# Increased Synthesis of Photosystem II in *Triticum vulgare* when Grown in the Presence of BAS 13-338

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Addition of BAS 13-338 (4-chloro-5-dimethylamino-2-phenyl-3(2H)-pyridazinone) to a suspension of chloroplast thylakoids caused an increase in the $I$ level of chlorophyll fluorescence induction without affecting the $F_0$ level and with a slight decrease in the $F_{max}$ level in a manner similar to the addition of DCMU to a thylakoid suspension. Addition of BAS 13-338 also inhibited the rate of Hill reaction $H_2O \rightarrow$ dichlorophenol indophenol with 50% inhibition occurring at about 10 μM BAS 13-338. The inhibition was not reversed by diphenyl carbazide used as an artificial electron donor to photosystem II. These results suggest that the site of inhibition by BAS 13-338 is between Q (next to the primary electron acceptor) and plastoquinone.

When the plants were grown in the presence of sublethal dose of BAS 13-338, the following changes were noted in the thylakoids of the treated plants as compared to the thylakoids isolated from the control plants: The $F_0$ and the normalized variable fluorescence ($\Delta F/F_0$) levels increased, chlorophyll $a/b$ ratio decreased, chlorophyll/P700 ratio increased. Furthermore, the rate of photosystem II electron transport both under saturated intensity and the limiting intensity of illumination increased, and the ratio of plastoquinone to Q decreased. These observations have been interpreted as due to an increase in the ratio of photosystem II to photosystem I in plants grown in the presence of BAS 13-338.

## Introduction

Substituted pyridazinone compounds have received considerable attention in recent years in the study of chloroplast development and function ([1] for review). Among various substituted pyridazinones known to have interaction with plant systems, 4-chloro-5-dimethylamino-2-phenyl-3(2H)-pyridazinone or BAS 13-338 (also known as SAN 9785) needs special mention because of its maximum ability in increasing the ratio of saturated to unsaturated fatty acids with no or very little change in the pigment content in the plants grown in the presence of pyridazinone compounds [2, 3]. This property of BAS 13-338 (hereafter mentioned as BAS for simplicity) offers a potential of inducing heat resistance in plants. However, before attempting to test this potential, it is necessary to examine other changes, if any, induced in the structure and function of the photosynthetic apparatus by BAS. This compound is also known as an inhibitor of photosystem II electron transport, although the site and the mode of action are not known clearly [4, 5]. Both of these aspects are dealt with in this communication.

*Abbreviations:* FeCN, potassium ferricyanide; DAD, diaminodurene; DCIP, dichlorophenol indophenol; DCMU (diuron), 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; PS, photosystem (II or I); Q, quencher (= primary electron acceptor of PS II); PQ, plastoquinone; Chl, chlorophyll ($a + b$).

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## Methods

The seeds of *Triticum vulgare* (CV: Pelisser Durum wheat) were surface sterilized with 0.1% mercuric chloride and seedlings were raised in glass petri plates (15 cm diameter) over three layers of crude filter papers. Various concentrations of BAS were prepared and 20 ml of a given solution was added once to a petri dish. Thereafter, watering was done only with distilled water. The seedlings were grown at room temperature under white cool illumination of 4000 lux. Chloroplasts were isolated according to Brewer *et al.* [6]. PS-II activity was measured spectrophotometrically following Darr *et al.* [7]. Chl/P700 ratio was estimated according to Mullet *et al.* [8]. Analysis of fluorescence induction was done according to Brewer *et al.* [6].

