Comparison of Characteristics Related to Photosynthesis between NAD-malic Enzyme *Panicum* Species Differing .in the Chloroplast Position in Bundle Sheath Cell

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Introduction

Tropical forage grasses which are classified as C_4 species based on their photosynthetic mechanism show a high bio-mass productivity, especially under high light intensity and high temperature conditions. However, the cultivation of tropical forage grasses, being restricted in western Japan, covers only 1% of the total cultivation area of pasture plants, since the climatic conditions in Japan are not generally favorable for the growth of C_4 species.

Studies in the past decade have demonstrated that the C_4 species exhibits wide variations in photosynthesis, leaf anatomy and environmental adaptation[2,3,5]. In order to promote introduction of well-adapted tropical forage grasses into Japan, the author investigated various characteristics relating to photosynthesis, leaf anatomy and environmental effect in a large number of tropical C_4 grasses which were collected in Africa, USA and Australia. The finding with special reference to the new relationship between the photosynthesis and the leaf anatomy was reported earlier[8]. This paper reviews the reports published since then on the comparison of the characteristics related to photosynthesis, leaf anatomy and environmental effect between the two *Panicum* species which have differences in leaf anatomy.

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Leaf anatomical variation in NAD-malic enzyme (ME) species in C_4 photosynthesis

The C_4 species were divided into three subgroups on the basis of the activity of C_4-acid decarboxylating enzymes in the bundle sheath cells (BSC) as follows: NADP-ME, NAD-ME and phosphoenolpyruvate carboxykinase (PEP-CK) species[3]. In the Gramineae, these decarboxylation types have been known to be related to various leaf anatomical characteristics. The NAD-ME species is readily distinguished from the other two decarboxylation types in that the former has centripetally-arranged chloroplasts in the BSC, while the latter two types have centrifugally-arranged chloroplasts[3].

Some *Panicum* NAD-ME species, however, were found to have centrifugal chloroplasts in the BSC similar to the other two decarboxylation types[7]. The NAD-ME species with centrifugal chloroplasts in the BSC

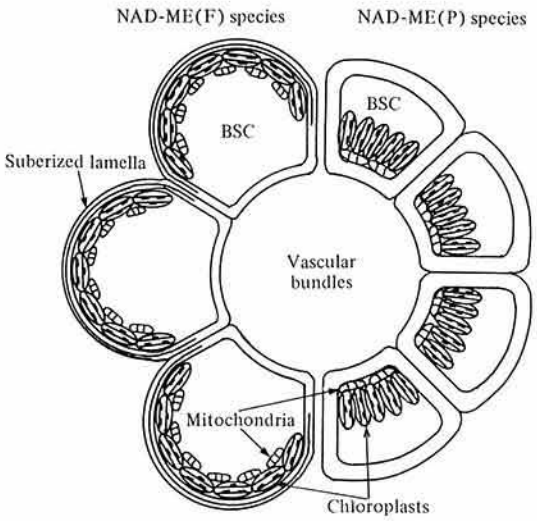

Fig. 1. Illustration of bundle sheath cells (BSC) of NAD-ME species with centrifugal chloroplasts in the BSC (NAD-ME (F) species, left side) and NAD-ME species with centripetal chloroplasts in BSC(NAD-ME(P) species, right side)

(designated as NAD-ME(F) species) were found in the *Dichotomiflora* group, a typical group of *Panicum*, which also contained the NAD-ME species with centripetal chloroplasts in the BSC (designated as NAD-ME(P) species)[12].

In addition to the chloroplast position, the NAD-ME(F) and NAD-ME(P) species differed from each other in some leaf anatomical characters (Fig. 1)[9]. The BSC of the NAD-ME(F) species, in comparison with that of NAD-ME(P) species, was larger in size with thin cell walls, which expanded widely into the mesophyll cell (MC) side, resulting in the increase of the area attached to the MC. It is likely that the shape of the BSC of the NAD-ME(F) species is associated with rapid transport of photosynthetic intermediate metabolites between the BSC and the MC.

Electron micrographs of transverse sections of the leaf blades of the NAD-ME(F) species revealed that mitochondria with well-developed cristae were arranged centrifugally and surrounded by chloroplasts, and that a suberized lamella was present in the BSC walls[12,14]. The relative position of chloroplasts and mitochondria in the BSC, i.e. the mitochondria inside and the chloroplasts outside for the NAD-ME(F) species was similar to that for the NAD-ME(P) species, although the suberized lamella was not present in the BSC walls of the NAD-ME(P) species. Therefore, the relative position of chloroplasts and mitochondria seems to be important for the NAD-ME type C_4 photosynthetic pathway to function, since the CO_2 released in the mitochondria is required to be trapped efficiently by the chloroplasts.

