C_4 Photosynthetic Characteristics of *Panicum* Species in the *Dichotomiflora* Group (Gramineae)

By RYU OHSUGI and TAKAO MURATA

Pasture Plants Division, National Grassland Research Institute
(Nishinasuno, Tochigi, 329-27 Japan)

Introduction

In C_4 photosynthesis, two different kinds of cells are needed to complete a series of photosynthetic process: KC* (chlorenchymatous bundle sheath cells) and MC. Atmospheric CO_2 is fixed and converted to C_4 acids (aspartate or malate) through phospho*enol*pyruvate carboxylase in MC. The C_4 acids are then transported to KC where they are decarboxylated through decarboxylating enzymes, and released CO_2 is refixed through ribulose-1, 5-bisphosphate carboxylase, to synthesize sucrose (Fig. 1). This CO_2 concentrating mechanism, called "CO_2 pump", is considered to be important for the adaptation of plants to hot or arid environments.[4]

The C_4 acid decarboxylating reaction in KC is known to be catalyzed by three different enzymes: NADP-ME located in chloroplasts, NAD-ME in mitochondria and PEP-CK in cytoplasm. Since the major operation of the different decarboxylating enzymes is specific to species, C_4 species can be divided into three subtypes: NADP-ME type, NAD-ME type and PEP-CK type species.[6,8]

In the Gramineae, the different subtypes have been closely correlated with distinct leaf anatomical and physiological characteristics

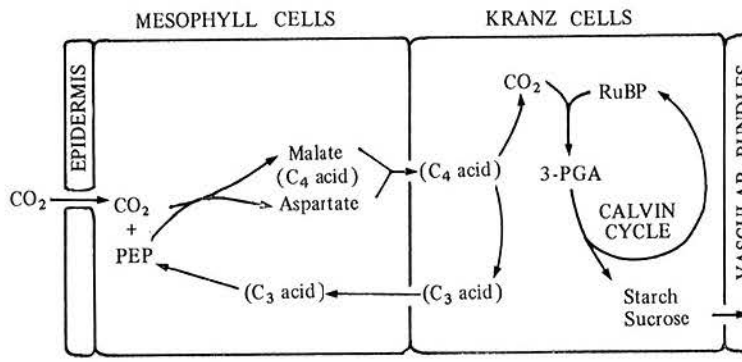

Fig. 1. A simplified scheme for C_4 photosynthesis[7]

(Table 1). NADP-ME type species lack mestome sheath, and chloroplasts in KC are located in the centrifugal position. Both NAD-ME type species and PEP-CK type species have mestome sheath. However, chloro-

* Abbreviations: KC: Kranz cells; MC: mesophyll cells; ME: malic enzyme; PEP-CK: phospho*enol*pyruvate carboxykinase; PIB: post-illumination CO_2 burst.

Table 1. Some characteristics of the species representing the C_4 subtypes

Characteristics	C_4 subtype		
	NADP-ME	NAD-ME	PEP-CK
Major decarboxylating enzyme	NADP-ME	NAD-ME	PEP-CK
Mestome sheath	Absent	Present	Preseut
Chloroplast location in Kranz cell	Centrifugal	Centripetal	Centrifugal
PIB	No	Yes (sharp)	Yes (broad)
Representative species	*Zea mays* *Panicum antidotale*	*Eleusine coracana* *Panicum miliaceum*	*Chloris gayana* *Panicum maximum*

plast location in KC is different between them, being in the centripetal position for NAD-ME type species and in the centrifugal position for PEP-CK type species. C_4 subtypes, therefore, have been estimated based on leaf anatomy.[5,9] NAD-ME type species are also characterized by a sharp pattern of PIB in less than 30 sec of darkness after the extinction of light.[1,3]

We have carried out various evaluations of C_4 grasses as pasture or fodder plants since 1978. In the work to elucidate photosynthetic aspects of C_4 grasses, we found out a new relationship among C_4 subtypes, leaf anatomy and PIB pattern in some species in the *Dichotomiflora* group of *Panicum*.[11,12] The present paper reports C_4 photosynthetic characteristics and some characters related to leaf anatomy and early growth of the species in the *Dichotomiflora* group.

Leaf anatomy, pattern of PIB and activity of C_4 acid decarboxylating enzymes

We have investigated the relationship between leaf anatomy and pattern of PIB in 7 genera 24 species of the Eragrostoideae and 16 genera 50 species of the Panicoideae, and found out that some species in the *Dichotomiflora* group of *Panicum* showed the sharp pattern of PIB, though their chloroplasts in KC were in the centrifugal position.

