

## Stimulant Effect of Plant Activator BION on Photosynthesis and its Inhibitory Effect on Pathogenic Fungi

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### Abstract

Spraying of wheat seedlings with aqueous preparations of CGA 245704 (BION®) caused a slight decrease in chlorophyll content accompanied by the stable increase in the rate of O<sub>2</sub> evolution per chlorophyll. Stimulant effect of BION on photosynthetic activity depended on both the ai concentration and number of treatments. Biophysical methods based on the registration of slow fluorescence induction and thermoluminescence of wheat leaves revealed the stimulation of photosystem II activity and the increase in the rate of electron transport between the photosystems in treated plants. It is also shown that BION itself can show a partial fungitoxic activity because it slowed down the germination of *Erysiphe graminis* f.sp. *tritici* fungi. This is obviously the reason for partial protective effect of BION when it was applied after the inoculation of wheat seedlings with the pathogen.

**Keywords:** fungitoxic activity; luminescence; phytopathogens

### INTRODUCTION

The plant activator CGA 245704 (BION®) is known as inducer of systemic activated resistance (SAR), one of the most important natural defence mechanisms of plants (RUESS *et al.* 1995). It is considered that the compound and its metabolites exhibit no direct antifungal activity against plant pathogens tested (FRIEDRICH *et al.* 1996). At the same time, full pattern of physiological changes in plant tissues under the treatment with BION remains still unclear.

This investigation consists of two parts. In the first one, we have studied the modifications of photosynthetic apparatus in wheat leaves treated with BION. Besides traditional physiological techniques, two biophysical methods allowing to follow the photosynthetic processes *in situ* were used in the experiments. These methods were based on the registration of so-called slow fluorescence induction (SFI) and thermoluminescence (TL) of the leaves. In the second part, we have tried to discover the fungitoxic activity of BION itself.

### MATERIALS AND METHODS

Seedlings of wheat (*Triticum aestivum* L. cv. Lyubava) were grown in a greenhouse at 20–22°C under natural daylight supplemented with incandescent lamps light. Seeds were sown in small laboratory pots with a volume of 250 cm<sup>3</sup>, 10–15 seeds per each pot. Seedlings were treated with BION two or three times, with the intervals of 3 or 4 days. The first treatment was carried out at the eighth day after sowing. Granules of BION WG 50 containing 50% of active ingredient were dispersed in distilled water just before treatments. Seedlings were sprayed with the preparations containing 0.5, 1 or 5 g ai/hl until run-off.

All the measurements were carried out with the first leaves of treated plants, at the second–third day after the last treatment. Chlorophyll content was estimated by standard spectrophotometrical method; the rate of O<sub>2</sub> evolution was measured according to Warburg (GAVRILENKO *et al.* 1975). Protein content was determined by the method of LOWRY *et al.* (1951);

the content of phenolic compounds was estimated as described in ZAPROMETOV (1975). Peroxidase activity in the leaves was assayed by the method proposed in BOYARKIN (1951).

The thermoluminescence curves were recorded with the instrument described previously (PLIQUETT & SOLNTSEV 1978). At the first stage, the leaf was pre-illuminated with 725 nm red light for 1 min at room temperature to oxidize the electron carriers between photosystems I and II. Then, the sample was chilled to  $-30^{\circ}\text{C}$  and illuminated for 3 min with a saturating white light ( $30\text{ W/m}^2$ ). After illumination, the sample was rapidly cooled down to  $-70^{\circ}\text{C}$  and then heated at an average rate 30 degrees/min. The TL signal was recorded during the heating of the sample up to the temperature  $+80^{\circ}\text{C}$ .

To measure the slow fluorescence induction the leaf was initially adapted to darkness for 5 min and then exposed to wide-band blue light ( $50\text{ W/m}^2$ ). Fluorescence intensity was recorded at the wavelength  $\lambda = 686\text{ nm}$  (the maximum of chlorophyll fluorescence band).