Carbon metabolism of the NAD-ME(F) species

The decarboxylation type of NAD-ME(F) species was identified only based on the activities of three kinds of decarboxylase: NADP-ME, NAD-ME and PEP-CK. In order to elucidate photosynthetic carbon metabolism of the NAD-ME(F) species, several experiments were conducted. In the analysis of the photosynthetic $^{14}CO_2$ fixation products for *P. dichotomiflorum*[13], a typical NAD-ME(F) species, the highest ^{14}C incorporation was observed in aspartate, one of the major C_4-dicarboxylic acid formed in the MC and transported to the BSC, at the end of pulse, while incorporation to malate, another C_4-dicarboxylic acid, was very small. During the chase, the ^{14}C was rapidly lost from aspartate, although the loss from malate was slow (Fig. 2). This result, together with the finding that no PEP-CK activity was detected in the whole leaf extract of the NAD-ME(F) species, indicates that the NAD-ME(F) species was an aspartate former and the basic photosynthetic mechanism for this species was identical with that for the NAD-ME(P) species. This result also indicates that there was no causal relationship between chloroplast position in the BSC and the NAD-ME type photosynthetic mechanism when assessed in a whole leaf.

On the other hand, intracellular distribution of the activity of sucrose phosphate synthase (SPS), which is a key enzyme in the regula-

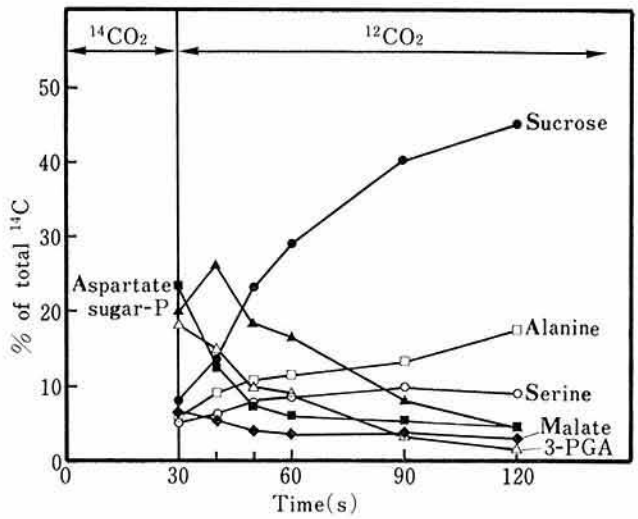

Fig. 2. Pulse-chase experiment with the leaves of *P. dichotomiflorum*, the NAD-ME (F) species
Leaves were exposed to $^{14}CO_2$-air for 30 sec and then transferred to $^{12}CO_2$-air in a closed system[13].

Table 1. Sucrose phosphate synthase activity in whole leaf extracts and percentage of activity located in mesophyll cells (MC) in NAD-ME (F) species and NAD-ME (P) species[1]

		Experiment 1 (Early reproductive)		Experiment 2 (Vegetative)		Experiment 3 (Early reproductive)	
		Activity in leaf	Percentage in MC	Activity in leaf	Percentage in MC	Activity in leaf	Percentage in MC
P. coloratum (cv. Kabulabula) (NAD-ME(F))	Light	105	58	111	62	70	114
	Dark	93	1	49	− 7	42	16
P. coloratum var. *makarikariense* (NAD-ME(P))	Light	138	33	127	50	158	25
	Dark	89	23	78	36	120	41

1) : Activity was measured at 12 : 00 (light) and at 23 : 00 (dark) and is expressed as umol g^{-1} fresh wt$\cdot h^{-1}$ [11].

tion of sucrose biosynthesis in leaves, was different between the NAD-ME(F) and NAD-ME(P) species (Table 1)[11]. In these two species, SPS activity was exhibited both in the MC and the BSC. In the light, however, the SPS activity was mainly detected in the MC for the NAD-ME(F) species, while mainly in the BSC for the NAD-ME(P) species. In the dark, for the NAD-ME(F) species, SPS activity in the MC decreased to a greater extent than that in the BSC, resulting in the major activity in the BSC similar to the NAD-ME(P) species. This result suggests an existence of difference in the compartmentation of sucrose biosynthesis process between the two species and a possible contribution of the chloroplast position in the BSC to this biochemical process.

Comparison of CO_2 gas exchange characteristics between the NAD-ME(F) species and NAD-ME(P) species

Carbon exchange rate in the leaves of the NAD-ME(F) species was relatively higher than that of the NAD-ME(P) species, although transpiration rate was not different from each other. The values of diffusive CO_2 conductance (Gc) and the ratio of intercellular CO_2 concentration to external CO_2 concentration (Ci/Ca) for the NAD-ME(F) species were also higher than those for the NAD-ME(P) species[6].

