | 430.0 ± $25.0^a$ | 105.0 ± $10.0^b$ |
| Chl *a* | 639.5 ± $34.2^a$ | 591.0 ± $30.4^a$ |
| Chl *b* | 191.0 ± $11.2^a$ | 184.6 ± $9.6^a$ |
| Car | 139.0 ± $5.7^a$ | 132.8 ± $4.4^a$ |
| $F_v/F_m$ | 0.80 ± $0.01^a$ | 0.74 ± $0.01^b$ |
| Φ | 0.67 ± $0.01^a$ | 0.62 ± $0.01^b$ |
| $q_p$ | 0.92 ± $0.01^a$ | 0.88 ± $0.01^b$ |
| $q_N$ | 0.30 ± $0.01^a$ | 0.17 ± $0.01^b$ |

In control plants, relative water content (RWC) was about 90 % during the whole ontogeny of primary leaves.

During water stress induced by cessation of watering for 4 d RWC decreased to 69 %. Simultaneously, stomata on both leaf sides closed almost completely and $P_N$ decreased to 25 % of the value of control plants. However, Chl *a*+*b* and carotenoid contents decreased only slightly (to 93 and 95 %, respectively). Similarly, the parameters of Chl fluorescence were only slightly affected, *e.g.*, $F_v/F_m$ decreased to 92 % (Table 2). Therefore, this water stress can be considered as mild water stress, as stomatal limitation to photosynthesis markedly prevailed to non-stomatal limitation. Much more important stomatal than non-stomatal limitation to photosynthesis under mild water stress was observed in many plant species (*e.g.*, Berkowitz 1998, Escalona *et al.* 1999, Montagu and Woo 1999).

After rehydration, all measured parameters returned to or nearly to the level of control (non-stressed) plants of the same age. This is another prove of mild water stress, because after recovery from severe water stress many parameters remain lower than in control plants (*e.g.*, Alvino *et al.* 1999). In addition, acceleration of leaf senescence due to water stress and rehydration was not observed in bean primary leaves. On day 17, no significant differences in RWC were found between control plants and stressed and rehydrated plants. Similarly, application of 1 μM BAP in the form of irrigation or sprayed on leaves to plants of both variants did not affect their RWC. However, decreased RWC was found in plants sprayed with 10 μM BAP (Fig. 1).

