STUDIES ON ISOLATED CHLOROPLAST IN SPINACH (*SPINACIA OLERACEA*): EFFECT OF CHANGE OF pH, TEMPERATURE AND CARBOHYDRATE METABOLITES ON THE RATE OF PHOTOSYNTHETIC ELECTRON TRANSPORT*

Introduction

THE carbohydrate content of the plant changes from season to season and from one plant to another[1,2]. This variation may be related directly to the change in the rate of photosynthesis[3,4]. An indirect correlation between the rate of photosynthesis and the level of carbohydrate metabolites in leaves was also reported[5,6]. Yamashita and Butler[7] have shown that the chloroplast activity may be inhibited by washing with higher concentration of Tris buffer and by heat treatment or exposure to U.V. light. In this communication, the influence of the pH, temperature and carbohydrates or their metabolites on the rate of photosynthetic electron transport in spinach chloroplast is reported.

Materials and Methods

Isolation of Chloroplast

Spinach (*Spinacia oleracea*) having soft tissue was chosen and the chloroplast was isolated by a modified method of Gee *et al*[8]. About 20 g of depetiolated, deveined leaves were taken in 100 ml of isotonic 0·05 M Tris-HCl buffer (pH 7·6) and homogenized for 6 sec, after a 5 sec gap, it was again homogenized for 6 sec. The homogenate was strained through 4 layers of fine voile cloth and the filtrate was centrifuged at 250 × g for 60 sec at 4° C and the supernatant was centrifuged again at 2,500 × g for 60 sec at 4° C. The supernatant was discarded and the residue was washed with 0·05 M Tris-HCl (pH 7·6) buffer and again centrifuged for 60 sec at 4° C. The residue containing chloroplast was extracted into the buffer. The chloroplast was subjected to microscopic examination for their intactness after their isolation. The chloroplast preparation was tested for oxygen evolution by Warburg apparatus[9] and for the activity of succinate dehydrogenase[10] to ensure that the preparation is free from mitochondrial contamination.

Absorption of Chloroplast

The chloroplast concentration was measured in terms of the chlorophyll by the method of Parsons and Schapiro[11]. The absorption of chloroplast with varying pH was recorded in SP 700 A Pye Unicam spectrophotometer.

The Rate of Photosynthetic Electron Transport

The rate of photosynthetic electron transport was studied on the reduction of 2,6-dichlorophenolindophenol as described by Vishniac[12]. 7·0 ml of 200 μM DPIP (pH 7·6) and 1·0 ml of chloroplast (100 μg chlorophyll/ml) were taken and the per cent transmission was measured at 620 nm after adjusting the chloroplast blank to 100% transmission. The sample was illuminated by 150 W 'Philips lamp' (250 V) from a distance of 4·5 cms and the precaution was taken to prevent heat radiations. The per cent transmission was recorded 6 times after an exposure of every minute. The influence of pH (7·2 to 8·4), the temperature (20° C to 40° C) and the different metabolites (0·0125 M) on the photosynthetic electron transport was studied.

Results and Discussion

Microscopic studies showed that the intactness of the chloroplast preparation varied from 75–80%. Photosynthetic oxygen evolution was shown by Warburg apparatus $Q_{O_2}^{Chl} = 200 \pm 21{\cdot}5$ mm³ of oxygen evolved/hr/mg chlorophyll. Absence of the activity of succinate dehydrogenase showed that the chloroplast preparation is free from mitochondrial contamination.

The absorption of chloroplast at λ 432 and λ 675 is maximum at pH 7·6. The rate of photosynthetic electron transport is also found to be maximum at pH 7·6. The inhibition of the system due to change of pH fell off rather sharply on the alkaline side as compared to a more gradual decline on the acid