## Results

When BAS was added to a chloroplast thylakoid suspension, the rate of Hill reaction of $H_2O \rightarrow$ DCIP, was inhibited in a manner shown in Fig. 1. The 50% inhibition of the rate occurred at about 10 μM BAS. The rate was not restored upon addition of diphenylcarbazide to the assay medium. The action of BAS as measured by fluorescence induction kinetics is shown in Fig. 2. Addition of BAS caused no increase in the $F_0$ level, but a marked increase in the $I$ level occurred. The $F_{max}$ level did not increase, rather, it decreased to some extent with increasing concentrations of BAS. A similar decrease with 5 μM DCMU was observed in these experiments as well as by others [9]. When the increase in $I$ level was expressed as $(\Delta F - \Delta I)/\Delta F$ and plotted as a function of concentration of BAS 13-338, the graph obtained was remarkably similar and overlapping with the inhibition curve of the Hill reaction (Fig. 1). In fact, when the percent decrease of $(\Delta F - \Delta I)/\Delta F$ was plotted as a function of percent rate of Hill reaction, an almost perfect correlation was observed (Fig. 2). This observation is similar to the earlier observation reported [6] that $(\Delta F - \Delta I)/\Delta F$ decreases linearly with the decrease in Hill reaction when atrazine (a symmetric triazine herbicide) was added to thylakoid suspension of *Amaranthus*. This similarity between DCMU and BAS effects implies that the site of inhibition by BAS lies between Q and plastoquinone. These observations also show that inhibition of $(\Delta F - \Delta I)/\Delta F$ can be monitored as a measure of inhibition of Hill reaction.

When plants were grown in the presence of various concentrations of BAS and the thylakoids were isolated from leaves of two week old seedlings the chlorophyll fluorescence kinetics were remarkably different from that of the control thylakoids (Fig. 3). First, the $F_0$ value increased markedly by 100% at 200 μM BAS treatment. The variable fluorescence $\Delta F$ increased more than the $F_0$ as shown in

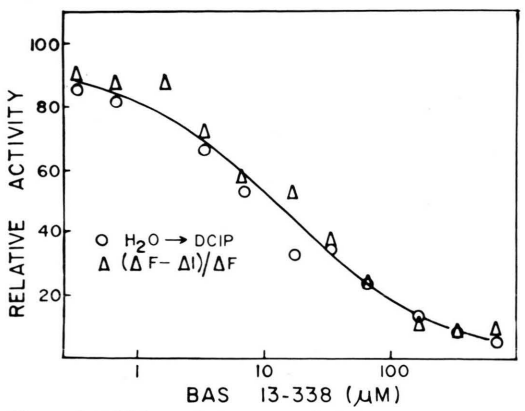


Fig. 1. Inhibition of the rate of electron transport and of $(\Delta F - \Delta I)/\Delta F$ as a function of BAS 13-338 concentration. Chloroplast thylakoids were isolated as described in Methods and BAS 13-338 was added to the assay medium to a given concentration prior to illumination. For assay conditions see Methods. $F_0$ is the ground level fluorescence intensity, $I$ is the intermediate level of fluorescence as determined in [6], $F_{max}$ is the maximum fluorescence intensity. $\Delta F = F_{max} - F_0$ and $\Delta I = I - F_0$.

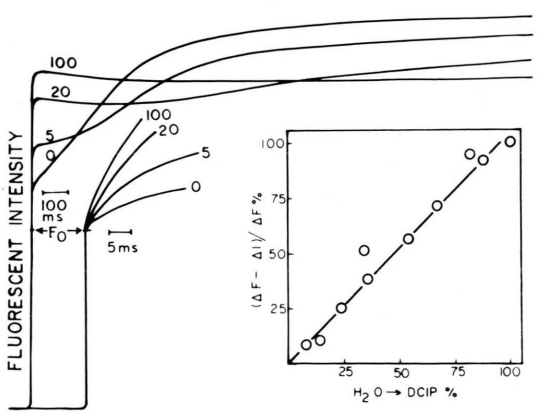


Fig. 2. Chlorophyll fluorescence induction curves in isolated chloroplast thylakoids in the presence of various concentrations of BAS 13-338. The function $(\Delta F - \Delta I)/\Delta F$ was estimated from induction curve as described in [6] and plotted against the rate of electron transport (inset) for a given concentration of BAS 13-338 (Fig. 1).

