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The Intercellular Distribution of Glycine Decarboxylase in Leaves of Cassava in Relation to the Photosynthetic Mode and Leaf Anatomy*

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Abstract : There have been conflicting reports on the photosynthetic mode of cassava (*Manihot esculenta* Crantz). It has been suggested that cassava is a C_3-C_4 intermediate plant and, alternatively, that it is a C_3 plant. Glycine decarboxylase (GDC), a photorespiratory enzyme, is known to exhibit different patterns of intercellular distribution between C_3-C_4 intermediate and C_3 leaves. In order to elucidate the photosynthetic mode of cassava, we investigated the localization of GDC in the leaves of three cultivars by immunogold electron microscopy and the anatomical structures of the leaves. The leaves of cassava are essentially hypostomatous, and the photosynthetic tissues consist of palisade mesophyll cells (PMCs), spongy mesophyll cells (SMCs) and bundle sheath cells (BSCs). Although the BSCs include centrifugally located chloroplasts, they show no increase in mitochondrial frequency. Labeling specific for GDC was found on all mitochondria of all three types of cell. However, the density of labeling in the PMCs was always higher than in the SMCs and BSCs. In leaves that developed under a water deficit, the difference in labeling density became even clearer. These data suggest that at least the cultivars of cassava examined here are not C_3-C_4 intermediates and should be regarded as C_3 plants. However, the intercellular differences in the level of accumulation of GDC seem to merit further investigation with respect to an internal gradient of photorespiratory capacity in the leaves.
Key words : Cassava, Glycine decarboxylase, Hypostomatous leaf, Immunogold localization, Photorespiration, Photosynthetic mode.

キャッサバ葉におけるグリシンデカルボキシラーゼの細胞間分布-光合成型並びに葉の解剖学的特性との関連

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要　旨：キャッサバ（*Manihot esculenta* Crantz）の光合成型については，C_3-C_4 中間型という報告と C_3 型という報告とがある．光呼吸の鍵酵素であるグリシンデカルボキシラーゼ（GDC）は，C_3-C_4 中間型と C_3 型との間で葉における細胞間分布のパターンが異なることが知られている．本研究ではこの点に着目して，キャッサバ3品種の葉内における GDC の分布を金コロイド免疫電子顕微鏡法により調査するとともに，葉の解剖構造を観察することにより光合成型を検討した．キャッサバの葉は基本的には下面気孔葉の特徴をもち，光合成組織は柵状葉肉細胞，海綿状葉肉細胞および維管束鞘細胞から構成されていた．維管束鞘細胞は遠心的に配列した葉緑体を含んでいたが，ミトコンドリアの密度の増加は観察されなかった．GDC の存在を表す金粒子の標識がこれらの光合成細胞のすべてのミトコンドリアに見出され，C_3 型の細胞間分布を示した．しかし，柵状葉肉細胞のミトコンドリアにおける金粒子の標識密度は，海綿状葉肉細胞や維管束鞘細胞のそれより高い値を示し，より濃密に GDC 蛋白質を蓄積しているものと考えられた．水不足の下で形成された葉では，この細胞間の標識密度の差はより顕著となった．以上の結果は，少なくとも実験した3品種のキャッサバは C_3-C_4 中間植物ではなく，むしろ C_3 植物であることを示している．なお，細胞間による GDC の蓄積量の違いについては，葉内部における光呼吸活性の勾配の点からさらに調査する価値があるものと考えられる．
キーワード：下面気孔葉，キャッサバ，グリシンデカルボキシラーゼ，光合成型，光呼吸，免疫電子顕微鏡法．

Cassava is an important root crop in tropical regions. There have been many reports on the photosynthetic traits and the dry-matter production of cassava[12,16,25]. Cassava is one of the most productive crops in dry regions with infertile soil[16]. El-Sharkawy et al. reported that the cassava plant is intermediate between C_3 and C_4 plants with respect to the nature of CO_2 gas exchange and the biochemical aspects of photosynthesis[4,7,8,9]. Other workers also investigated the photosynthetic traits of cassava, but they failed to find any traits

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typical of C_3-C_4 intermediate plants[1,5,12].