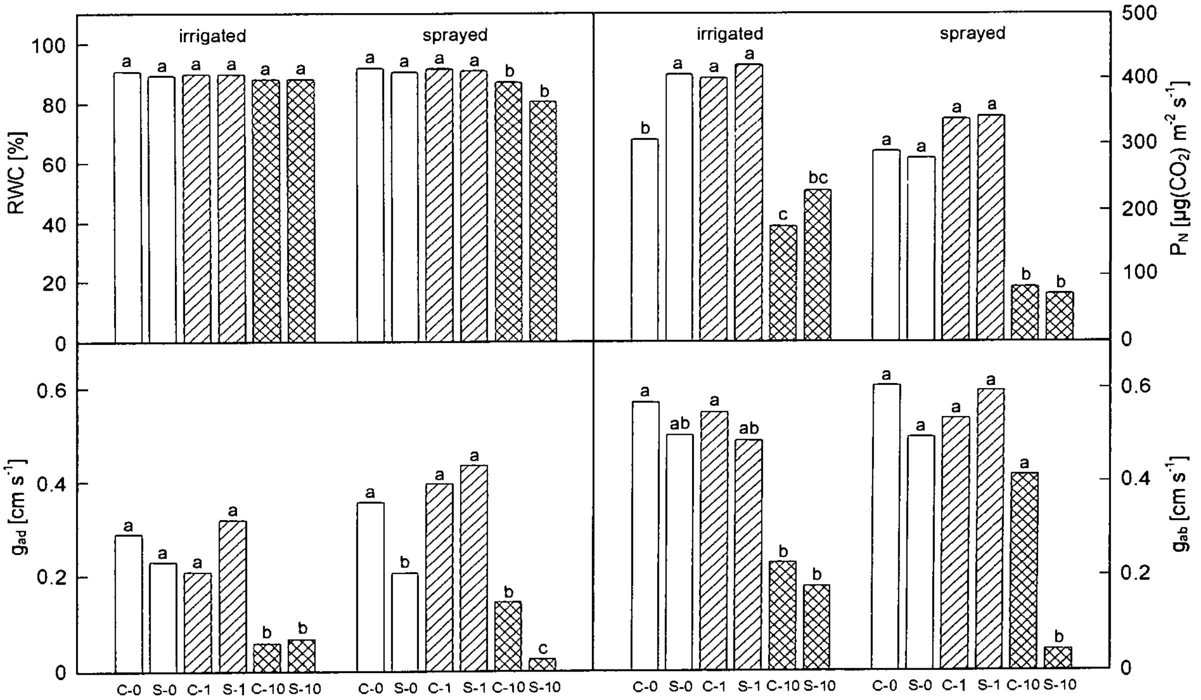

Fig. 1. Relative water content (RWC), net photosynthetic rate ($P_N$) at 600 mg(CO$_2$) m$^{-3}$, and adaxial and abaxial stomatal conductances ($g_{ad}$, $g_{ab}$) in control 17-d-old bean plants (C-0), water-stressed and rehydrated plants (S-0), and in control, and water-stressed and rehydrated plants treated by 1 μM BAP (C-1, S-1) or 10 μM BAP (C-10, S-10). BAP was applied either as irrigation or sprayed on the leaves. Means significantly different from the respective C-0 ($P = 0.05$) are marked by different letters.

Both adaxial and abaxial stomatal conductances ($g_{ad}$, $g_{ab}$) were slightly lower after water stress and rehydration than in control plants and application of 1 μM BAP to the substrate or sprayed on leaves increased them to the level of control plants (Fig. 1) or to the slightly higher level. Promotion of stomatal opening induced by application of CK was in accordance with the reviews of Incoll and Jewer (1987) and Incoll *et al.* (1990), and results of Blackman and Davies (1983), Meinzer *et al.* (1991), Morsucci *et al.* (1991), and Pharmawati *et al.* (1998). Todorov *et al.* (1998) observed increased transpiration rate in rehydrated plants treated by CK before water stress. Naturally occurring CK supplied to leaves *via* xylem increased transpiration rate and delayed senescence in wheat and oak (Badenoch-Jones *et al.* 1996). Fusseder *et al.* (1992) found promotion of $g_s$ with increased content of endogenous CK when amounts of soil water available to the plants were high. However, stomatal conductance was decreased after application of 10 μM BAP. In previous experiments with tobacco (Pospíšilová *et al.* 1993) we also found increased transpiration rate under lower concentration of BAP and decreased under higher concentration.

$P_N$ in water-stressed and rehydrated plants was similar or even higher than $P_N$ of control plants. Application of 1 μM BAP stimulated $P_N$ in both control or stressed and rehydrated plants. However, BAP at concentration 10 μM inhibited $P_N$ (Fig. 1). 1 μM BAP-induced increase in $g_{ad}$, $g_{ab}$, and $P_N$ in rehydrated plants was important for the whole plant recovery after water stress as these parameters were much more affected by water stress than other parameters measured.

Chl *a* and Chl *b* contents in rehydrated plants were similar to those in control plants. Application of 1 μM BAP delayed senescence-induced decrease in Chl content in control plants, but did not affect Chl content in stressed and rehydrated plants. After application of 10 μM BAP, Chl content was decreased (Table 3). Application of BAP ameliorated negative effect of salinity on Chl content in wheat (Mumtaz *et al.* 1997). Similarly 4-PU-30 ameliorated negative effect of water stress on Chl content in maize but did not affect Chl content in rehydrated plants (Todorov *et al.* 1998). Total carotenoid content in rehydrated plants was similar or even slightly higher than in control plants. It was not affected by application of 1 μM BAP but slightly decreased after application of 10 μM BAP (Table 3).

