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PHOTOSYNTHETIC SYSTEM OF *LEPTOCHLOA FUSCA* (L.) KUNTH.

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Abstract

Photosynthetic system of *Leptochloa fusca* (L.) Kunth., was determined by observing its leaf anatomy, distribution of starch grains, measurement of CO_2-compensation point and $^{13}C/^{12}C$ ratios. All diagnostic characters revealed that this grass has a C-4 pathway of photosynthesis.

Introduction

It has been known that many grasses possess the C-4 dicarboxylic acid pathway of photosynthesis instead of reductive pentose pathway (C-3) found generally in plants (Kortschak *et al.,* 1965; Hatch & Slack, 1970). Grasses possessing C-4 pathway utilize their available nitrogen more efficiently in producing biomass than grasses having C-3 pathway (Black *et al.,* 1978). The fact that all the grasses in which associative N_2-fixation has been reported except rice and wheat possess C-4 pathway, lead Dobereiner *et al.,* (1972) to propose a relationship between C-4 photosynthetic pathway and dinitrogen fixation in the root zones of the plant. Malik *et al.,* (1980, 1981) has already reported nitrogenase activity based on acetylene reduction assays (ARA) in the excised roots of Kallar grass (*Leptochloa fusca* (L.) Kunth.), growing in saline areas. Present investigations were carried out to know the photosynthetic system of this grass.

Materials and Methods

Collection: Plant material of *Leptochloa fusca* (L.) Kunth. (Synonym *Diplachne fusca* (L.) Beauv.) commonly known as Kallar grass was collected from field of NIAB campus, Faisalabad.

Leaf anatomy: Free hand sections of leaves of Kallar grass were placed in 40% commercial sodium hypochlorite solution (v/v) in water for one hour in order to decolourise the plant material. Water mounts were made and examined under the Microlux-11 photomicroscope. Distribution of starch grains in the parenchymatous cells was determined according to the method of Downton & Tregunna (1968). For staining purpose, leaves were collected in the afternoon of a sunny day. Sections were dipped for one hour in small vial containing Gram's iodine (Iodine-1 gm, KI-2 gm, distl. H_2O, 100 ml). Sections were examined under microscope for the distribution of stained starch grains and photographed.

CO_2-compensation point: CO_2-compensation point for Kallar grass was measured by an infra red gas analyser (IRGA-120). A quantity of fresh green leaves were enclosed in a growth chamber having light intensity of 30,000 Lux and temperature of $30 \pm 2^{o}C$. The decrease in CO_2 concentration was recorded. In addition to Kallar grass, known C-3 plants namely *Vicia faba* and *Triticum aestivum* were also studied.

Carbon isotope ratios: Leaf tissue was collected from the field and dried in a forced air oven at $80^{o}C$ for 24 hrs. The dried tissue (5-10 mg) was combusted at $750^{o}C$ in an excess of oxygen and isotopic ratio ($^{13}C/^{12}C$) of the CO_2 evolved was measured on a mass spectrometer as described by Osmond *et al.,* (1978).

This method is based on the observation that plants discriminate against ^{13}C during photosynthesis in ways which reflect plant metabolism and environment (Benedict, 1978; O'Leary, 1981). Atmospheric CO_2 contains about 1.1% of the heavier isotope ^{13}C and 98.9% of the lighter isotope ^{12}C. The discrimination of ^{13}C in favour of ^{12}C has been highly correlated with the C_3 and C_4 pathways of photosynthetic metabolism. This characteristic when considered in relation to leaf anatomy, provides the most reliable criterion for distinguishing these two photosynthetic pathways (Smith & Brown, 1973). A theory is developed by Farguhar *et al.,* (1982) to explain the carbon isotope composition of plants which is based on diffusion of gaseous CO_2 and carboxylation.

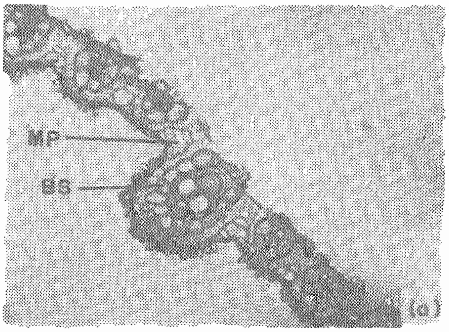
The isotopic composition is specified as $\Delta^{13}C$ values.

$$\Delta^{13}C‰ = (\frac{R_{sample}}{R_{standard}} - 1) \times 1000$$

where R sample and R standard are the $^{13}C/^{12}C$ ratios of the sample and the standard. As absolute isotope ratios are troublesome to obtain and for most purposes it is adequate to give $\Delta^{13}C‰$ values in relation to some standard. The standard in general use is PDB (belemnite from Pee Dee Formation in South Carolina, USA) with $^{13}C/^{12}C$ ratio = 0.01124 (Craig, 1958) and can be obtained from U.S. National Bureau of Standards In addition to Kallar grass a known C-4, *Saccharum officinarum* and aC-3 *Rosa indica* were also analysed for $^{13}C/^{12}C$ ratios.