Direct effect of BION on pathogenic fungi was studied in the experiments of two different types. In the first ones, conidia of *Erysiphe graminis* f.sp. *tritici* Maron fungi were grown at the surface of 2% hungry agar with the addition of BION solutions. Yeast *Saccharomyces cerevisiae* Meyen was used for comparison; it was grown in aqueous medium with the addition of 1 g ai/hl solution and sugar. In the second ones, wheat seedlings were inoculated with the suspension of *E. graminis* conidia, and 6 h later they were sprayed by BION preparations as described above.

Similar results were obtained in three series of experiments performed with the plants sown at intervals. The maximum error of the results presented in Tables 1 and 2 does not exceed 5%.

## RESULTS AND DISCUSSION

Spraying of wheat seedlings with aqueous preparations of BION (three treatments) caused a slight but well reproducible decrease in the chlorophyll content (Table 1). Content of protein and phenolic compounds as well as peroxidase activity in wheat leaves slightly decreased too, indicating a more accelerated senescence in BION-treated plants as compared with control ones. These data agree with the results presented in STADNIK and BUCHENAUER (1999) and WENDEHENNE *et al.* (1998). Peroxidase is known to participate in the dismutation of  $\text{H}_2\text{O}_2$  to water and to play an important role in the detoxication of  $\text{H}_2\text{O}_2$  in chloroplasts (IVANOV 1998), therefore the decreases in the peroxidase activity and in the chlorophyll content seem to be related with each other. It is interesting that the decrease in the chlorophyll content was accompanied by a well-pronounced increase in the photosynthetic activity ( $\text{O}_2$  evolution per chlorophyll, Table 1). This is obviously the result of compensatory mechanisms maintaining the intensity of photosynthesis per biomass unit.

Biophysical methods of thermoluminescence and fluorescence revealed the stimulation of PS II activity and the increase in the rate of electron transport between the photosystems in treated leaves (see below).

Table 1. Physiological and luminescent characteristics of wheat leaves treated with BION

	Control ( $\text{H}_2\text{O}$ )	Concentration (g ai per hl)		
		0.5	1.0	5.0
Content of chlorophyll, mg/(g fr wt)	0.78 (100%)	0.69 (88%)	0.69 (88%)	0.71 (91%)
$\text{O}_2$ evolution, ml $\text{O}_2$ per mg of chlorophyll in 1 h	1.60 (100%)	2.03 (127%)	1.89 (118%)	1.74 (109%)
Relative fluorescence decay ( $F_M - F_T$ )/ $F_T$ , rel. units	1.15 (100%)	1.43 (124%)	1.35 (117%)	1.26 (110%)
Relative light sum of TL band A, $S_A/S_{\text{tot}}$ , rel. units	0.41 (100%)	0.51 (125%)	0.47 (115%)	0.44 (107%)
Relative light sum of TL band C, $S_C/S_{\text{tot}}$ , rel. units	0.18 (100%)	0.13 (72%)	0.16 (89%)	0.20 (111%)
Content of protein, mg/(g fr wt)	35.9 (100%)	33.7 (94%)	31.2 (87%)	28.7 (80%)
Content of phenolics, arb units/(g fr wt)	12.5 (100%)	11.6 (93%)	10.6 (85%)	10.1 (81%)
Peroxidase activity, arb units/(g fr wt h)	14.5 (100%)	13.9 (96%)	12.9 (89%)	12.2 (84%)