1) Leaf anatomy

As to leaf anatomy, we observed the presence or absence of mestome sheath between the metaxylem vessel elements and the laterally adjacent KC in the primary lateral vascular bundles[9] and chloroplast location in KC. Cross sections of leaf blades are shown in Plate 1. Plate 1A is for *P. coloratum* cv. Kabulabula and Plate 1B is for *P. coloratum* var. *makarikariense*. Both accessions had mestome sheath. However, chloroplasts in KC of *P. coloratum* cv. Kabulabula were located in the centrifugal position, attached to the inner wall adjacent to vascular bundles. On the other hand, chloroplasts in KC of *P. coloratum* var. *makarikariense* were located in the centripetal position, attached to the inner wall adjacent to MC. All the accessions examined were definitely divided into those with centrifugal chloroplasts and those with centripetal chloroplasts (Table 2). In *P. coloratum*, especially, both types of chloroplast location were observed depending on cultivars.

2) Pattern of PIB

PIB is a rapid, transient evolution of CO_2 from leaves, following a change from light to darkness. For the first time this phenomenon was found in C_3 species and the photorespired CO_2 was identified to be a source for CO_2 evolved in the PIB. Some C_4 species exhibit a pronounced PIB though they show little photorespiration.[1,3] According to recent investigations, the presence or absence of PIB in C_4 species seems to be correlated to C_4 subtypes: that is, NADP-ME type species lack the PIB. NAD-ME type species exhibit a sharp pattern of PIB in less than 30 sec of darkness following illumination. PEP-CK type

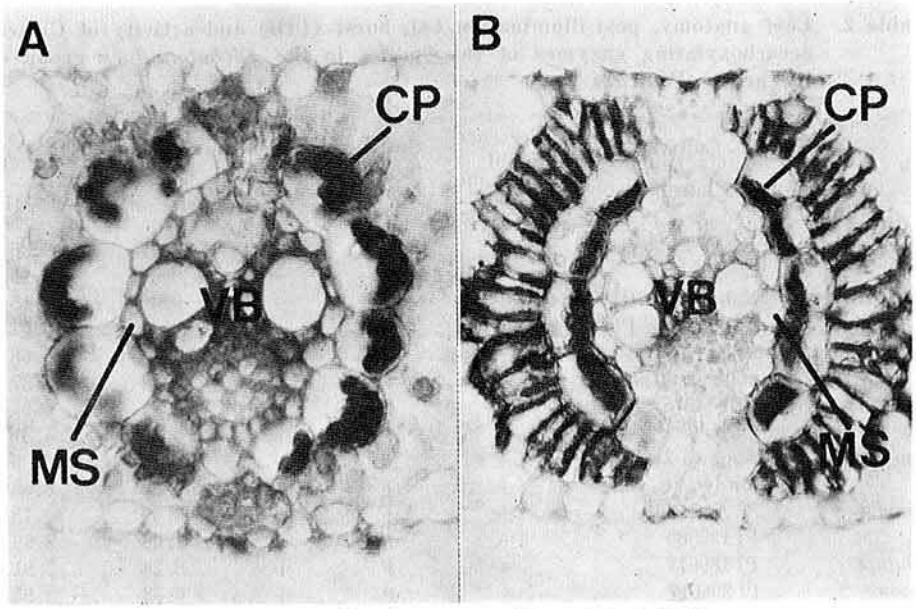

Plate 1. Light micrographs of cross sections of leaf blades
A: *P. coloratum* (cv. Kabulabula PI253240). Mestome sheath (MS) is present. Chloroplasts (CP) in Kranz cells are located in the centrifugal position. ×540
B: *P. coloratum* var. *makarikariense* (strain CPI14375). Mestome sheath (MS) is present. Chloroplasts (CP) in Kranz cells are located in the centripetal position. ×410
VB: Vascular bundles

species exhibit a broad pattern of PIB between 60 and 120 sec of darkness.[13]

Fig. 2 shows the pattern of PIB of *P. coloratum* cv. Kabulabula with centrifugal chloroplasts in KC. The sharp pattern of PIB was practically the same as that observed in the NAD-ME type species with centripetal chloroplasts in KC.[1] All the accessions examined showed the sharp pattern of PIB, irrespective of the difference in chloroplast location in KC (Table 2). The result of PIB pattern suggests that NAD-ME may operate mainly in decarboxylating process in KC of all the accessions examined.