Table 3. Contents of chlorophyll (Chl) *a*, Chl *b*, and carotenoids [mg $m^{-2}$] in control 17-d-old bean plants (C-0), water-stressed and rehydrated plants (S-0), and in control and rehydrated plants treated by 1 μM BAP (C-1, S-1) or 10 μM BAP (C-10, S-10). BAP was applied either as irrigation or sprayed on the leaves. Means ± SE, $n$ = 10. Means significantly different from the respective C-0 ($P = 0.05$) are marked by different letters.

| | Chl *a* | | Chl *b* | | Carotenoids | |
|---|---|---|---|---|---|---|
| | irrigated | sprayed | irrigated | sprayed | irrigated | sprayed |
| C-0 | $263.4 \pm 38.0^{ab}$ | $393.7 \pm 32.7^{a}$ | $90.9 \pm 14.6^{ab}$ | $124.3 \pm 12.2^{a}$ | $93.9 \pm 5.6^{a}$ | $103.5 \pm 6.9^{ab}$ |
| S-0 | $253.3 \pm 19.2^{ab}$ | $391.4 \pm 24.1^{a}$ | $80.4 \pm 6.6^{ab}$ | $123.2 \pm 6.1^{a}$ | $103.0 \pm 8.3^{a}$ | $106.0 \pm 5.5^{ab}$ |
| C-1 | $345.1 \pm 38.0^{a}$ | $451.3 \pm 37.1^{a}$ | $106.4 \pm 10.9^{a}$ | $141.6 \pm 5.0^{a}$ | $98.9 \pm 6.9^{a}$ | $121.8 \pm 4.9^{a}$ |
| S-1 | $248.5 \pm 25.7^{ab}$ | $348.8 \pm 26.9^{ab}$ | $79.3 \pm 7.6^{ab}$ | $114.4 \pm 9.2^{ab}$ | $85.5 \pm 6.1^{ab}$ | $101.3 \pm 5.5^{ab}$ |
| C-10 | $219.6 \pm 17.0^{ab}$ | $269.3 \pm 69.6^{b}$ | $71.8 \pm 5.5^{b}$ | $88.6 \pm 16.1^{b}$ | $97.3 \pm 4.7^{a}$ | $91.6 \pm 12.1^{ab}$ |
| S-10 | $170.3 \pm 12.9^{c}$ | $267.0 \pm 51.1^{b}$ | $60.8 \pm 4.7^{b}$ | $79.0 \pm 12.4^{b}$ | $72.6 \pm 4.0^{b}$ | $78.8 \pm 6.6^{b}$ |

Table 4. Chlorophyll *a* fluorescence kinetic parameters [photochemical efficiency ($F_v/F_m$), actual quantum yield of photosystem 2 photochemistry (Φ), photochemical ($q_P$) and nonphotochemical ($q_N$) quenching] in control 17-d-old bean plants (C-0), water-stressed and rehydrated plants (S-0), and in control and rehydrated plants treated by 1 μM BAP (C-1, S-1) or 10 μM BAP (C-10, S-10). BAP was applied either as irrigation or sprayed on the leaves. Means ± SE, $n$ = 5 - 10. Means significantly different from the respective C-0 ($P = 0.05$) are marked by different letters.