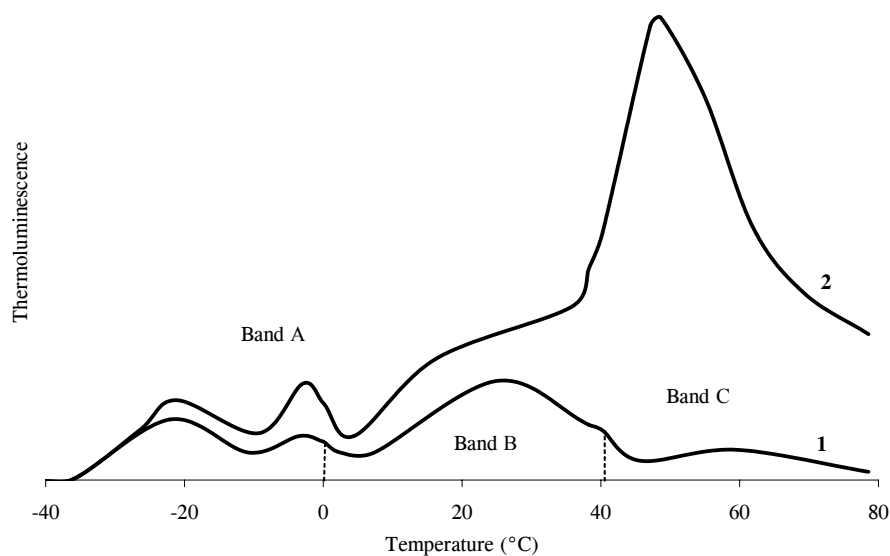


Figure 1. Typical TL curves of wheat leaves: 1 – control plants, 2 – plants treated with BION 10 g ai/hl

Typical curve of TL is shown in Figure 1. This curve is characterized by three bands, A, B, and C. Bands A and B originate from the recombination of electrons from reduced acceptors of PS II with the “holes” in the oxidized S-states in  $O_2$ -evolving system (DEMETER & GOVINDJEE 1989). Earlier, we have shown that the relative light sum of band A,  $S_A/S_{tot}$ , correlates with the photosynthetic activity (YURINA *et al.* 1992). The treatment of plants with BION caused the increase in the TL band A (Table 1); this result is in accordance with the data obtained by the method of fluorescence induction. Band C is supposed to reflect the destruction of chloroplast membranes during freezing of the samples; the intensity of this band was shown to increase under unfavorable growth conditions (SOLNTSEV 1989). Thus, the increase in  $S_C/S_{tot}$  with ai concentration (Table 1) indicate the progressive decrease in membranes stability.

The typical pattern of the SFI curve is presented in Figure 2. Fast rise of the leaf fluorescence up to the maximal value  $F_p$  is due to the reduction of primary electron acceptors of PS II. Slow decrease  $F_p \rightarrow F_T$  is supposed to be associated with a number of the regulatory processes of photosynthesis. In general,

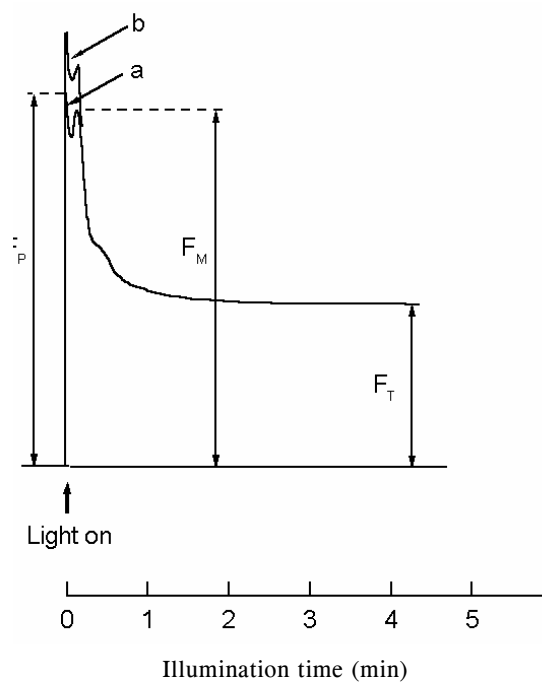


Figure 2. Typical pattern of slow fluorescence induction in wheat leaves: a – control plants, b – plants treated with BION (5 g ai/hl)

Table 2. Antifungal properties of plant activator BION in experiments *in vitro* and *in vivo*