3) *Activity of three C_4 acid decarboxylating enzymes*

Table 2 shows activity of three different C_4 acid decarboxylating enzymes in whole leaf extracts. NADP-ME activity was low with Mg^{2+} and PEP-CK activity was not detected at all. On the other hand, NAD-ME activity was high, ranging from 3.59 to 6.66, and was stimulated by fructose bisphosphate or coenzyme A. (The results with coenzyme A are shown in Table 2.) The activator-stimulated activity of NAD-ME observed here was comparable to that observed in the whole leaf extracts of the typical NAD-ME type species.[6,8]

Based on the results of leaf anatomy, pattern of PIB, and activity of C_4 acid decarboxylating enzymes, it is apparent that NAD-ME type species have two leaf anatomical variations: those with centrifugal chloroplasts in KC and those with centripetal chloroplasts in KC. In the following text, they are referred to NAD-ME(F) type species and NAD-ME (P) types species, respectively. The difference in chloroplast location in KC has been considered to reflect some functional differences in photosynthesis of C_4 subtypes. However, the occurrence of NAD-ME(F) type

Table 2. Leaf anatomy, post-illumination CO_2 burst (PIB) and activity of C_4 acid decarboxylating enzymes of the species in the *Dichotomiflora* group of the genus *Panicum*

Species	Cultivars or strains	Presence of mestome sheath	Chloroplast position in Kranz cell	PIB	Activity of C_4 acid decarboxylating enzymes[3]		
					NADP-ME	NAD-ME	PEP-CK[4]
Panicum coloratum	Klein PI 364948	+	P[1]	+[2]	0.47	3.96	0
	73-294	+	P	+	0.46	3.95	0
	Kabulabula CPI 17446	+	F	+	0.46	5.15	0
	Kabulabula PI 253240	+	F	+	0.43	5.07	0
	Solai GR-23	+	F	+	0.41	4.66	0
	Solai Yukijirushi	+	F	+	0.52	5.27	0
P. coloratum var. *makarikariense*	CPI 14375	+	P	+	0.81	5.57	0
	N 780	+	P	+	0.71	4.81	0
P. dichotomiflorum	Kagawa-kei	+	F	+	0.87	6.66	0
	Mitoyo-kei	+	F	+	0.80	6.55	0
P. laevifolium	S. Africa-kei	+	F	+	0.69	4.13	0
P. lanipes	PI 185560	+	P	+	1.08	3.59	0
P. longijubatum	PI 189614	+	F	+	0.24	3.91	0
P. stapfianum	PI 208017	+	P	+	0.58	3.95	0
	PI 208246	+	P	+	0.79	4.58	0

1) P: Centripetal position, F: Centrifugal position
2) The sharp pattern of PIB is observed in less than 30 sec of darkness following illumination.
3) All enzyme activity values are expressed as μmoles/min/mg chlorophyll.
4) PEP-CK activity of leaves of *Chloris gayana* used as the control plant is 9.36.

species indicates that centripetal location of chloroplasts in KC is not essential to C_4 photosynthetic metabolism of NAD-ME type species and suggests that chloroplast location in KC may be correlated to other environmental factor(s), such as moisture or temperature, rather than photosynthesis itself.

Differences in the characters related to leaf anatomy and early growth between NAD-ME(F) type species and NAD-ME(P) type species in the *Dichotomiflora* group

The genus *Panicum* is unusual in that it comprises C_3 species, all C_4 subtypes and even C_3–C_4 intermediate species and are divided into some sections and groups.[2] The *Dichotomiflora* group has been considered to have typical *Panicum* species. As shown in the preceding section, both NAD-ME(F) type species and NAD-ME(P) type species are included in the *Dichotomiflora* group. In the following study we investigated some characters related to leaf anatomy and early growth to show the difference between both type species.

1) Difference in the characters related to leaf anatomy

NAD-ME(F) type species examined are *P. coloratum* (cv. Kabulabula and cv. Solai), *P. dichotomiflorum* and *P. laevifolium*. NAD-ME (P) type species are *P. coloratum* (cv. Klein), *P. coloratum* var. *makarikariense* and *P. stapfianum*. The youngest fully expanded leaf blades were sampled for the measurements of leaf anatomy from plants grown outdoors at their vegetative stage. The result is shown in Table 3. Leaf width and leaf thickness of NAD-ME(F) type species were larger than those of NAD-ME(P) type species, although leaf length of both type species was almost similar in size. Most distinct difference between two type species was observed in the ratio of longitudinal length to radial width of Kranz cells: 1.2 for NAD-ME(F) type species and 0.8 for NAD-ME(P) type species. That is, NAD-ME

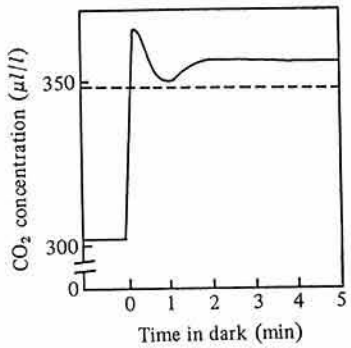

Fig. 2. The pattern of PIB of *P. coloratum* (cv. Kabulabula PI253240, leaf area: 9.4 cm²)
The sharp pattern is shown in less than 30 sec of darkness following illumination. The solid line indicates CO_2 concentration in air after passage through the chamber before and after the lights are turned off. The broken line indicates CO_2 concentration in air before passage through the chamber.

