

## Effect of *meta*-topolin and bohemine derived from benzylaminopurine on PSII function in artificially senescing wheat leaves

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### Introduction

Compounds with cytokinin activity (such as BA) are known to slow down chlorophyll and protein degradation and to conserve the photosynthetic function during leaf senescence. Their effect depends on concentration used, plant age and species, and is particularly distinct in case of exogenous application on detached leaves kept at dark conditions. An effect of BA on leaf senescence is relatively well described in literature. However, the molecular mechanism of its action is still largely unknown. We compared the effect of BA and its two derivatives - *meta*-topolin (with cytokinin activity) and bohemine (acting as a cyclin-dependent kinase inhibitor) on photosynthetic pigment content and photosystem II (PSII) function in wheat (*Triticum aestivum* L. cv. Hereward) leaves during artificial senescence.

### Materials and methods

Plants of winter wheat (*Triticum aestivum* L. cv. Hereward) were grown in the growth chamber at 25°C and under light regime 8 h dark/16 h light with PAR intensity of 50  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . After 7 days, in the growth phase 1.2 according to Feeke (1941), tip sections (approximately 35 mm long) were cut off from the primary leaves and immersed with the cut edge in distilled water or in a  $10^{-4} \text{ mol l}^{-1}$  solution of i) 6-benzylaminopurine (BA), ii) *meta*-topolin (mT) and iii) bohemine (boh) in wells of microtiter polystyrene plates. BA was purchased from Sigma (USA), mT (6-(3-hydroxybenzylamino)purine) was prepared by method described in Holub et al. (1998), and boh (6-benzylamino-2-(3-hydroxypropylamino)-9-isopropylpurine) was a gift from Dr. Libor Havlíček, Institute of Nuclear Medicine, Medical Faculty I, Charles University, Prague, Czech Republic. The BA, mT and boh solutions were prepared by dissolving of the substances in 0.5% dimethylsulfoxide (DMSO) and diluted by distilled water to  $10^{-4} \text{ mol l}^{-1}$  concentration. It has been confirmed that 0.5% DMSO had no significant effect on studied parameters. The plates with the segments were inserted into plastic boxes lined with paper tissues soaked with distilled water and kept at 25°C under dark conditions for 7 days to induce the artificial senescence.

The content of chlorophyll *a* and *b* and a sum of carotenoids were determined in 80% acetone according to Lichtenthaler (1987) using a spectrophotometer Lambda 40 (Perkin-Elmer, USA).

Chl *a* fluorescence was measured at room temperature with a fluorometer PEA (Hansatech, UK) and modulation fluorometer PAM 2000 (Walz, Germany), from the adaxial side of the

leaf segments. An O-J-I-P fluorescence transient (Strasser and Govindjee 1991) was measured with PEA (excitation intensity of  $6000 \mu\text{mol m}^{-2} \text{s}^{-1}$ , time of detection 2 s) and a relative height of the J step was evaluated as  $V_J = (F_J - F_0)/(F_P - F_0)$ , where  $F_0$  was the minimal Chl fluorescence intensity and  $F_J$  and  $F_P$  were intensities at J and P steps, respectively. Using PAM 2000, the  $F_V/F_M$  ratio ( $= (F_M - F_0)/F_M$ ) was determined, where  $F_M$  was maximal fluorescence intensity measured using saturation pulse of light. Induction kinetics of  $I - q_P$  ( $= 1 - (F'_M - F_t)/(F'_M - F_0)$ ) were examined after switching on the actinic PAR of intensity of about  $90 \mu\text{mol m}^{-2} \text{s}^{-1}$ .  $F'_M$  was the maximal and  $F_t$  was the actual Chl fluorescence intensity at the time  $t$  of the actinic illumination. A general statistical description (medians and quartiles) was used in a case of the Chl fluorescence parameters (Lazár and Nauš 1998).

