A SCREENING OF THE DICOTYLEDONOUS WEED FLORA FOR THE OCCURRENCE OF C_4 DICARBOXYLIC ACID PATHWAY OF PHOTOSYNTHESIS

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Abstract

Using the criteria of leaf anatomy, carbon dioxide compensation point and photosynthetic rate under reduced oxygen tension, a number of the local herbaceous and dicotyledonous weeds were screened for the occurrence of the C_4 pathway of photosynthesis. Of the thirty-two species, belonging to nine families seventeen species revealed the characteristics of the C_4 plants. All of the plants surveyed here were hitherto not tested and therefore the present results extend the number of plants already known to possess the Hatch-Slack pathway of photosynthesis. Another noteworthy feature of the investigation was the finding of interspecific differences within the genera *Heliotropium*, *Alternanthera*, *Mollugo* and *Euphorbia* in the presence or absence of the C_4 pathway.

Introduction

Ever Since the discovery of an alternative pathway of photosynthetic carbon fixation (Kortschalk *et al.*, 1965; Hatch and Slack, 1966) to that of the classical type of Calvin cycle was made, higher plants are being classified into two distinct categories. Plants that possess Calvin cycle mechanism are known as the C_3 plants while the others predominantly tropical in distribution and which fix carbon dioxide during photosynthesis through the more recently discovered C_4 dicarboxylic or the Hatch-Slack pathway are called the C_4 plants. Initially the C_4 pathway was thought to be confined mainly to the tropical grasses. Subsequently it has been shown to occur among dicotyledonous plants as well (Osmond, 1967; Crookston and Moss, 1970; Downton, 1971).

The C_4 plants are known to possess a characteristic type of leaf anatomy described as the 'Kranz' type which involves the occurrence of a chlorenchymatous bundle sheath in the leaves. These plants also consistently exhibit low carbon dioxide compensation point, while the C_3 plants usually compensate at higher levels of carbon dioxide. It is therefore possible to distinguish the C_4 plants from the others based on the characteristics of leaf anatomy, and carbon dioxide compensation point. The present study was undertaken in order to make a survey of the locally available plants for the occurrence of the Hatch-Slack pathway among them. Hence only those plants hitherto not investigated by other workers elsewhere were included in the present work. In this investigation a relatively simple and rapid method for quantitative determination of carbon dioxide compensation point was developed and was applied here for the screening of the plants chosen for study.

MATERIALS AND METHODS

All the plants studied are common weeds occurring in the campus and were collected from their natural habitat. The leaf anatomy was investigated by preparing free hand sections which were examined under microscope for the presence of chlorenchymatous bundle sheath. Staining with I-KI solution was used to test the specialisation of bundle sheath cells for starch accumulation.

For the determination of carbon dioxide compensation point a relatively simple technique was used here. The principle involved in this method also is the measurement of residual carbon dioxide content after photosynthesis in a closed system. Residual carbon dioxide in the experimental chamber (Fig. 1) was estimated by absorbing into Barium hydroxide solution of suitable normality and its content was calculated from the volume of the chamber used.

Either a twig or a single leaf (in case of compound leaf) from the plant to be studied was cut carefully under water and was placed in a 100 ml. beaker of water. It was then introduced inside the belljar. A petriplate with solid Lithium chloride was also placed inside the belljar for absorption of moisture released during transpiration which otherwise accumulated on the top of the belljar. For experiments under anaerobic atmosphere the tube from the flask A was disconnected and the air was allowed to enter through another flask having 100 ml. of 10% pyrogallol solution. After evacuation for 5 minutes, by closing the stopcock F, the air was run continuously opening F into the belljar for 15 minutes.

With F and G closed, photosynthesis was allowed for 1 hour, the illumination being provided by an incandescent bulb. The light was filtered through water and the intensity at the level of plant chamber was 20,000 lux. After one hour the stopcock G was opened, and the suction was applied for 15 minutes. Then the stopcock F was also opened and CO_2 free air was flushed through the plant chamber for further 15 minutes to ensure the complete removal of residual CO_2 from the chamber. The contents of flask C were titrated against 0·01 N HCl. A suitable blank titration was also performed to determine the initial amount of CO_2 absorbed by Barium hydroxide solution from the gas phase of the flask C.