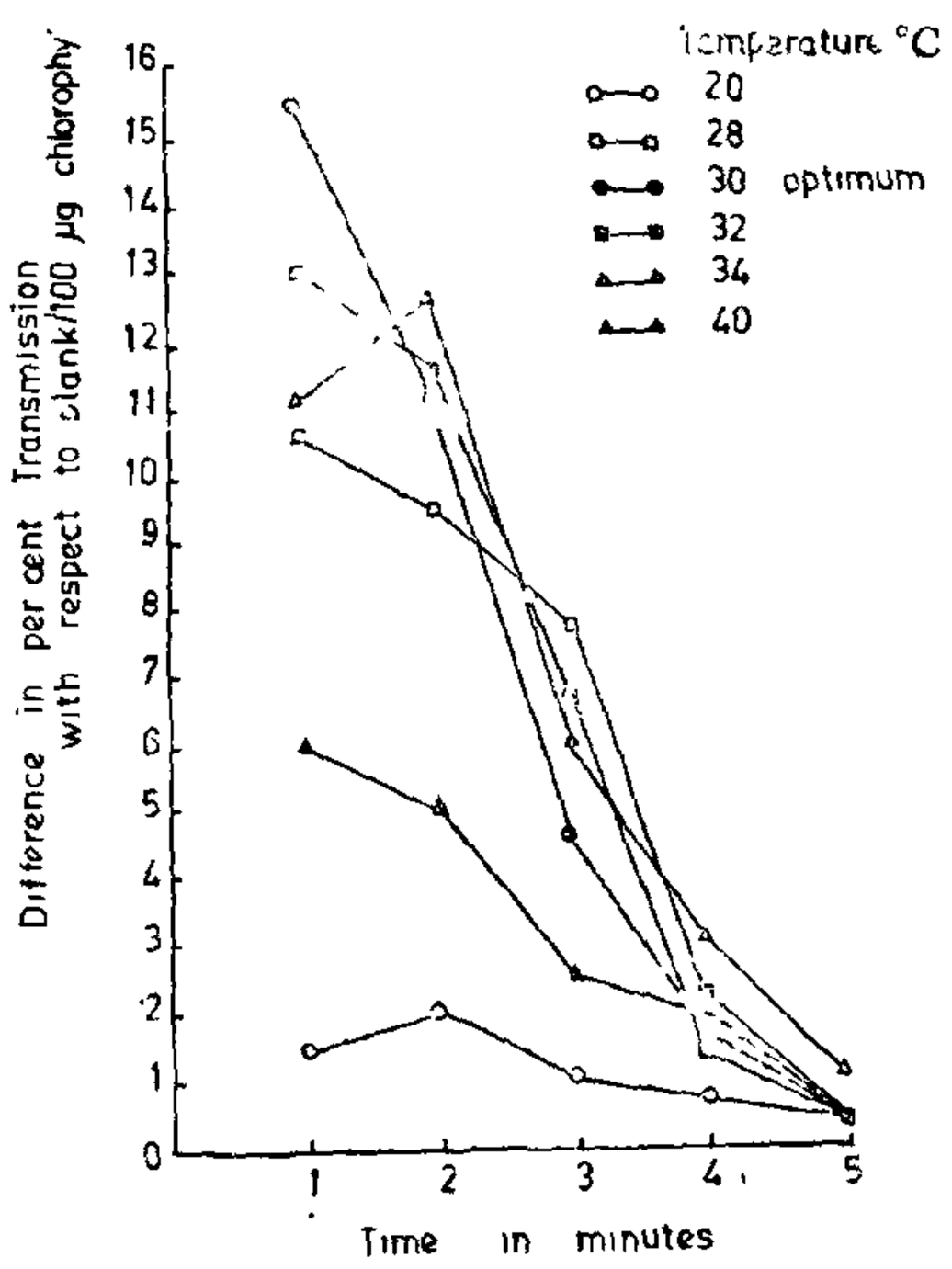

FIG. 1. Effect of temperature on the reduction of 2,6-dichlorophenolindophenol by isolated chloroplast from Spinach.

side. The rate at pH 7·2 and 7·4 was almost similar to that of pH 7·6, The pH optimum agrees well with the pH of the extract of the leaves. The maximum absorption of chloroplast at pH 7·6 may be related to the photosynthetic electron transport activity in normal plants. Similar influence of hydrogen ion concentration on CO_2 fixation was reported by Werden *et al*[13].

The change of temperature also changes the rate of photosynthetic electron transport. Figure 1 shows the reduction of DPIP by chloroplast is maximum at 30° C. It is greatly reduced at temperatures beyond 34° C and less than 28° C.

Figure 2 shows the inhibition of photosynthetic electron transport due to different carbohydrate metabolites (0·0125 M) and the maximum inhibition was found to be due to citrate. The other metabolites inhibit the reaction in the following order: citrate, tartrate, sucrose and fructose. Neales and Incoll[5] have reported the accumulation of sucrose in leaves when the rate of photosynthetic reaction is reduced. An increase in the concentration of sugars in the leaves was also reported after the removal of fruits by Moss[6]. The inhibition of chloroplast activity due to different carbohydrate metabolites may regulate the photosynthetic electron transport reaction.