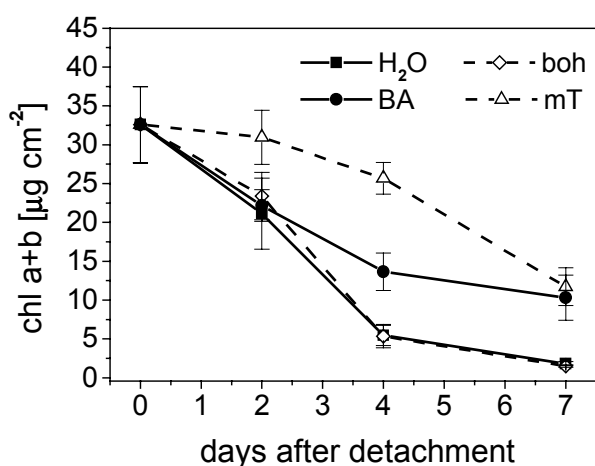
## Results

The chlorophyll content decreased with time after detachment in all the leaf segments (Fig. 1). Also the content of the sum of carotenoids decreased, but the decrease was slower than that of the chlorophyll content as the chl/car ratio decreased from about 5 to 1 during 7 days of senescence. mT and BA slowed down the decrease in both chlorophyll (Fig. 1) and carotenoid contents in the leaf segments in comparison with the segments kept in water and boh solution.

From the 4<sup>th</sup> day after detachment, an increase in a relative height of the J step ( $V_J$ ) in the O-J-I-P fluorescence transient was found in the leaf segments kept in water and boh solution (Table 1). An  $V_J$  increase is usually observed under mild stress conditions (including early stage of senescence – Matoušková et al. 1996) and it is thought to reflect an accumulation of  $Q_B$ -nonreducing reaction centres of PSII (Lazár et al. 1997). In the leaf segments kept in mT and BA solutions, the  $V_J$  increase was delayed – it was observed after 7 days of senescence (Table 1).

The  $F_V/F_M$  ratio reflecting the efficiency of PSII photochemistry (Krause and Weis 1991) decreased with time of the artificial senescence (Table 1). The lowest  $F_V/F_M$  ratio was found 7 days after detachment in the leaf segments kept in water and boh solution whereas in the leaf segments kept in mT and BA solutions the decrease was relatively milder.

Changes in the induction kinetics of  $I - q_P$  upon actinic illumination were followed during senescence of the leaf segments (Fig. 2). The  $I - q_P$  parameter reflects a proportion of the primary quinone acceptor of PSII, *i. e.*,  $Q_A$ , in the reduced state (Schreiber et al. 1986). The onset of actinic illumination brings about a rapid reduction (within 2 s) of  $Q_A$  resulting in an increase of  $I - q_P$ . As the electron transport and Calvin cycle become activated,  $Q_A^-$  is reoxidized and  $I - q_P$  decreases (Schreiber et al. 1986). In the leaf segments kept in water, a gradual retardation of the  $I - q_P$  decrease was found with time after detachment (Fig. 2) which indicated an inhibition of  $Q_A^-$  reoxidation. Similar changes were observed in the segments kept in boh. mT and BA reduced the retardation of the  $I - q_P$  decrease during senescence (Fig. 2). The effect of mT was less pronounced.



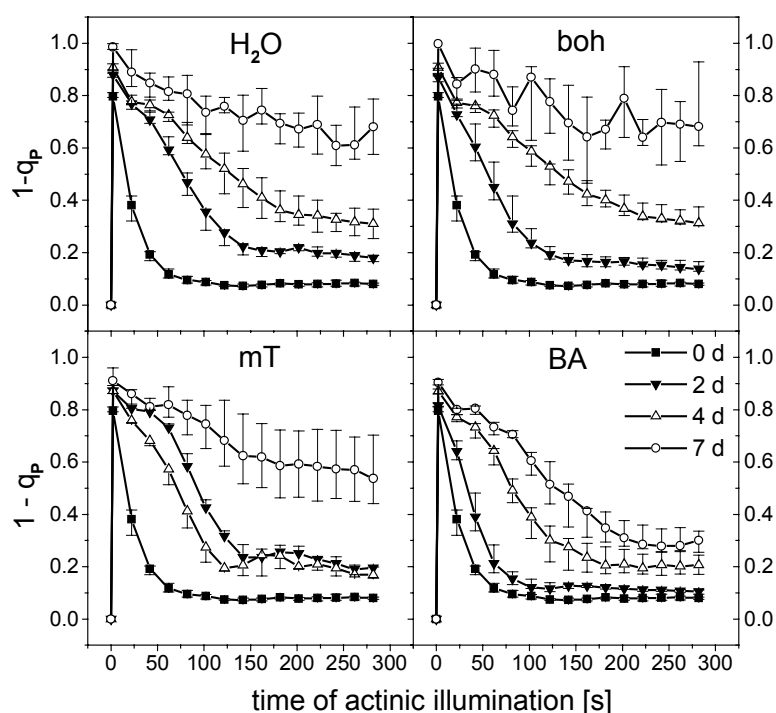
**Fig. 1** Changes in the chlorophyll content per leaf area in segments of wheat primary leaves. The segments were kept at dark in distilled H<sub>2</sub>O or in  $10^{-4} \text{ mol l}^{-1}$  solutions of bohemine (boh), 6-benzylaminopurine (BA), and *meta*-topolin (mT). Means and SD are shown ( $n=7$ ).