(F) type species have a paradermally longer Kranz cell than NAD-ME(P) type species. NAD-ME(F) type species had high percentage of the area of KC to the total cross-sectional area of leaf blade and low percentage of epidermis plus sclerenchyma and of vascular bundles, compared to NAD-ME(P) type species. The ratio of the area of MC to KC of NAD-ME(F) type species and of NAD-ME(P) type species were 1.3 and 2.0, respectively. Recently, the significance of leaf anatomy in determining digestibility has been demonstrated.[15] The differences in tissue proportions in leaf cross-sectional area and in the MC : KC area ratio may result in the difference in digestibility of leaves between NAD-ME(F) type species and NAD-ME(P) type species.

2) *Difference in the characters related to early growth*

NAD-ME(F) type species and NAD-ME(P) type species examined are similar to those used in the above experiment. Plants grown in 1/5000 a pots in a glass house were sampled at the 6th–7th leaf stage and 12 days later for the measurements of some characters related to growth of terrestrial part, and growth analysis during a 12-day period was applied. The result is shown in Tables 4 and 5. At the 6th–7th leaf stage, NAD-ME(F) type species were greater in all of the quantitative characters related to growth than NAD-ME(P) type species. Especially, leaf area of NAD-ME(F) type species was 2.7 times larger than that of NAD-ME(P) type species. Specific leaf area, 0.400, of NAD-ME(F) type species was 1.4 times higher than 0.286 of NAD-ME(P) type species. Relative vegetative growth rate is 0.190 for NAD-ME(F) type species and 0.144 for NAD-ME(P) type

Table 3. Mean of some characters related to leaf anatomy

Characters		NAD-ME(P)	NAD-ME(F)	NAD-ME(F) / NAD-ME(P)
Leaf length	(cm)	41.5	44.9	1.08
Leaf width	(cm)	0.9	1.4	1.56
Leaf thickness	(μm)	174.4	216.4	1.24
Interveinal distance	(μm)	197.3	203.5	1.03
Longitudinal length/radial width of Kranz cell		0.8	1.2	1.50
Epidermis plus sclerenchyma[1)]	(%)	31.1	23.1	0.74
Mesophyll cells[1)]	(%)	39.1	38.6	0.99
Kranz cells[1)]	(%)	20.1	30.5	1.52
Vascular bundles[1)]	(%)	9.7	7.7	0.79
(Mesophyll cells)/(Kranz cells)		2.0	1.3	0.65

1) Percentage of the area of different tissue portions to the total cross-sectional area of leaf blade.

Table 4. Mean of some characters related to early growth of terrestrial part at the 6th-7th leaf stage

Characters		NAD-ME(P)	NAD-ME(F)	MAD-ME(F) / MAD-ME(P)
No. of leaves		6.6	6.6	
Plant height	(cm)	38.8	47.0	1.21
Leaf area	(cm^2)	27.7	74.7	2.70
Leaf dry weight	(mg)	99.3	188.9	1.90
Plant top dry weight	(mg)	152.7	302.9	1.98
Specific leaf area[1)]	(cm^2/mg)	0.286	0.400	1.40
Percentage dry weight	(%)	16.1	10.9	0.68

1) (Leaf area) / (Leaf dry weight)

Table 5. Growth analysis during a 12-day period from the 6th-7th leaf stage

Variates		NAD-ME(P)	NAD-ME(F)	NAD-ME(F) / NAD-ME(P)
RGR	(mg/mg/day)	0.144	0.190	1.32
RLGR	(cm^2/cm^2/day)	0.156	0.166	1.06
NAR	(mg/cm^2/day)	0.794	0.875	1.10
LAR	(cm^2/mg)	0.182	0.231	1.27

RGR: Relative vegetative growth rate, RLGR: Relative leaf area growth rate, NAR: Net assimilation rate, LAR: Leaf area ratio

species throughout a 12-day period. This high relative vegetative growth rate of NAD-ME (F) type species seems to be caused by high leaf area ratio (1.27 times) rather than net assimilation rate (1.10 times). The result indicates that NAD-ME(F) type species show high productivity in early growth, particularly, high rate of leaf development, compared to NAD-ME(P) type species.