	Control ( $H_2O$ )	Concentration (g ai/hl)		
		0.5	1.0	5.0
Length of <i>Erysiphe graminis</i> hyphae ( $\mu m$ )	43 (100%)	40 (93%)	38 (88%)	24 (56%)
Mean % of infected leaf area	90	87	75	56

this decrease is caused by photochemical quenching (partial oxidation of the primary electron acceptors in PS II under low light illumination), and the non-photochemical one (redistribution of excitation energy from PS II to PS I; generation of the proton gradient,  $\Delta pH$ , across the thylakoid membrane; and non-radiative energy dissipation under high light illumination) (KRAUSE & WEISS 1991; LAZAR 1999). Earlier, we showed that the relative fluorescence decay, ratio  $(F_M - F_T)/F_T$ , was correlated with the photosynthetic activity (KARAVAEV *et al.* 1998). The treatment of plants with BION caused the increase in  $(F_M - F_T)/F_T$  ratio (Table 1) due to the enhancement of the  $F_M$  values (Figure 2). The increase in the level of  $F_M$  can be associated with lower  $\Delta pH$  values as the result of more active ATP synthesis at the first seconds of illumination. An active synthesis of ATP leads to the increase both in the rate of electron transport and in the total photosynthetic activity.

It must be noted that all the above mentioned changes depended both on the ai concentration (Table 1), number of treatment, and season. In particular, in one of the experiments with two treatments (spring-time) we failed to register the decrease in protein and

phenols content. At the same time, the increase in photosynthetic activity in treated plants was significantly higher and equal to about 150% as compared to control plants (data not shown).

In the second part of our work, we have demonstrated that plant activator BION can reveal the fungitoxic activity itself. It slowed down both the germination of parasitic fungi *E. graminis* (Table 2), and the growth of the yeast *S. cerevisiae* (Figure 3). Direct inhibition of pathogenic fungi is obviously the reason for partial protective effect of BION, when it was applied after the inoculation (Table 2).

### Conclusion

Thus, we conclude that BION can produce a stimulant effect on the photosynthetic activity of wheat seedlings. Other physiological characteristics registered in our model experiments could not reveal any stimulant effect of BION on the metabolism of healthy plants. However, this is not in discrepancy with a well-recognized role of BION as SAR inducer.

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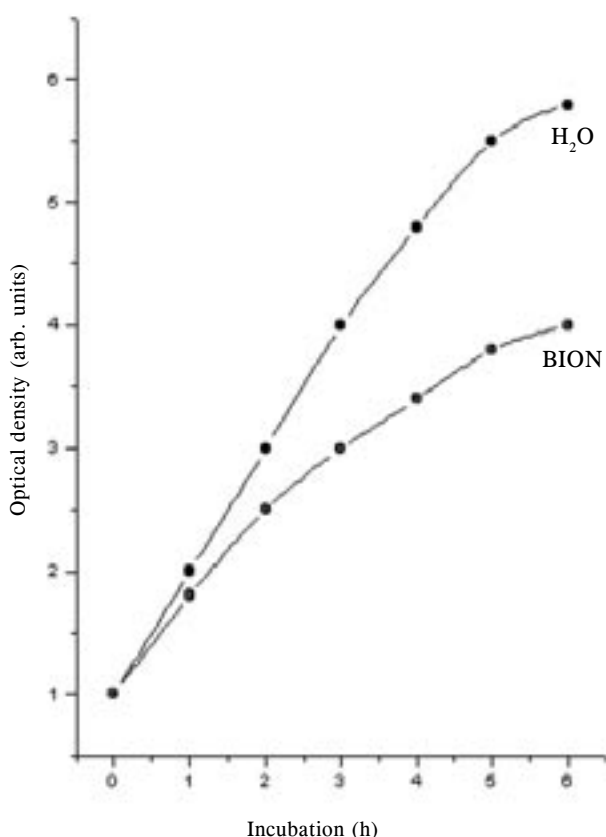


Figure 3. Impact of plant activator BION on the growth of *Saccharomyces cerevisiae*

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