| | | $F_v/F_m$ | Φ | $q_P$ | $q_{NP}$ |
|---|---|---|---|---|---|
| C-0 | irrigated | $0.75 \pm 0.007^{a}$ | $0.575 \pm 0.011^{a}$ | $0.883 \pm 0.007^{a}$ | $0.269 \pm 0.016^{a}$ |
| | sprayed | $0.77 \pm 0.006^{a}$ | $0.635 \pm 0.014^{a}$ | $0.867 \pm 0.012^{a}$ | $0.221 \pm 0.011^{a}$ |
| S-0 | irrigated | $0.69 \pm 0.013^{b}$ | $0.568 \pm 0.015^{a}$ | $0.885 \pm 0.021^{a}$ | $0.300 \pm 0.010^{a}$ |
| | sprayed | $0.75 \pm 0.006^{a}$ | $0.617 \pm 0.009^{a}$ | $0.870 \pm 0.013^{a}$ | $0.234 \pm 0.014^{a}$ |
| C-1 | irrigated | $0.74 \pm 0.011^{a}$ | $0.598 \pm 0.013^{a}$ | $0.874 \pm 0.012^{a}$ | $0.251 \pm 0.018^{a}$ |
| | sprayed | $0.84 \pm 0.014^{b}$ | $0.717 \pm 0.019^{b}$ | $0.888 \pm 0.011^{a}$ | $0.270 \pm 0.023^{a}$ |
| S-1 | irrigated | $0.69 \pm 0.012^{b}$ | $0.570 \pm 0.016^{a}$ | $0.909 \pm 0.019^{a}$ | $0.253 \pm 0.012^{a}$ |
| | sprayed | $0.79 \pm 0.011^{a}$ | $0.621 \pm 0.013^{a}$ | $0.877 \pm 0.015^{a}$ | $0.227 \pm 0.022^{a}$ |
| C-10 | irrigated | $0.76 \pm 0.006^{a}$ | $0.622 \pm 0.006^{a}$ | $0.882 \pm 0.010^{a}$ | $0.247 \pm 0.022^{a}$ |
| | sprayed | $0.74 \pm 0.018^{a}$ | $0.600 \pm 0.013^{a}$ | $0.865 \pm 0.015^{a}$ | $0.200 \pm 0.018^{a}$ |
| S-10 | irrigated | $0.70 \pm 0.021^{b}$ | $0.546 \pm 0.018^{a}$ | $0.872 \pm 0.019^{a}$ | $0.225 \pm 0.007^{a}$ |
| | sprayed | $0.77 \pm 0.020^{a}$ | $0.623 \pm 0.020^{a}$ | $0.863 \pm 0.013^{a}$ | $0.209 \pm 0.016^{a}$ |

As mentioned above, water stress did not affect markedly Chl *a* fluorescence kinetic parameters. However, in some cases photochemical efficiency ($F_v/F_m$) did not recover completely. The recovery after rehydration was not affected by application of 1 μM BAP. Actual quantum yield of photosystem 2 photochemistry (Φ), photochemical ($q_P$) and nonphotochemical ($q_N$) quenching, were not significantly different in control and stressed and rehydrated plants, and also the recovery after rehydration was not affected by 1 μM BAP. These parameters were only very slightly adversely affected by 10 μM BAP. In contrast, Metwally *et al.* (1997) found higher photochemical activity of chloroplasts isolated from control, stressed, and rehydrated bean plants sprayed with 100 $\mu$M BAP. Yordanov *et al.* (1999) observed positive effect of 4-PU-30 on $q_p$ in bean leaves during water stress.

In rice seedlings, content of endogenous CK decreased during root drying and increased after rewatering (Bano *et al.* 1993). Similar results we found in primary bean leaves and the CK content increase after rehydration was promoted by application of BAP (preliminary results).

The above results showed only slight positive effects (in terms of delay of leaf senescence or improvement of recovery during rehydration) of 1 $\mu$M BAP on parameters of water relation and photosynthesis of primary bean leaves, but negative effects of 10 $\mu$M BAP. Thus the effects of BAP were strongly dependent on concentration, but they were only very slightly dependent on the way of application. The confirmation of the above stated hypothesis needs further experiments on bean plants under more severe water stress and on another plant species.

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