Conclusion

As shown in the above experiments, the *Dichotomiflora* group of *Panicum* has a newly-found NAD-ME(F) type species as well as NAD-ME(P) type species and there are many differences in the characters related to leaf anatomy and early growth between NAD-ME (F) type species and NAD-ME(P) type species, suggesting that two type species may be adapted to different environmental conditions. Hsu[10)] demonstrated that the species in the *Dichotomiflora* group were dominant around ponds and wet places. Ohta and Ochi[14)] showed that *P. coloratum* cv. Kabulabula and *P. dichotomiflorum* were tolerant to high soil moisture. NAD-ME(F) type species therefore may be adapted particularly to high moisture conditions. We have succeeded in the hybridization between *P. coloratum* cv. Kabulabula [NAD-ME(F) type species] and *P. coloratum* var. *makarikariense* [NAD-ME (P) type species] and many characters related to photosynthesis, cytology and productivity of the progenies are now under investigation.

References

1) Brown, R. H. & Gracen, V. E.: Distribution of the post-illumination CO_2 burst among grasses. *Crop Sci.*, **12**, 30–33 (1972).
2) Brown, W. V.: The Kranz syndrome and its subtypes in grass systematics. *Mem. Torrey Bot. Club*, **23**, 1–97 (1977).
3) Downton, W. J. S.: Preferential C_4-dicarboxylic acid synthesis, the post-illumination

CO_2 burst, carboxyl transfer step, and grana configurations in plants with C_4-photosynthesis. *Can. J. Bot.*, 48, 1795–1800 (1970).
4) Edwards, G. E. & Walker, D.: C_3,C_4:mechanisms, and cellular and environmental regulation, of photosynthesis. Blackwell Scientific Publications, London (1983).
5) Ellis, R. P.: Distribution of the Kranz syndrome in the Southern African Eragrostoideae and Panicoideae according to bundle sheath anatomy and cytology. *Agroplantae*, 9, 73–110 (1977).
6) Gutierrez, M., Gracen, V. E. & Edwards, G. E.: Biochemical and cytological relationships in C_4 plants. *Planta (Berl.)*, 119, 279–300 (1974).
7) Hatch, M. D.: The C_4 pathway photosynthesis: mechanism and function. *In* CO_2 metabolism and plant productivity. Ed. Burris, R. H. & Black, C. C., University Park Press, Baltimore, 59–81 (1976).
8) Hatch, M. D., Kagawa, T. & Craig, S.: Subdivision of C_4-pathway species based on differing C_4 acid decarboxylating systems and ultrastructural features. *Aust. J. Plant Physiol.*, 2, 111–128 (1975).
9) Hattersley, P. W. & Watson, L.: C_4 grasses: an anatomical criterion for distinguishing between NADP-malic enzyme species and PCK or NAD-malic enzyme species. *Aust. J. Bot.*, 24, 297–308 (1976).
10) Hsu, C. C.: The classification of *Panicum* (Gramineae) and its allies, with special reference to the characters of lodicule, stylebase and lemma. *J. Fac. Sci. Univ. Tokyo, Sect. 3. Bot.*, 9, 43–150 (1965).
11) Ohsugi, R. & Murata, T.: Leaf anatomy, post-illumination CO_2 burst and NAD-malic enzyme activity of *Panicum dichotomiflorum*. *Plant Cell Physiol.*, 21, 1329–1333 (1980).
12) Ohsugi, R., Murata, T. & Chonan, N.: C_4 syndrome of the species in the *Dichotomiflora* group of the genus *Panicum* (Gramineae). *Bot. Mag., Tokyo*, 95, 339–347 (1982).
13) Ohsugi, R., Murata, T. & Shimizu, N.: Relationship among leaf anatomy, post-illumination CO_2 burst and C_4 subtypes of C_4 species of the Eragrostoideae and the Panicoideae (Gramineae). *Bull. Natl. Grassl. Res. Inst.*, 26, 39–51 (1983) [In Japanese with English summary].
14) Ohta, K. & Ochi, M.: Comparison of high soil moisture tolerance in seven warm season grasses. *Bull. Natl. Grassl. Res. Inst.*, 25, 37–47 (1983) [In Japanese with English summary].
15) Wilson, J. R., Brown, R. H. & Windham, W. R.: Influence of leaf anatomy on the dry matter digestibility of C_4, C_3 and intermediate types of *Panicum* species. *Crop Sci.*, 23, 141–146 (1983).

(Received for publication, November 8, 1984)