THE CYTOPLASMIC FACTOR IN ATTENUATING THE ULTRAVIOLET RADIATION INDUCED INHIBITION OF PHOTOSYNTHESIS IN ISOLATED LEAF CELLS

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ABSTRACT

The level of inhibition by ultraviolet-C (UV-C) radiation on Hill activity of mesophyll cells isolated from a variety of plant species differ markedly. Such variation on the degree of inhibition appears to be related to the presence of UV absorbing compounds. Spectral analysis of the cell extract indicate that the UV resistant plants contain more of UV absorbing compounds in the cytoplasm.

INTRODUCTION

THE mechanism of ultraviolet-C (UV-C) inhibition of photosynthetic reaction has been the subject of a number of investigations[1]. Several *in vitro* experiments clearly indicate UV-C irradiation brings about rapid inactivation of Hill reaction by acting at various sites in the electron transport chain[2-6]. Murphy *et al*[7] have reported that some cells of *Rosa damascena* in cultures develop unusual resistance to UV radiation. Such resistance was attributed to production of large quantities of UV absorbing compounds. This paper describes variations in the extent of UV-C inactivation of mesophyll cells isolated from divergent group of plants and the possible factor producing such variations.

EXPERIMENTAL

Fresh young leaves of *Phaseolus mungo L., Musa paradisiaca L., Zinnia elegans* Jacq. and *Ipomaea pentaphylla* Jacq. were collected from the field grown plants. Mesophyll cells were isolated by mechanical grinding according to the method of Gnanam and Kulandaivelu[8]. Type II broken chloroplasts were isolated as described earlier[9].

Mesophyll cells and/or chloroplasts were suspended in a medium containing 400 mM sucrose, 10 mM NaCl, 5 mM $MgCl_2$ and 20 mM Tris-HCl, pH 7.8 at a final chlorophyll concentration of 100 μg/ml. The suspension was uniformly spread in a petri dish (thickness of the suspension was about 1 mm) and exposed to UV-C radiation from a Phillips 15 W germicidal lamp. The energy fluence rate was 26 W/m^{-2}. To prevent the loss of photosynthetic activity due to aging, the temperature during irradiation was maintained at $5 \pm 2°$ C. Control samples were covered with aluminum foil and treated in the same way.

The rate of O_2 evolution was continuously monitored with a YSI O_2 electrode at 28 ° C under red light (> 620 nm RG 620, Schott). Fluence rate at the sample surface was 100 W/m^{-2}. The basal reaction mixture contained 20 mM Tris-HCl, pH 7.8, 400 mM sucrose, 10 mM NaCl, 5 mM $MgCl^2$ and 2 mM *p*-benzoquinone (BQ).

RESULTS AND DISCUSSION

Changes in the rate of Hill activity ($H_2O \rightarrow$ BQ) were followed after UV-C treatment in mesophyll cells isolated from divergent group of plants to understand their relative tolerance to radiation. Under identical conditions of UV-C treatment, leaf cells from divergent plants exhibited large variation in the degree of inactivation (figure 1a). While complete inactivation of O_2 evolution had occurred in the cells of *Phaseolus* in 20 min, approximately 3-fold longer duration of UV-C-treatment was required for *Ipomaea*. Contrary to this when the chloroplast preparations of these plants were exposed to UV radiation no variation in the degree of inactivation was seen (figure 1b). This clearly indicate that the variations observed in UV sensitivity in mesophyll cells of different plants are due to some factor present in the cytoplasm.

