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SHORT COMMUNICATION

PREPARATION OF ACTIVE CHLOROPLASTS FROM *PINUS PINEA*

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Abstract—The preparation of photoreducing chloroplasts from *Pinus pinea* is described.

AS FAR as we know, preparations of photoreducing chloroplasts have been obtained only from angiosperms and from algae.[1-3] Nothing has been reported on their preparation from conifers. For this reason we have examined the preparation[4] of active chloroplasts from cotyledons of forty-day-old seedlings of *Pinus pinea*. The chloroplast preparations obtained showed photoreducing activity against NADP (with ferredoxin), cytochrome C and indophenol. The results with NADP are shown in Fig 1. It can be seen that with our preparation there is a direct ratio of the change in absorptivity with the time of illumination and/or quantity of chloroplast suspension added to reaction mixture. Similar results were obtained with the other oxidants. The presence of chloroplasts in the suspension was confirmed by e.m. micrographs.

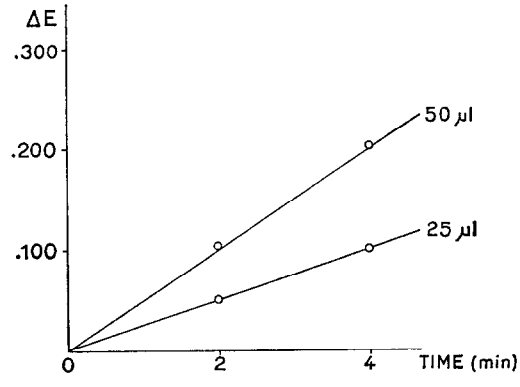

FIG. 1. REDUCTION OF NADP BY *Pinus pinea* CHLOROPLASTS IN PRESENCE OF *Pinus pinea* FERREDOXIN.

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EXPERIMENTAL

Cotyledons from seedlings of *Pinus pinea*, 40 days old, germinated on moistened sand at room temperature (about 22°) and illuminated by day-light (experimental period March–April) were used as source material. Chloroplasts were prepared according to the method of Davenport,[4] using a MSE homogenizer for 30 sec at 0°. The photoreducing activity of chloroplasts obtained in this way, was determined by their ability to reduce, after illumination, NADP, cytochrome C and 2,6-dichlorophenol indophenol according to the method of Jagendorf,[5] at 366 nm, 546 nm and 578 nm respectively.[5-7] For the photoreduction of NADP we used ferredoxin (protein content 1·8 mg/ml) prepared from *Pinus pinea*.[8] The composition of reaction mixture in a final volume of 1·5 ml was: chloroplast suspension 25 or 50 μl; 16 μmoles $MgSO_4$; 100 μmoles TRA buffer pH 7·8; 0·3 μmoles NADP or 0·2 μmoles cytochrome C or 0·1 μmoles 2,6-dichlorophenol indophenol. In the NADP reduction experiments 20 μl of ferredoxin were added. The illumination system for the photochemical activity measurements was that described by Davenport.[4] The chlorophyll content in the chloroplast suspensions (1·95 mg/ml) was determined, after extraction with 80 per cent acetone, according to MacKinney.[9]

Chemicals

TRA (Triethanolamine-hydrochloride, buffer), NADP and cytochrome C were purchased from C. F. Boehringer and Soehne G.m.b.H., Mannheim, Germany. 2,6-dichlorophenol indophenol was a Merck product. All other chemicals were from commercial sources.

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