C_3-C_4 intermediate species have been found in some genera, such as *Flaveria*[5,21]. Among crop plants, only cassava has been reported to be a C_3-C_4 intermediate, although there are conflicting reports. C_3-C_4 intermediate plants exhibit reduced apparent photorespiration, as compared with C_3 plants. In C_3 plants, the efficiency of carbon assimilation is reduced by high levels of photorespiration[5,21]. C_3-C_4 intermediate photosynthesis provides plants with an advantage at high leaf temperatures, compared to C_3 plants[13]. In order to understand the physiological traits of cassava, it is important to determine whether cassava is a C_3-C_4 intermediate plant or a C_3 plant.

It has recently been demonstrated that, in leaves of C_3-C_4 intermediate plants and C_4 plants, a photorespiratory enzyme, glycine decarboxylase (GDC), is present predominantly in the mitochondria of the bundle sheath cells (BSCs) but is absent from those of the mesophyll cells (MCs)[10,14]. In leaves of C_3 plants, GDC accumulates in the mitochondria of the MCs as well as in those of the BSCs[10,24]. GDC has a pivotal role in the photorespiratory glycolate pathway and it catalyzes the conversion of glycine to serine, CO_2 and NH_3[17]. It is generally accepted that the accumulation of GDC in the BSCs is the primary factor responsible for the reduced apparent photorespiration of C_3-C_4 intermediate species[21].

In order to elucidate the photosynthetic mode of cassava, we investigated the intercellular localization of GDC in the leaves by immunogold electron microscopy. In addition, we examined the anatomical structures of leaves of cassava. The results obtained suggest that examined cassava plants should not be regarded as C_3-C_4 intermediates. They demonstrate that cassava has a unique pattern of intercellular accumulation of GDC of a type that has not previously been reported. Possible relationships between the anatomical features of leaves and the intercellular distribution of GDC are discussed.

Materials and Methods

1. Plant materials and growth conditions

Three cultivars of cassava (*Manihot esculenta* Cranz) were supplied as stem cuttings by the Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan. In these cultivars, Rayong 3 is a recommended cultivar in Thailand. Others are cultivars that were introduced from Southeast Asia and have been conserved in JIRCAS, and they were tentatively named Okinawa and Izu[15]. The stem cuttings were planted in 1/2,000a or 1/5,000a Wagner pots filled with field soil and were grown outdoors in Tsukuba in the summer of 1995. Plants were watered daily and supplied with full strength Hoagland nutrient solution at weekly intervals. After 1.5 months (mid August), young and fully expanded leaves were used for experiments.

Cassava plants (Rayong 3), planted in 1/5,000a Wagner pots, were also grown in a naturally illuminated growth cabinet that was maintained at 25°C/20°C (day/night temperature), and watered daily. Full strength Hoagland nutrient solution (500 m*l*/pot) was supplied at weekly intervals. A water deficit was achieved by withholding water and the nutrient solution for 3 weeks. The leaf water potential was measured for fully expanded leaves at weekly intervals with a psychrometer (C-52 sample chamber and HR-33T dew point microvoltmeter; Wescor Inc., USA). Leaves that had newly expanded during this treatment were used for the examination.

Three C_3 species, *Spinacia oleracea* L., *Chenopodium album* L. and *Oryza sativa* L. (cv. Nipponbare), a C_3-C_4 intermediate, *Flaveria anomala* B.L. Robinson, and a C_4 species, *Echinochloa crus-galli* (L.) Beauv., were used as controls for immunocytochemical examination and assay of phosphoenolpyruvate (PEP) carboxylase. Their seeds were sown in 1/5,000a Wagner pots that had been filled with field soil. Plants were grown in a greenhouse during May and August. Fully expanded leaves were examined.

