Phytochemistry, Vol 22, No 1, pp 297–298, 1983
Printed in Great Britain

0031–9422/83/010297–02$03 00/0

EFFECT OF CARBON DIOXIDE ON LIPID METABOLISM IN *CROTALARIA JUNCEA* POLLEN SUSPENSION CULTURE

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(*Revised received* 17 *June* 1982)

Key Word Index—*Crotalaria juncea*, Leguminosae, pollen tube growth, polar lipids, acetate incorporation, carbon dioxide

Abstract—The effect of carbon dioxide (1 %) on the incorporation of [1-^{14}C]acetate into polar and non-polar lipids was studied in the growing pollen tubes of *Crotalaria juncea* Carbon dioxide stimulated the incorporation of ^{14}C into non-polar (except sterols) fractions and decreased the incorporation into polar (except phosphatidic acid) fractions It is suggested that carbon dioxide could regulate membrane biosynthesis

INTRODUCTION

Carbon dioxide is known to stimulate pollen tube growth [1–7], a marked stimulation being observed in the case of *Crotalaria juncea* [7] Carbon dioxide fixation in germinating pollen grains is reported to occur via a non-photosynthetic pathway [1–3] involving the participation of PEP carboxylase [3, 4] The objective of the present study was to examine whether carbon dioxide also affected lipid biosynthesis in some way

Table 1 Effect of CO_2 (1 %) on [1-^{14}C]acetate incorporation into polar lipids

Polar lipids	Radioactivity incorporated (10^{-2} cpm/30 mg pollen/3 hr in the dark)	
	Basal medium (control)	CO_2 (1 %)
PA	284 8 ± 9	157 ± 3
PC	208 2 ± 4	394 ± 7
PI	52 ± 1 8	72 ± 1
PE	35 ± 1 2	131 ± 1
PS + MGDG	300 ± 11	387 ± 18
PG + DGDG	64 ± 1	125 ± 1

Table 2 Effect of CO_2 (1 %) on [1-^{14}C]acetate incorporation into various non-polar lipid components

Non-polar lipids	Radioactivity incorporated (10^{-2} cpm/30 mg pollen/3 hr in the dark)	
	Basal medium (control)	CO_2 (1 %)
Sterols	468 ± 11	449 ± 2
Free fatty acids	1265 ± 17	220 ± 3
Triglycerides	1689 ± 28	689 ± 4
Sterol esters	386 ± 10	33 ± 1

RESULTS AND DISCUSSION

When expressed as cpm [1-^{14}C]acetate incorporated by 30 mg pollen during a 3 hr dark incubation period, the results indicated that carbon dioxide enhanced the incorporation of label into total lipids (1 53 × 10^6 cpm) over the control (1 00 × 10^6 cpm) Among the polar lipids, all fractions showed increased incorporation with carbon dioxide treatment except the PA fraction (Table 1) The data on non-polar lipids (Table 2) show that except for sterols, which were only marginally affected, carbon dioxide markedly decreased the label in triglycerides, free fatty acids and sterol esters

Carbon dioxide promotion of polar membrane lipids synthesis appears to be comparable with the effect of growth regulators on lipid biosynthesis [8] Like GA_3, carbon dioxide also stimulated acetate incorporation into MGDG and DGDG The precise role of these two glycolipids is not clear in this non-chlorophyllous system since they are reportedly present in the plastid membrane Pollen and pollen tubes have proplastids and the glycolipids might serve to store sugars The reduced incorporation of label into the triglycerides and FFA may indicate the utilization of these components in membrane lipid synthesis These effects of carbon dioxide are reported here for the first time though the precise mode of action remains obscure There is a possibility that carbon dioxide affects the carboxylation of acetyl CoA to malonyl CoA Pollen grains of several species are known to fix carbon dioxide non-photosynthetically (e g through PEP carboxylase) into oxaloacetate (OAA) and then into malate [5, 9] Non-photosynthetically fixed carbon dioxide is incorporated into the total lipids [1, 5, 9] Assuming dark carbon dioxide fixation could provide the carbon skeleton for lipid biosynthesis, the fact that the response of all the fractions did not parallel the controls, tempts us to suggest that carbon dioxide has some other role in lipid biosynthesis

EXPERIMENTAL

Materials [1-^{14}C]Acetate (1 μCi/ml) was obtained from the Radioisotopes Division of BARC, Trombay (India) Some lipid standards were purchased from Sigma and the Biochemical Unit, VP Chest Institute, New Delhi

Crotalaria juncea L (sun hemp) plants were raised in the Botanical Garden, Punjab Agricultural University, Ludhiana, India

Culture conditions Pollen grains (30 mg) were cultured in 4 ml basal medium (3 % sucrose + 20 μg/ml H_3BO_3) containing 1 μCi [2-^{14}C]acetate per ml of culture medium and incubated at 28 $\pm$2° in the dark Cultures were harvested after 3 hr and were centrifuged at 3000 *g* for 15 min at 10° to remove the culture sols The pellet was washed with H_2O (several times) until no residual activity was detected in the supernatants

Lipid extraction and analysis Lipids were extracted from the pellets by the method of Folch *et al* [10] The $CHCl_3$–MeOH phase was subsequently evaporated at low temp and the lipids redissolved in 3 ml $CHCl_3$–MeOH (2 1) 0 5 ml of this preparation was taken to measure the quantity of radioactivity incorporated in the total lipids The remaining 2 5 ml was used for the detection of the amount of radiolabel incorporated in the various polar and non-polar lipids The lipid types were separated by the solvent partitioning method of Nichols [11] and were evaporated at 37° They were dissolved in $CHCl_3$ and petrol, respectively Individual polar and non-polar lipids were separated by Si gel G TLC using $CHCl_3$–MeOH–7 N NH_3 (70 20 1 5) and petrol–Et_2O–HOAc (80 20 1) as the solvent systems, respectively Spots were visualized with I_2 vapour and marked Identifications of different spots were by comparison with the R_f values of standards [12] Individual polar and non-polar lipid components were eluted in $CHCl_3$ and petrol, respectively The radioactivity was measured in a liquid scintillation spectrometer using a toluene-based scintillation fluid [4 g PPO (2,5-diphenyl oxazole), 200 mg POPOP (1,4-bis-2,5-phenyloxazolylbenzene) in 1 l toluene] The incorporation studies were repeated at least $\times$ 4 and the data in Tables 1 and 2 represent mean values $\pm$ s e

Generation of CO_2 CO_2 was generated in a 50 ml conical flask closed with Parafilm by injecting lactic acid into an aq soln of Na_2CO_3 Appropriate vols of CO_2 were withdrawn with an air-tight syringe and injected into the air space above the pollen suspension cultures which had been closed with Parafilm immediately prior to injection of CO_2 The cultures were shaken gently after every 30 min interval

Acknowledgements—Financial assistance to J C from the C S I R, New Delhi in the form of a Senior Research Fellowship is gratefully acknowledged We also thank Dr I S Bhandal for helpful suggestions

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