**Table 1.**  $V_J$  and  $F_V/F_M$  in the mature wheat leaves and in the leaf segments kept for 4 and 7 days in distilled water ( $H_2O$ ) and  $10^{-4}$  mol  $l^{-1}$  solutions of bohemia (boh), *meta*-topolin (mT), and 6-benzylaminopurine (BA). Medians and quartile-median differences (in parentheses) are shown ( $n=7-10$ ). Significant differences at  $P=0.05$  are marked by different letters.

	mature	days	$H_2O$	boh	mT	BA
$V_J$	0.48	4	0.65 <sup>a</sup>	0.68 <sup>a</sup>	0.41 <sup>b</sup>	0.43 <sup>b</sup>
	(0.01, 0.01)		(0.04, 0.07)	(0.03, 0.03)	(0.02, 0.02)	(0.07, 0.03)
		7	0.62	0.67	0.55	0.65
			(0.05, 0.06)	(0.03, 0.03)	(0.08, 0.09)	(0.06, 0.05)
$F_V/F_M$	0.793	4	0.650 <sup>a</sup>	0.681 <sup>a</sup>	0.786 <sup>b</sup>	0.751 <sup>b</sup>
	(0.002, 0.002)		(0.030, 0.030)	(0.007, 0.020)	(0.001, 0.004)	(0.016, 0.015)
		7	0.316 <sup>a</sup>	0.250 <sup>a</sup>	0.538 <sup>b</sup>	0.687 <sup>b</sup>
			(0.070, 0.060)	(0.055, 0.056)	(0.126, 0.103)	(0.017, 0.086)

## Discussion

Senescence of the wheat leaf segments kept at dark conditions in water was characterized by a rapid degradation of chlorophylls and carotenoids (Fig. 1). A degradation of photosynthetic pigments (preferentially chlorophylls) is a typical feature of leaf senescence. The  $V_J$  increase (Table 1) indicated an accumulation of the  $Q_B$ -nonreducing RCII (Lazár et al. 1997) that are not able to transfer electrons from  $Q_A^-$  to  $Q_B$ . This interpretation is supported by the retarded kinetics and increased steady-state value of  $1 - q_P$  during senescence (Fig. 2) reflecting an inhibition of  $Q_A^-$  reoxidation and a higher proportion of  $Q_A$  in reduced state, respectively (Schreiber et al. 1986). The accumulation of the  $Q_B$ -nonreducing RCII was also suggested in wheat plants senescing in darkness (Lu and Zhang 1998). The very pronounced decrease in  $F_V/F_M$  found in the leaf segments on the 7<sup>th</sup> day after detachment reflected a marked inhibition of PSII photochemistry (Krause and Weis 1991).



**Fig. 2** Changes in the induction kinetics of  $1 - q_P$  during artificial senescence of segments of the primary wheat leaves. The segments were kept at dark in distilled  $H_2O$  or in  $10^{-4}$  M solutions of bohemia (boh), *meta*-topolin (mT), and 6-benzylaminopurine (BA), and measured on the 2<sup>nd</sup> (2 d), 4<sup>th</sup> (4 d), and 7<sup>th</sup> (7 d) day after detachment. 0 d – mature leaves. Measured with a PAM 2000 fluorometer, medians and quartiles are shown ( $n=7$ ).

Both mT and BA had a positive effect on maintenance of both photosynthetic pigment content (Fig. 1) and PSII function (Table 1, Fig. 2). They slowed down the process of the artificial senescence of the wheat leaves. In the case of boh, no positive effect was found and the senescence process was not retarded in comparison with the leaf segments kept in water. Future research is desirable to reveal more details of boh action during leaf senescence.

### Acknowledgements

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