2. Light and electron microscopy and immunocytochemical staining

Light and electron microscopic observations and immunocytochemical examination were performed as described previously[27,28]. Immunostaining was performed with the antiserum described below and a suspension of Protein A-colloidal gold particles (E.Y. Lab. Inc., San Mateo, CA, USA). The density of labeling was determined by counting the gold particles on micrographs enlarged to a magnification of 24,000 X and calculating the num-

ber per unit area (μm^2). Between 18 and 40 individual mitochondria were examined on several immunolabeled sections in each case.

3. Antiserum

Antiserum that had been raised against the P protein of GDC from mitochondria of pea leaves was generously provided by Dr. D.J. Oliver (University of Idaho, Moscow, ID, USA). In order to examine the cross-reactivity of the antiserum with enzyme proteins from leaves of cassava, SDS-polyacrylamide gel electrophoresis and western blotting were performed as described by Ueno[28]. The antiserum gave strong bands on the blots, indicating that it was a reliable tool for the immunocytochemical localization of GDC in sections of leaves.

4. Assay of enzymatic activity and quantitation of chlorophyll

The activity of PEP carboxylase and the chlorophyll content of samples were determined as described previously[26].

Results

1. Anatomical features

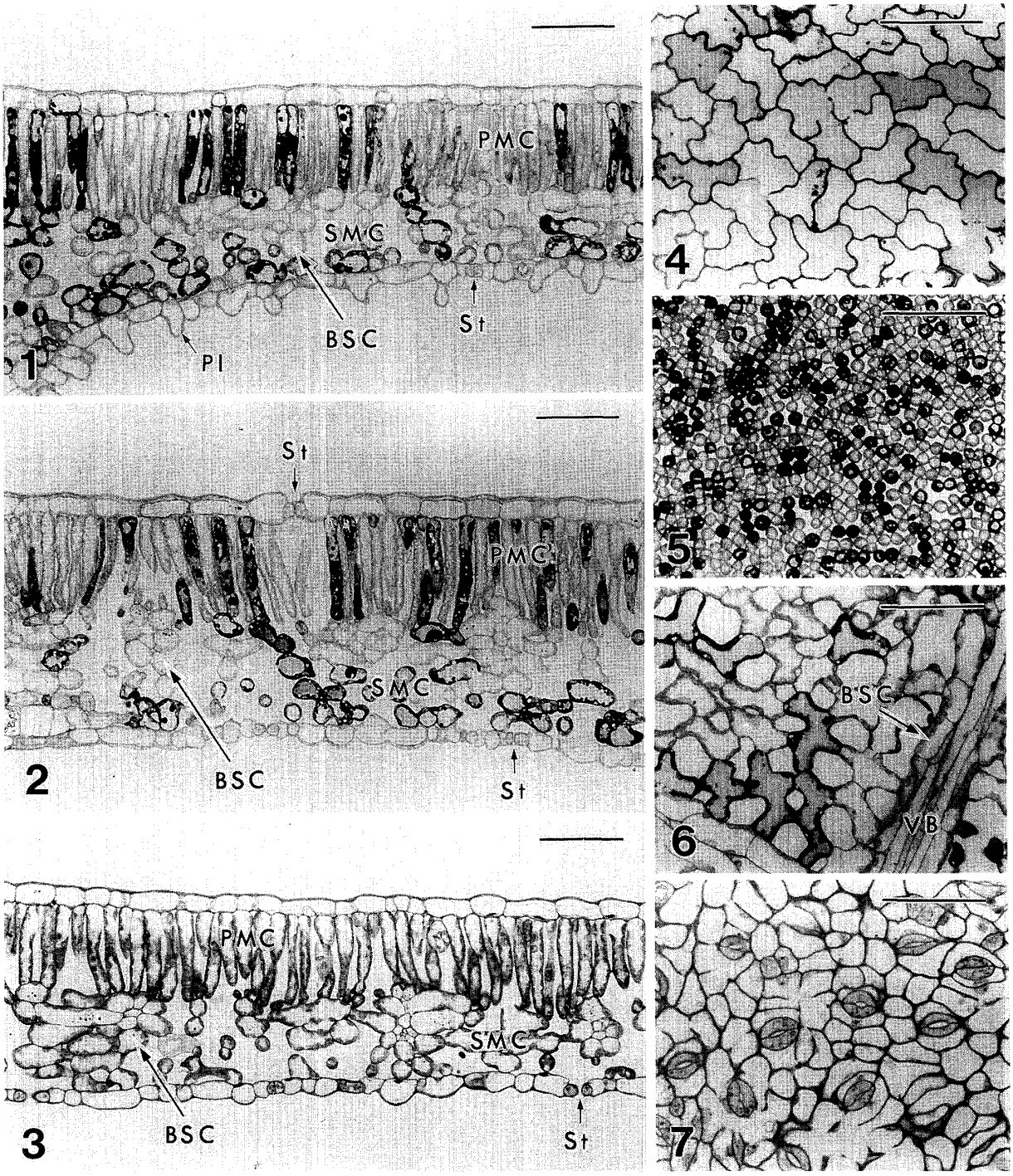
The photosynthetic tissues of leaves of cassava were composed of three kinds of cell, namely, the palisade mesophyll cells (PMCs), the spongy mesophyll cells (SMCs) and the BSCs (Figs. 1-3). The PMCs and the SMCs were not arranged radially. The PMCs which were shaped like narrow cylinders were densely packed below the adaxial epidermis. The length of the PMCs was equal to approximately one half of the thickness of the leaf. Intercellular spaces were very small, as was more clearly recognizable in paradermal sections (Fig. 5). The SMCs formed several layers below the PMCs, with large intercellular spaces. In paradermal sections, the SMCs were irregular in shape