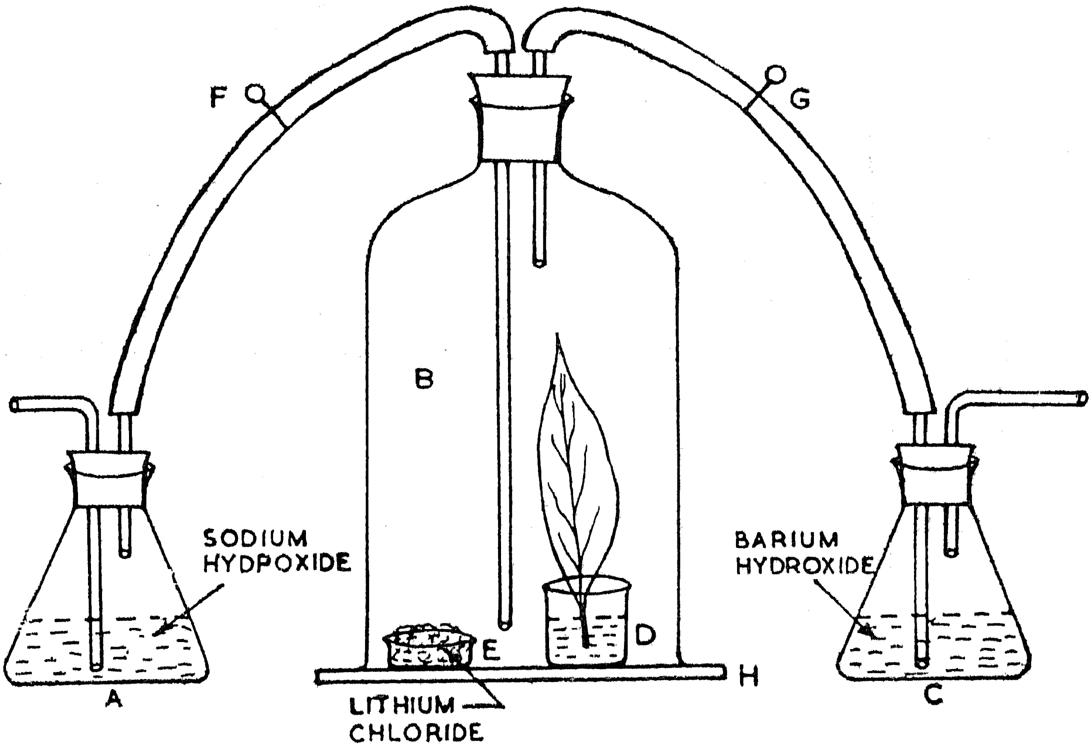

FIG. 1. The experimental set up for the determination of carbon dioxide compensation point of the plant.

The compensation point was calculated by using the formula.

$$\text{Compensation point (in parts per million by volume)} = \frac{D \times N \times 11127600}{V}$$

Where D = Difference in titrations

N = Normality of HCl.

V = Volume of the belljar with appropriate corrections for beaker having the plant and petriplate. (3,300 c.c. in this case)

11127600 = a factor derived as follows

$$22 \times \frac{1000}{1977} \times 1000000$$

where 22 = equivalent weight of CO_2

1977 = weight of CO_2 in mg per litre

1000/1977 = to convert into c.c.

1000000 = per million.

The same apparatus was used to determine the photosynthetic rates under aerobic and anaerobic conditions. Air was allowed to flow continuously through the system with and without the plant. Barium hydroxide (0·1 N) solution was used to absorb CO_2. Difference obtained in the titrations with and without the plant was used in the formula for calculation of rate of photosynthesis

mg of CO_2 fixed = $D \times N \times 22$

where D = the difference in titrations

N = normality of HCl

22 = equivalent weight of CO_2

RESULTS

The present survey of locally occurring dicotyledonous weeds showed that many of them exhibit the typical characteristics of C_4 plants. Kranz type leaf anatomy, occurrence of specialised bundle sheath for starch accumulation, a low compensation point of 5 and below and lack of response to lowered oxygen tension in the rate of photosynthesis, all being characteristic of C_4 pathway were evident in several plants as listed in Table I.

The genera *Heliotropium*, *Mollugo*, *Alternanthera* and *Euphorbia* exhibited interspecific differences in their photosynthetic characteristics, as shown in Table II.