The $\delta^{13}C$ value, which is an indicator of discrimination of ^{13}C against ^{12}C is known to be different in the three decarboxylation types in the order of NADP-ME > PEP-CK > NAD-ME species[1]. The $\delta^{13}C$ values in the leaves of the NAD-ME(F) species were significantly higher than those of the NAD-ME(P) species, suggesting that CO_2 leakage from the BSC for the NAD-ME(F) species be lower than the case for the NAD-ME(P) species (Fig. 3)[14].

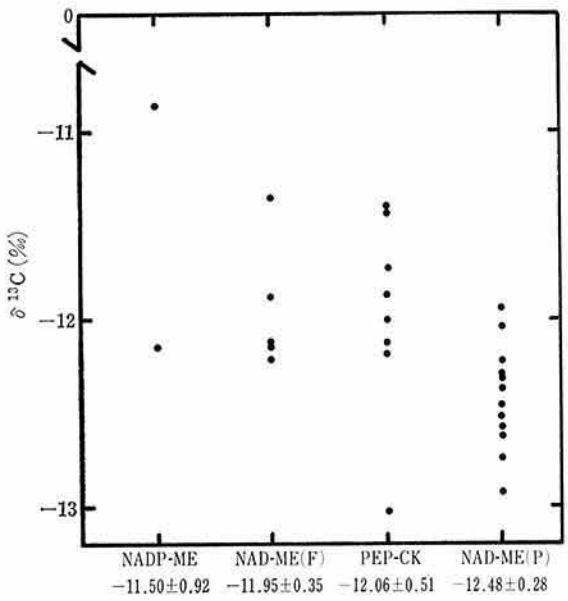

Fig. 3. Distribution of $\delta^{13}C$ values in C_4 *Panicum* species
Numbers are means and s. d. for the respective C_4 subtypes.
The differences between the NAD-ME (F) and NAD-ME (P) species were significant at 5 % level (student t test)[14].

Those values of Gc, Ci/Ca and $\delta^{13}C$ and the existence of suberized lamella in the BSC walls indicate that the NAD-ME(F) species fix CO_2 more efficiently than the NAD-ME(P) species do, by maintaining high intercellular CO_2 concentration through high Gc and minimizing CO_2 leakage from the BSC by the existence of the suberized lamella.

Comparison of growth and water requirement between the NAD-ME(F) species and NAD-ME(P) species

At the vegetative stage, the plants of the NAD-ME(F) species showed a more active growth in seedling, a higher relative growth rate and a more rapid development of succulent and thick leaves, compared to the plants of the NAD-ME(P) species. At the heading stage, the plants of the NAD-ME(F) species continued to grow at a higher rate. Therefore, the NAD-ME species produced a greater dry matter on a plant basis than the NAD-ME(P) species did[10].

Under low (65–70% of field moisture capacity) and high (85–90%) soil moisture conditions, water requirement for the dry matter production in the NAD-ME(F) species was larger than that in the NAD-ME(P) species (Table 2)[6]. Particularly, with the increase of soil moisture more water was required in the NAD-ME(F) species, while no change of water requirement took place in the NAD-ME(P) species. The dry matter increase in the top portion of the plants was greater under the high soil moisture condition than under the low moisture condition in both the NAD-ME(F) and NAD-ME(P) species, although the rate of increase was high in the former species. These results suggest that the NAD-ME(F) species be more adapted to high soil moisture conditions, compared to the

Table 2. Water requirement of NAD-ME(F) and NAD-ME(P) species of *Panicum*

	Low soil moisture[2]			High soil moisture[2]		
	Dry matter increase[3] (g/plant)	Water consumption[3] (ml/plant)	Water requirement (ml/g)	Dry matter increase[3] (g/plant)	Water consumption[3] (ml/plant)	Water requirement (ml/g)
1. NAD-ME (F)						
LAE (2)[1]	3.96	554.5	139.9	4.86	724.8	149.1
DIC (2)	4.34	518.7	119.6	4.50	576.7	129.0
KAB (2)	4.56	490.0	107.4	5.39	631.3	117.1
SOL (2)	2.52	303.9	120.5	4.16	540.6	130.0
mean±s.d.	3.85±0.92	466.8±112.0	121.9±13.4	4.73±0.53	618.4±80.2	131.3±13.2
2. NAD-ME (P)						
COL (2)	1.98	212.5	107.1	2.28	249.0	109.3
MAK (1)	1.66	162.8	98.2	1.85	171.4	92.8
STA (1)	1.19	118.4	99.7	1.50	124.0	82.8
mean±s.d.	1.61±0.40	164.6±47.1	101.7±4.8	1.87±0.39	181.5±63.1	95.0±13.4
1.—2.			20.2			36.3[4]