and were often branched (Fig. 6). In the two cultivars Okinawa and Izu, both the PMCs and the SMCs frequently contained dense dark materials (Figs. 1 and 2). The BSCs surrounded vascular bundles and were similar in appearance to the SMCs. In paradermal views, the BSCs were elongated parallel to the vascular bundles and were distinguishable in terms of shape from the SMCs (Fig. 6). A few stomata were observed, only around veins, on the adaxial epidermis (Figs. 2 and 4), as reported previously[15]. Below the stomata, the arrangement of the PMCs was disorganized as a result of the formation of substomatal cavities (Fig. 2). Many stomata were seen on the abaxial epidermis (Figs. 1-3, 7). Therefore, the leaves of the three cultivars appeared to be basically hypostomatous. In the Okinawa cultivar, the abaxial epidermis formed papillae (Fig. 1).

2. Ultrastructural features

The PMCs and the SMCs contained many chloroplasts with dense stroma (Figs. 8-11). The chloroplasts tended to be slender in shape in the PMCs and lenticular in the SMCs. As a result, the chloroplasts in the PMCs were longer than those in the SMCs. Some PMCs and SMCs accumulated electron-dense materials in their cytosol (Fig. 8). However, organelles in these cells were similar in terms of structure to those in the PMCs and SMCs without such materials. The BSCs also contained a considerable number of chloroplasts, which tended to be located centrifugally in each cell (Fig. 8). The chloroplasts contained grana and were similar in shape to the chloroplasts in the SMCs (Fig. 9). The chloroplasts in the three types of photosynthetic cell contained starch grains in varying numbers (Figs. 8-11). The epidermal cells contained small chloroplasts (Fig. 12). The BSCs of C_3-C_4 intermediate plants have been reported to contain large numbers of mitochondria[3,10]. However, the BSC of cassava did not contain many mitochondria (Fig. 8), and they were indistinguishable in terms of the number of mitochondria from the SMCs. The BSCs lacked suberized lamellae in their cell walls, as did the PMCs and the SMCs (Fig. 9).