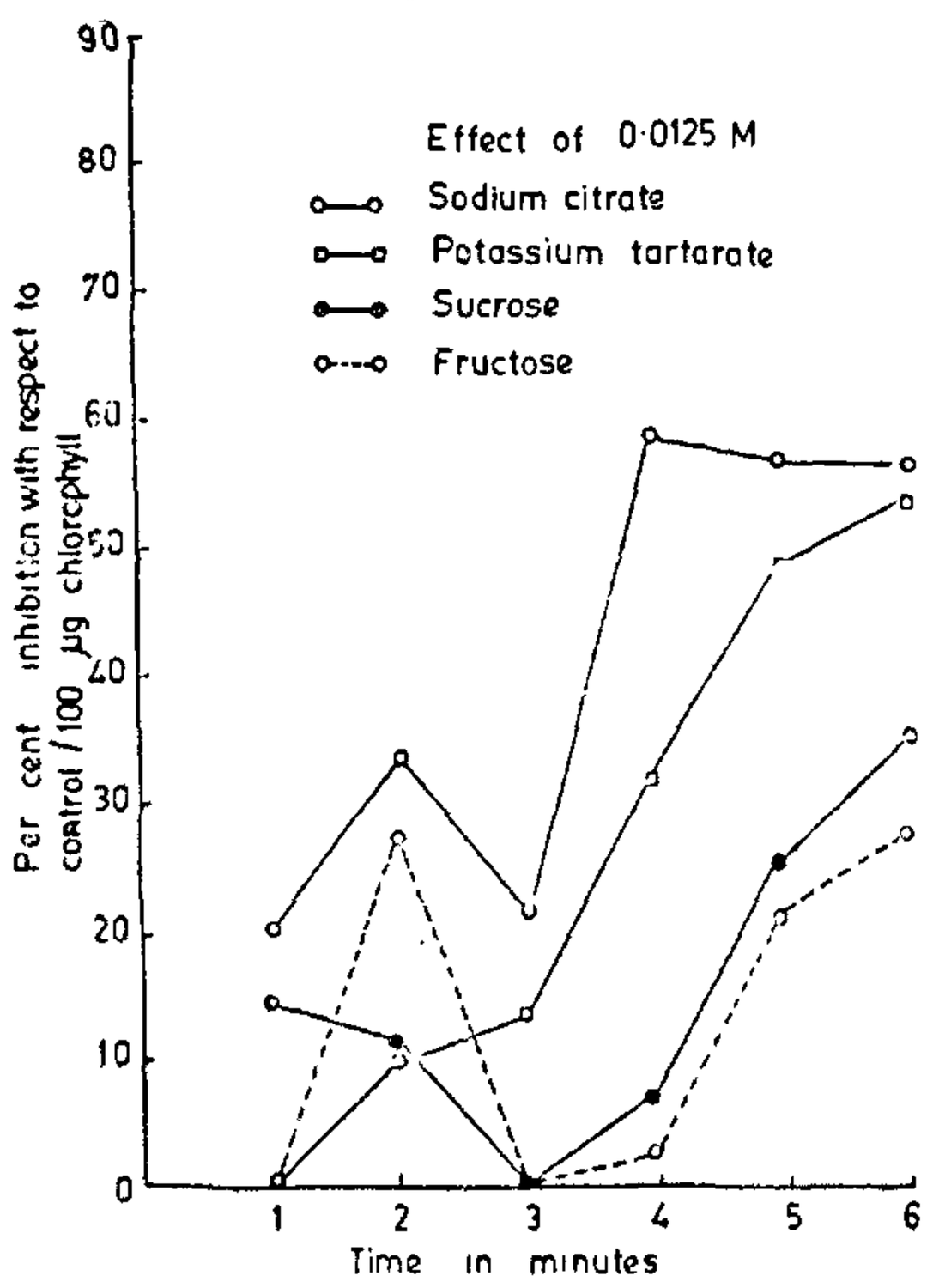

FIG. 2. Effect of different carbohydrate metabolites (0·0125 M) on the reduction of 2,6-dichlorophenol-indophenol by isolated chloroplast from Spinach.

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LEPIDIUM SATIVUM, LINN.—A NEW HOST RECORD FOR *ALTERNARIA ALTERNATA* (FR.) KEISSLER

A SEVERE leaf spot disease is observed at Almora on the leaves of *Lepidium sativum*, Linn. which later on infects the whole plant. The disease first makes its appearance as small oval discoloured lesions which become irregular in shape with increase in size and brown in colour. A light yellow zone surrounds the spot. Lowermost leaves of the plants along irrigation channels receive the infection first. Later on similar symptoms are also observed on the stem and seed coat. The diseased portions of the stem and seed are sterilized (with 0·1% $HgCl_2$ solution) and