1) : LAE, *P. laevifolium;* DIC, *P. dichotomiflorum;* KAB, *P. coloratum* cv. Kabulabula; SOL, *P. coloratum* cv. Solai; COL, *P. coloratum* (excluding cv. Kabulabula and Solai); MAK, *P. coloratum* var. *makarikariense;* STA, *P. stapfianum.*

2) : Low (65—70% of field moisture capacity) and high (85—90% of field moisture capacity) soil moisture treatments were applied.

3) : Dry matter increase and water consumption were measured during 11-days and 14-days for the NAD-ME (F) and NAD-ME(P) species, respectively[6].

4) : $P < 0.05$.

NAD-ME(P) species. This is confirmed by the finding that some NAD-ME(F) species were tolerant to high soil moisture with a well developed aeration from root to shoot[15].

Discussions and conclusion

As listed in Table 3, the NAD-ME(F) species newly identified in the *Dichotomiflora* group of *Panicum* differed from the NAD-ME(P) species in various characteristics in addition to their chloroplast position. It is especially worthy of noting that the compartmentation of SPS activity in the light is different between the above two species. In the NADP-ME and PEP-CK species whose chloroplasts are located in the centrifugal position in the BSC, the SPS activity in the light is considered to be mainly in the MC as in the case of the NAD-ME(F) species[11]. These results suggest a high correlation of the chloroplast position with the compartmentation of sucrose biosynthesis, although the causal relationship between them remains unidentified.

Some of the NAD-ME(F) species are known to produce a high yield, comparable to the main tropical forage grasses in Japan such as guineagrass or rhodesgrass. The major difference in environmental effect between the two species is shown in their water requirement; the NAD-ME(F) species seem to be more adapted to high moisture condition. It is, therefore, expected that the NAD-ME(F) species, such as *P. coloratum* (cv. Kabulabula and Solai) and *P. dichotomiflorum*, are promising tropical forage grasses in an ill-drained field in the western part of Japan.

It might possibly be assumed that the chloroplast position in the BSC is differentiated as a result of adaptation to moisture conditions. The recent finding on the NAD-ME(F) species in *Eragrostis* (Chlorideae) confirms this postulation since this species predominates in the high-rainfall regions as

Table 3. Comparison of various characteristics between the NAD-ME (F) and NAD-ME (P) species of *Panicum* (Gramineae)[6]

Characteristics	NAD-ME (F)	NAD-ME (P)
Leaf anatomy		
Chloroplast position in BSC	Centrifugal	Centripeta
Suberized lamella in BSC walls	Present	Absent
MC/BSC area ratio	1.3±0.4	2.0±0.1
Photosynthesis		
Decarboxylating enzyme	NAD-ME	NAD-ME
Carbon exchange rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$, at 265 ppm CO_2)	32.3±1.0	26.5±1.4
Diffusive conductance (mol $CO_2 \cdot m^{-2} \cdot s^{-1}$)	0.24±0.02	0.16±0.02
δ ^{13}C value (‰)	−11.95±0.35	−12.48±0.28
Post-illumination CO_2 burst	Sharp peak	Sharp peak
Sucrose biosynthesis		
Sucrose phosphate synthase ($\mu mol \cdot gFW^{-1} \cdot h^{-1}$)	66.4±7.4	116.3±11.8
SPS distribution in MC and BSC Light	MC>BSC	MC<BSC
Dark	MC<BSC	MC<BSC
Dry matter production		
Early growth (up to 6–7 leaf stage) (g)	0.30±0.06	0.15±0.04
Yield at heading stage (g/plant)	149.4±30.7	50.7±2.9
Percentage dry weight at heading stage (%)	17.7±3.3	20.8±2.4
Water requirement (ml/g)	122—131	95—102
Representative species		
Species in the *Dichotomiflora* group	*P. coloratum* (cv. Kabulabula) (cv. Solai)	*P. coloratum* (cv. Klein)
	P. dichotomiflorum	*P. coloratum* var. *makarikariense*
	P. laevifolium	*P. stapfianum*
Species in the other group		*P. capillare*
		P. miliaceum

well as in the humid coastal areas of Australia[16]. However, in order to identify the causal relationship between the chloroplast position in the BSC and adaptation to moisture condition, further studies are required, including investigations on a route of water movement in the leaf and on progenies obtained from hybridazation between the NAD-ME (F) and the NAD-ME (P) species.

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