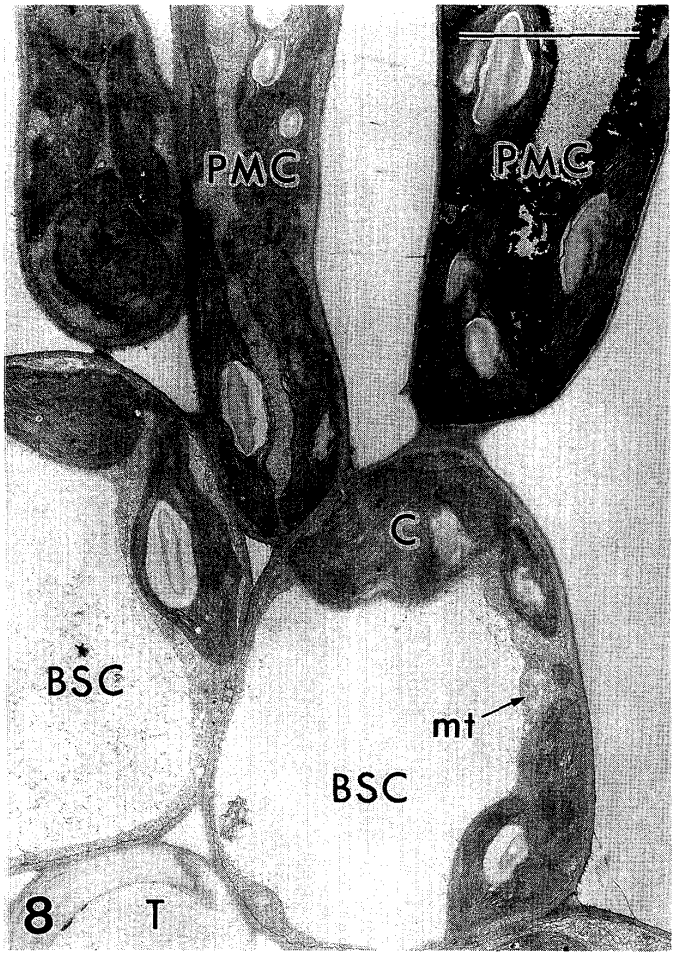
3. Immunogold localization of GDC

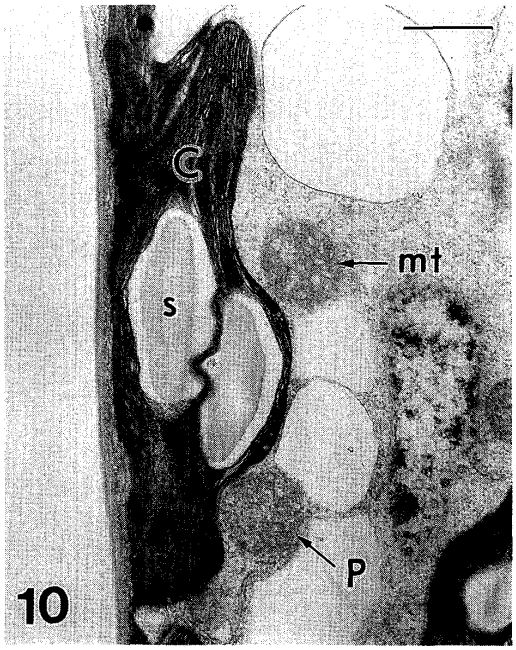
Representative species with different modes of photosynthesis were examined as controls for the immunocytochemical localization of GDC. When sections of leaves of a C_3 plant, spinach, were incubated with the antiserum against GDC, labeling of gold particles specific for GDC was found on all mitochondria of the MCs and the BSCs (data not shown). In leaves of a C_3-C_4 intermediate species, *Flaveria anomala*, the gold labeling specific for GDC was recognized only on the mitochondria of the BSCs (Fig. 14) and not on those of the MCs (Fig. 13). The leaves of a C_4 species, *Echinochloa crus-galli*, showed the same pattern of labeling of GDC as those of *F. anomala*