Results and Discussions

Anatomical sections of leaves of *Leptochloa fusa* were found to be of Kranz type (Fig. 1a & b). This name was given by Meser (1934) to such leaf structural arrangement in which a chlorenchymatous sheath of large, thick walled cells surrounds vascular bundles. There are dense concentration of chloroplasts, mitochondria and peroxisomes in the bundle sheath cells as compared to mesophyll cells which are often radially arranged (Laetsch, 1971). The Kranz syndrome is a remarkable example of structure related to function with the functional aspects of photosynthetic carbon fixation being correlated with the anatomy of the leaf. Well developed bundle sheath cells were observed around vascular bundle. Adaxial bundle sheath cells of primary lateral bundles are enlarged and devoid of chloroplasts (Fig. 2b). Earlier Kranz syndrome in this grass growing in Southern Africa have been reported by Ellis (1977). Smith & Brown (1973) surveyed the family Gramineae for the occurrence of Kranze syndrome which was based on $^{13}C/^{12}C$ ratios



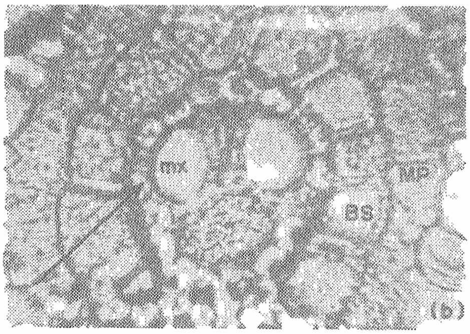


Fig. 1a. Hand cut sections of leaf of Kallar grass showing bundle sheath (BS) and Mesophyll (MP) chlorenchymatous cells (x 25.0).

b. A portion at higher magnification (x 100). Intervening cells between metaxylem vessel (mx) and bundle sheath of primary vascular bundle are indicated by arrow.

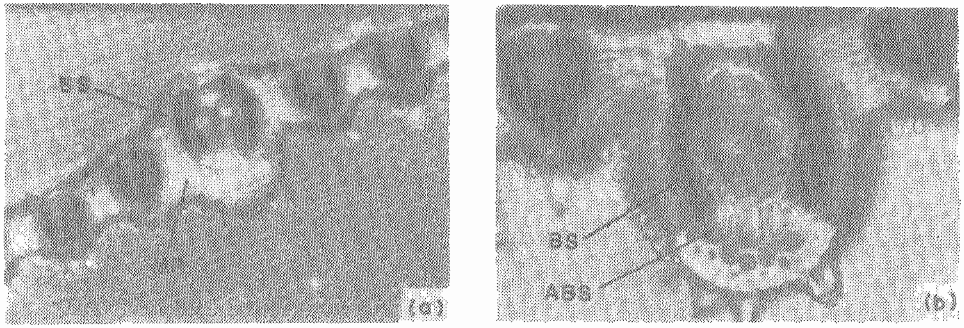


Fig. 2a. IKI-stained dark coloured bundle sheath cells (BS) of Kallar grass. Mesophyll (MP) cells are light in colour as they do not contain starch (x 25.0).

b. Same section at higher magnification (x 100). Empty adaxial cells (ABS) of primary lateral bundle are also evident.

and reported *L. fusca* to be of Kranze type. They did not measure the carbon isotopic ratio of this specie and cited the reference of Chen *et al.*, (1971). A search of literature revealed that no reference of *L. fusca* or its synonym *Diplachne fusca* exists in that report. Our observations confirmed the report of Ellis (1977).

Three sub groups of C-4 photosynthesis are currently recognized as NADP-ME type (NADP-malic enzyme species), NAD-ME type (NAD-malic enzyme species), and PCK type (PEP-carboxykinase species), depending on the reation sequence for C-4 acid decarboxylation in the bundle sheath cells of leaf blades (Hatch *et al.,* 1975). Presence (Xy MS+) or absence (Xy MS–) of cells intervening between metaxylem vessel elements and laterally adjacent chlorenchymatous bundle sheath cells of primary lateral vascular bundle is related to sub grouping in C-4 species by Hatterslay & Watson (1976). They observed a perfect correlation; NADP-Malic enzyme species being Xy MS– while Xy MS+ species were found to be NAD-Malic enzyme or PCK type. Anatomical sections of Kallar grass were found to be Xy MS+ (Fig. 1b) and thus belongs to NAD-Malic enzyme or PCK type. From its anatomy Ellis (1977) categorised it as NAD-Malic enzyme species, however, biochemical studies are needed to confirm the exact C-4 sub group of this grass.