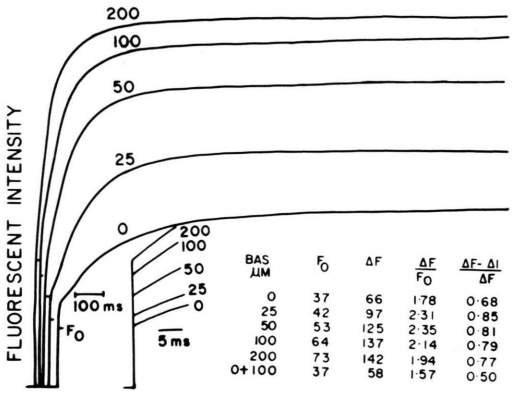


Fig. 3. Chlorophyll fluorescence induction curves in chloroplast thylakoids isolated from plants grown in the presence of various concentrations of BAS 13-338. For assay conditions see Methods.

the normalized variable fluorescence ($\Delta F/F_0$). The value of $\Delta F/F_0$ increased from 1.78 in the control to 2.35 at 50 μM BAS treatment and decreased to 1.94 at 200 μM. Second, the value of $(\Delta F - \Delta I)\,\Delta F$ also increased from 0.68 in the control to 0.85 at 25 μM BAS). As expected, addition of 100 μM BAS to the control did not increase any of these three parameters (last row in the table of Fig. 3).

When the area above the fluorescence induction curves in the presence of 5 μM DCMU was analyzed in thylakoids isolated from the control and the treated plants, the relative ratio of PQ to Q was found to be less in the thylakoids of treated plants (Table I). Since the amount of PQ per chlorophyll did not change in treated samples (data not shown), results indicated a 36% increase in the amount of Q in the thylakoids of treated plants. Studies in the Hill reactions showed that the rate of electron transport in the thylakoids of treated plants was higher than the control rate. Table II shows that in case of $H_2O \rightarrow$ DCIP Hill reaction about 30–40% increase in the light-saturated rate in the light-limited rate occurred in thylakoids of plants treated with 50 μM BAS. A similar increase in the rate was observed with DAD (+ FeCN), and DAD (+ FeCN) + DBMIB mediated Hill reactions. Table I also shows that the chlorophyll *a*/*b* ratio in these thylakoids decreased markedly; the degree of decrease depended on the concentration of BAS in the growth medium. The chlorophyll content in leaf (area or fresh weight basis) decreased only marginally (< 10%).

Table I. Effect of BAS 13-338 treatment on chlorophyll content, Chl *a*/Chl *b* ratio and PQ/Q ratio.

| Treatment | Total Chl [mg/mm²] | Chl *a*/ Chl *b*-ratio | PQ / Q | Chl / P700 |
|---|---|---|---|---|
| Control | 2.80 | 3.26 | 10.8 | 263 |
| 50 μM | 2.67 | 2.55 | – | 424 |
| 100 μM | 2.53 | 2.32 | 8.0 | 519 |

## Discussion

A decrease in the Chl *a*/*b* ratio and an increase in the chlorophyll fluorescence yield are generally regarded as a consequence of an increase in the unit size of PS II and commonly referred to as shade-plant characteristics. An increase in the Chl/P700 ratio is an obvious consequence of the increase in the unit size of PS II. It should be noted, however, that all of these observations can alternatively be interpreted in terms of an increase in the number of PS II units. Further observations, namely, an increase in the rate the PS-II electron transport measured under high light intensity and an increase in Q/PQ ratio exclude the former possibility and support the latter. In the case of an increase in the unit size alone, the Q/PQ ratio should not change and the light-saturated rate (on chlorophyll basis) of PS II should decrease. Another observation (to be reported elsewhere) that the $I_{50}$ value DCMU inhibition of PS-II Hill reaction is increased (by about 40%) in the treated plants is another evidence supporting this conclusion. Since the chlorophyll content is not affected on leaf area (or fresh weight) basis, the increase in the number of PS II units has occurred not only at the thylakoid level but also in the leaf as a whole.

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Table II. Effect of BAS 13-338 treatment on PS-II electron transport rates with different electron acceptors. The rates are expressed as μmoles of acceptor reduced/mg Chl × hr.

| Treatment | $H_2O \rightarrow$ DCIP | | $H_2O \rightarrow$ DAD FeCN | $H_2O \rightarrow$ DAD DBMIB + FeCN |
|---|---|---|---|---|
| | Saturating light intensity | Limiting light intensity | | |
| Control | 229 | 40 | 256 | 69 |
| 50 μM | 300 | 55 | 355 | 100 |
| 100 μM | 255 | 50 | 387 | 108 |

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