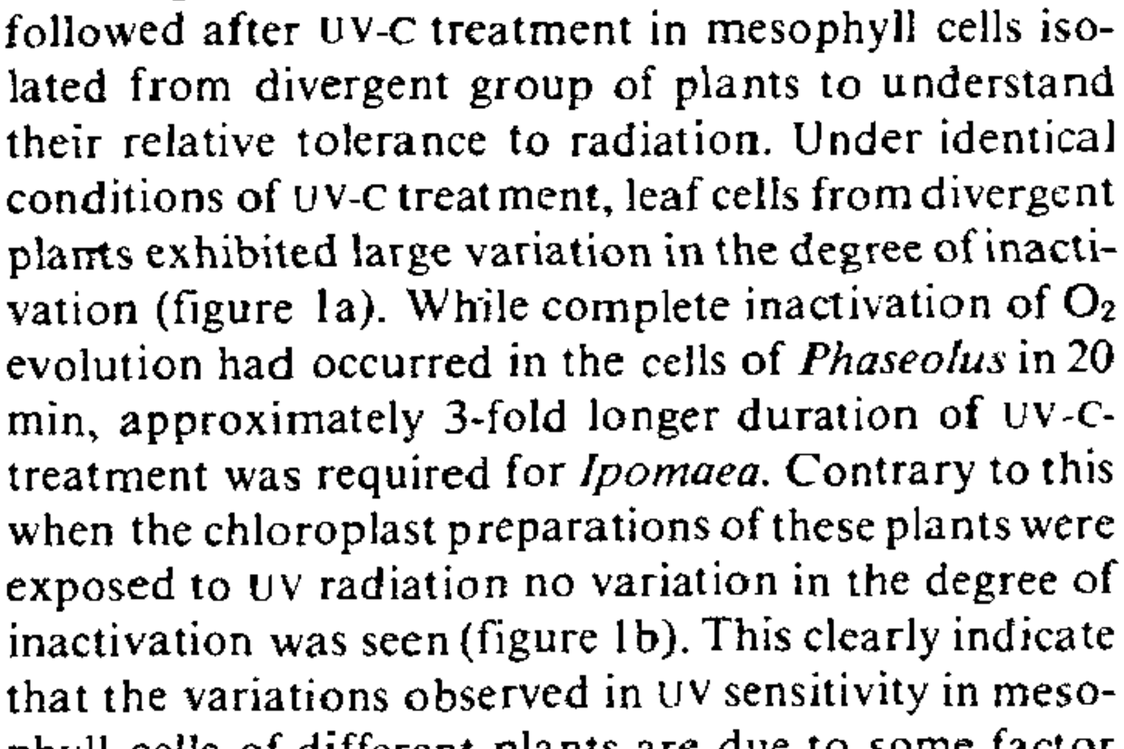

Figure 1. Relative sensitivity of photosynthetic O_2 evolution ($H_2O \rightarrow$ BQ) to UV-C irradiation in mesophyll cells (a) and chloroplasts (b) isolated from different plants. For other details see method. The 100% levels in μmoles O_2. mgChl. hr are: (Δ) *Phaseolus*, 230; (●) *Musa* 180; (□) *Zinnia*, 190 and (○) *Ipomaea* 245. Average of 3 measurements.

It has been demonstrated that cultured cells of *Rosa damascena* develop unusual resistance to UV-C radiation which was attributed to the production of UV absorbing pigments[1]. In order to find out if the difference in resistance to UV-C irradiation observed in the mesophyll cells of different plant species is due to any change in the level of UV absorbing compounds, absorption spectra of the aqueous cell extracts from the mesophyll cells showing the highest (*Ipomaea*) and lowest (*Phaseolus*) UV resistance were taken in the UV region. The spectra clearly reveal that the cell extract of *Ipomaea* contains relatively more of UV absorbing compounds. This is further strengthened by the difference (*Ipomeae–Phaseolus*) spectrum obtained from the cell extract.