IKI-staining: Clear, well defined bundle sheath cells turned dark after staining with IKI solution as shown in Fig. 2a & b. Rhoades & Carvalho (1944) have shown that for maize and sorghum (C_4 plants) the sheath cells contain specialized plastids concerned with starch formation and no starch was formed in the outer chlorenchymatous cells.

In C_3 species starch is accumulated in the mesophyll cells during the day (Rhoades & Carvalho, 1944) whereas in C_4 species it is only formed in the Kranz sheath cells and not in the mesophyll cell.s Downton & Tregunna (1968) determined the correlation between CO_2-compensation and leaf anatomy alongwith starch distribution. They observed that plants having high CO_2 compensation point (C_3) have starch granules present in whole mesophyll. On the other hand the plant species with low CO_2 compensation point (<10 ppm, C_4) have starch granules restricted to bundle sheath.

CO_2-compensation point: The result of the study made on Kallar grass regarding its compensation point is presented in Fig. 3. CO_2 concentration in the chamber containing Kallar grass leaves decreased from 300 ppm to about 4 ppm in an hour as compared to 70 ppm in case of *Vicia faba* (C_3-dicot) and 45 ppm in wheat (C_3-monocot). Chen *et al.*, (1970) included *L. fusca* in the list of plants having low CO_2-compensation point and thus has C-4 photosynthesis. Our results reconfirm the earlier report.

Carbon isotope ratio: The difference in isotopic composition has become one of the standard methods by which C-4 plants can be distinguished from C-3 plants.

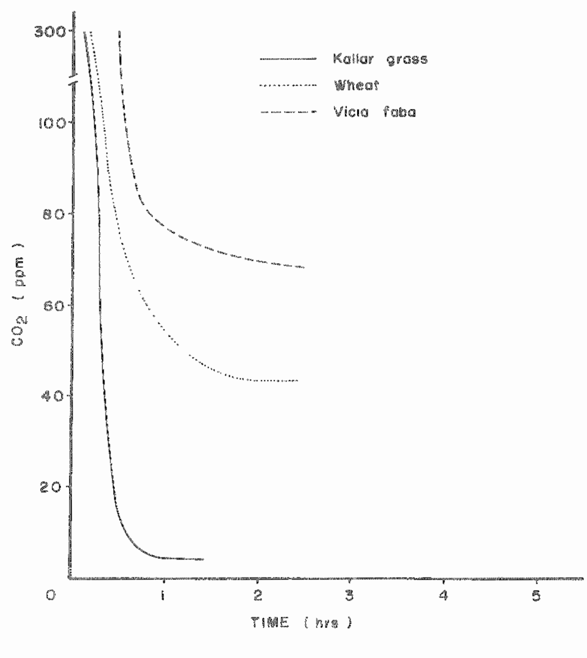


Fig. 3. CO_2 concentration in an enclosed vessel (ul/L) is plotted against time (h). Sealed vessels containing plants were placed in an illuminated growth chamber fitted with an automatic gas sampler for infra red gas analyser and a recorder. Chart speed was 2 mm/min and initial conc. of CO_2 was 300 ppm.

Table 1. $\Delta^{13}C$‰ **values of plants collected from NIAB-campus. Standard deviation is ±0.2%.**

Name of plant	$\Delta^{13}C$‰
Saccharum officinarum (Sugarcane C-4)	–12.5
Rosa indica ('Wild rose-C-3)	–26.7
Leptochloa fusca (Kallar grass)	–15.9

O'Leary, (1981) reported mean $\Delta^{13}C$‰ values –13.5±1.5 and –28.1±2.5 for C-4 and C-3 plants respectively. The $\Delta^{13}C$‰ values of Kallar grass and other plants are presented in Table 1. The $\Delta^{13}C$‰ value for *L. fusca* (15.9±0.2%) is higher but it is known that $\Delta^{13}C$ value is influenced by salinity, dry and hot weather and CO_2 composition in the environment (O'Leary, 1978). Bender (1971) has reported $\Delta^{13}C$‰ value of –15.3 for *Cynodon dactylon* (L.) Pers. which is a C-4 grass and this figure is quite close to the observed value of Kallar grass. $\Delta^{13}C$‰ readings for sugarcane and wild rose are well in the range of already reported values from other locations. The $\Delta^{13}C$‰ value long with other observations are sufficient to conclude that *Leptochloa fusca* which is commonly known as Kallar grass is a C-4 grass.

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