TABLE 1

The photosynthetic characteristics of the local dicotyledonous weeds

Sl. No.	Name of the plant	Leaf anatomy Kranz (K) type or normal (N)	Specialisation of bundle sheath for starch accumulation	CO_2 Compensation point	Enhancement in photosynthetic rate by the removal of oxygen (as %)
	CARYOPHYLLACEAE				
1.	*Polycarpaea corymbosa*, Lam.	K	+	0	0
2.	*Polycarpaea aurea*, W & A.	K	+	0	0
	PORTULACACEAE				
3.	*Portulaca quadrifida*, L.	K	+	2	+ 2
4.	*Portulaca tuberosa*, Roxb.	K	+	2	+ 4
	AIZOACEAE				
5.	*Gisekia pharnaceoides*, L.	K	+	2	− 2
6.	*Mollugo lotoides*, O. Kze.	N	−	57	+39
7.	*Mollugo nudicaulis*, L.	K	+	8	+ 6
8.	*Mollugo pentaphylla*, L.	N	−	52	+47
9.	*Trianthema decandra*, L.	K	+	0	− 4
	RUBIACEAE				
10.	*Borreria hispida*, K. Sch.	N	—	24	+22
	BORAGINACEAE				
11.	*Coldenia procumbens*, L.	N	−	47	+36
12.	*Heliotropium indicum*, L.	N	−	58	+42
13.	*Heliotropium scabrum*, Retz.	K	+	2	0
14.	*Heliotropium zeylanicum*, Lam.	K	+	2	0
15.	*Trichodesma indicum*, R. Br.	N	−	38	+42

TABLE I—(*Contd.*)

Sl. No.	Name of the plant	Leaf anatomy Kranz (K) type or normal (N)	Specialisation of bundle sheeth for starch aecumulation	CO_2 Compensation point	Enhancement in photosynthetic rate by the removal of oxygen (as %)
	ACANTHACEAE				
16.	*Justicia prostrata*, Gamb.	N	—	18	+29
	AMARANTACEAE				
17.	*Aerva lanata*, Juss.	N	—	27	+26
18.	*Aerva Monsoniae*, Mart.	N	—	38	+28
19.	*Alternanthera pungens*	K	+	2	+ 2
20.	*Alternanthera sessiles*, R. Br.	N	—	52	+23
21.	*Amaranthus paniculatus*, L.	K	+	4	+ 2
22.	*Amaranthus polygamus*, L.	K	+	5	+ 4
23.	*Amaranthus spinosus*, L.	K	+	3	+ 6
24.	*Gomphrena decumbens*, Jacq.	K	+	0	0
	NYCTAGINACEAE				
25.	*Boerhaavia diffusa*, L.	K	+	0	+ 2
	EUPHORBIACEAE				
26.	*Croton sparsiflorus*, Mor.	N	—	42	+35
27.	*Euphorbia heterophylla*, L.	N	—	46	+37
28.	*Euphorbia hirta* L.	K	+	0	0
29.	*Euphorbia pulcherrima*, Willd.	N	—	62	+33
30.	*Euphorbia thymifolia*, L.	K	+	2	0
31.	*Phyllanthus niruri*, L.	N	—	48	+38
32.	*Phyllanthus maderaspatensis*, L.	N	—	38	+29

DISCUSSION

The present finding of the occurrence of the characteristics of C_4 pathway in a number of plants hitherto unreported suggests that the C_4 pathway is much more widespread than is so far known. It has also become clear that the pathway is associated with annual herbs. Most of the weeds investigated here and found to possess the C_4 pathway belong to families placed under the order Centrospermae as per the system of classification of Engler and Prantl. This observation confirms the finding of Crookston and Moss (1970) who has also reported similar pattern of distribution of the pathway among dicotyledonous plants. However the plants belonging to Caryophyllaceae were not found to have the characteristics of C_4 plants earlier. The occurrence of the C_4 characteristics in the plants belonging to Boraginaceae of the order Tubiflorae as found in the present work strongly suggests that the pathway is not restricted to a single taxonomic group within the dicotyledonae but is scattered over a much larger scale.

TABLE II

Interspecific differences in the photosynthetic characteristics

Sl. No.	Family	Genus	Species having the characteristics of	
			C_4 pathway	C_3 pathway
1.	Amarantaceae	*Alternanthera*	*A. pungens*	*A. sessiles*
2.	Euphorbiaceae	*Euphorbia*	*E. hirta* *E. thymifolia*	*E. heterophylla* *E. pulcherrima*
3.	Boraginaceae	*Heliotropium*	*H. scabrum* *H. zeylanicum*	*H. indicum*
4.	Aizoaceae	*Mollugo*	*M. nudicaulis*	*M. lotoides* *M. pentaphylla*

An invariable association of the occurrence of chlorenchymatous bundle sheath with low CO_2 compensation point has not been shown previously (Crookston and Moss, 1970). In this investigation all those plants that exhibited chlorenchymatous bundle sheath were also specialised for starch accumulation and invariably possessed low compensation point.

The finding of interspecific differences in a number of genera not reported earlier is highly interesting from a taxonomic standpoint and further studies may lead to a better understanding of the evolution of the C_4 pathway of photosynthesis.

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