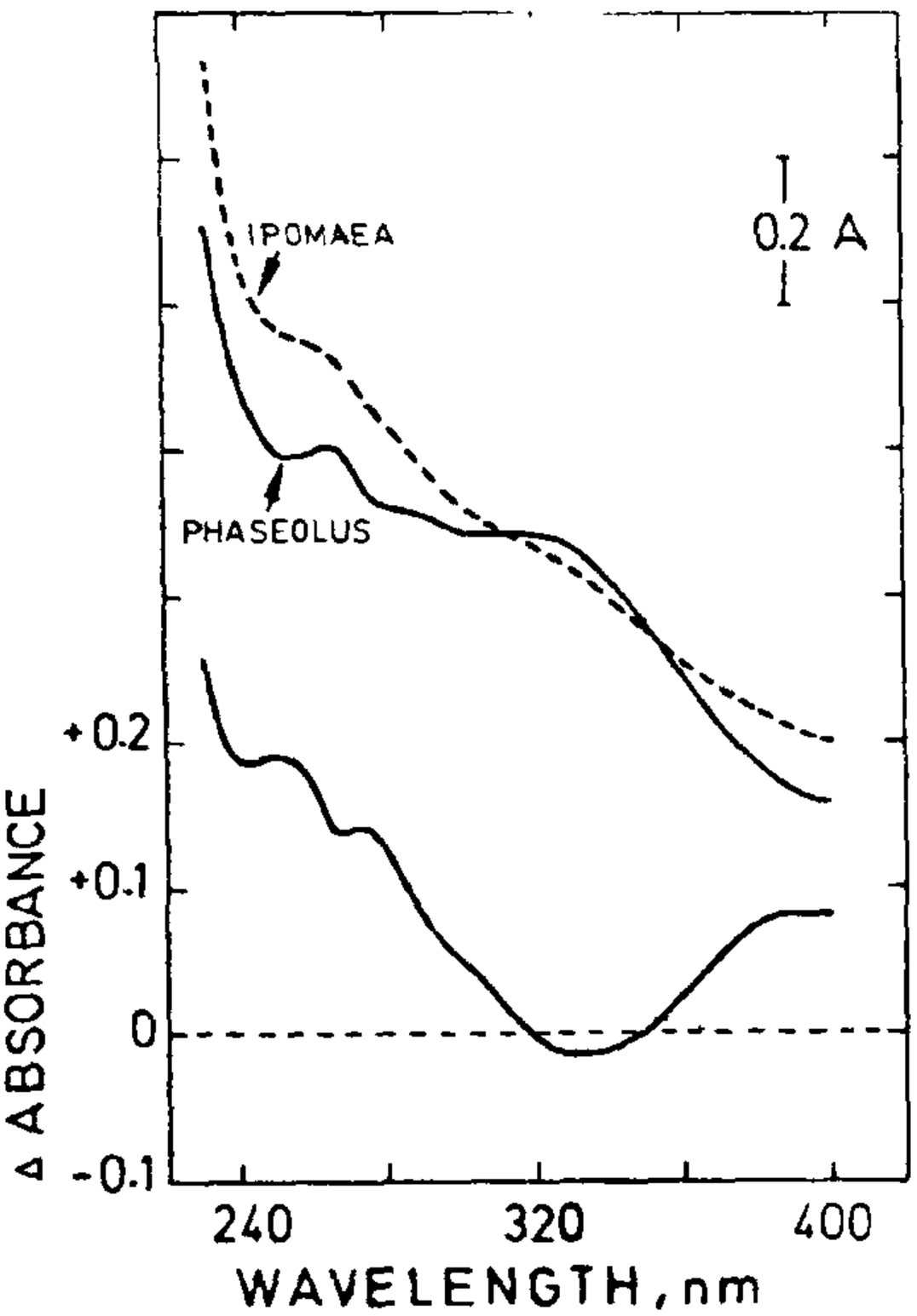

Figure 2. Absorption and difference spectra of the aqueous extrats of *Ipomaea* and *Phaseolus* mesophyll cells. The concentration of both extracts was adjusted at 340 nm. For difference spectrum (*Ipomaea* minus *Phaseolus*) the absorbance scale was amplified by 2-fold. Spectra were recorded in Hitachi 557 spectrophotometer.

The presence of phenolic compounds in plants is known to act as strong absorbers of UV radiation. Hence the contrasting difference in UV resistance observed between *Ipomaea* and *Phaseolus* could be the result of higher content of phenolic compounds in the cytoplasm. Further work is underway to identify the UV absorbing compounds in these plants (figure 2).

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