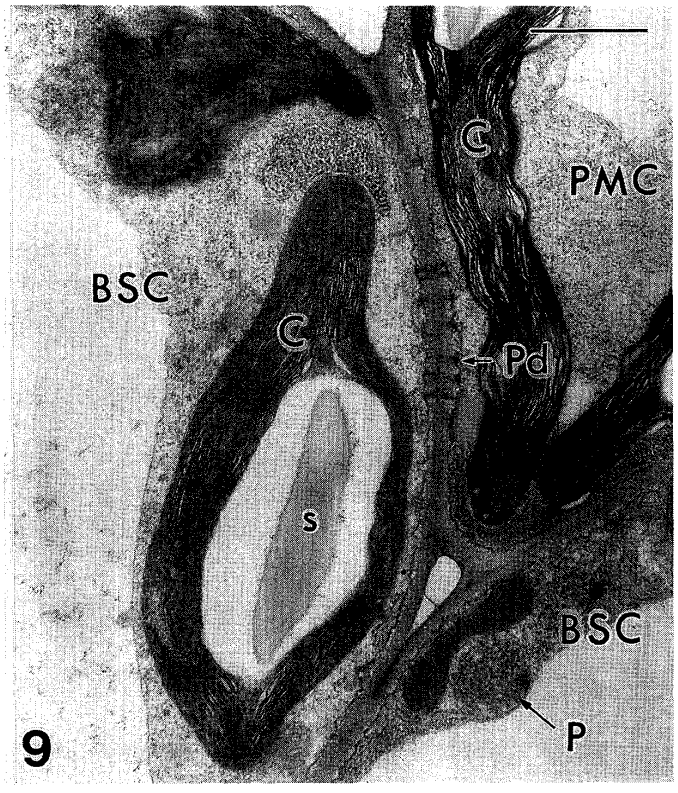


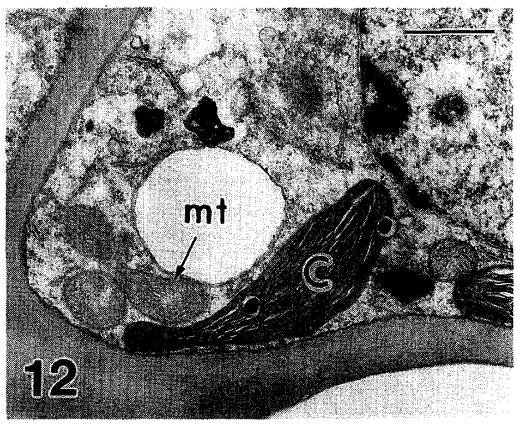
Figs. 1-7. Anatomical structures of leaves of cassava. Fig. 1. Cross section of a leaf of cv. Okinawa. Note papillae on the abaxial epidermis. Fig. 2. Cross section of a leaf of cv. Izu. Note a stoma on the adaxial epidermis. Fig. 3. Cross section of a leaf of cv. Rayong 3. Figs. 4-7. Paradermal sections of a leaf of cv. Okinawa. Fig. 4. Adaxial epidermis without stomata. Fig. 5. Palisade mesophyll cells. Fig. 6. Spongy mesophyll cells and bundle sheath cells. Fig. 7. Abaxial epidermis with stomata. Bars = 50 μm. PMC, Palisade mesophyll cell; SMC, spongy mesophyll cell; BSC, bundle sheath cell; Pl, papilla; St, stoma; VB, vascular bundle.

Figs. 8-12. Inner ultrastructure of leaves of cassava (cv. Okinawa). Fig. 8. Bundle sheath cells and palisade mesophyll cells. Note a palisade mesophyll cell (right) that contains electron-dense materials.









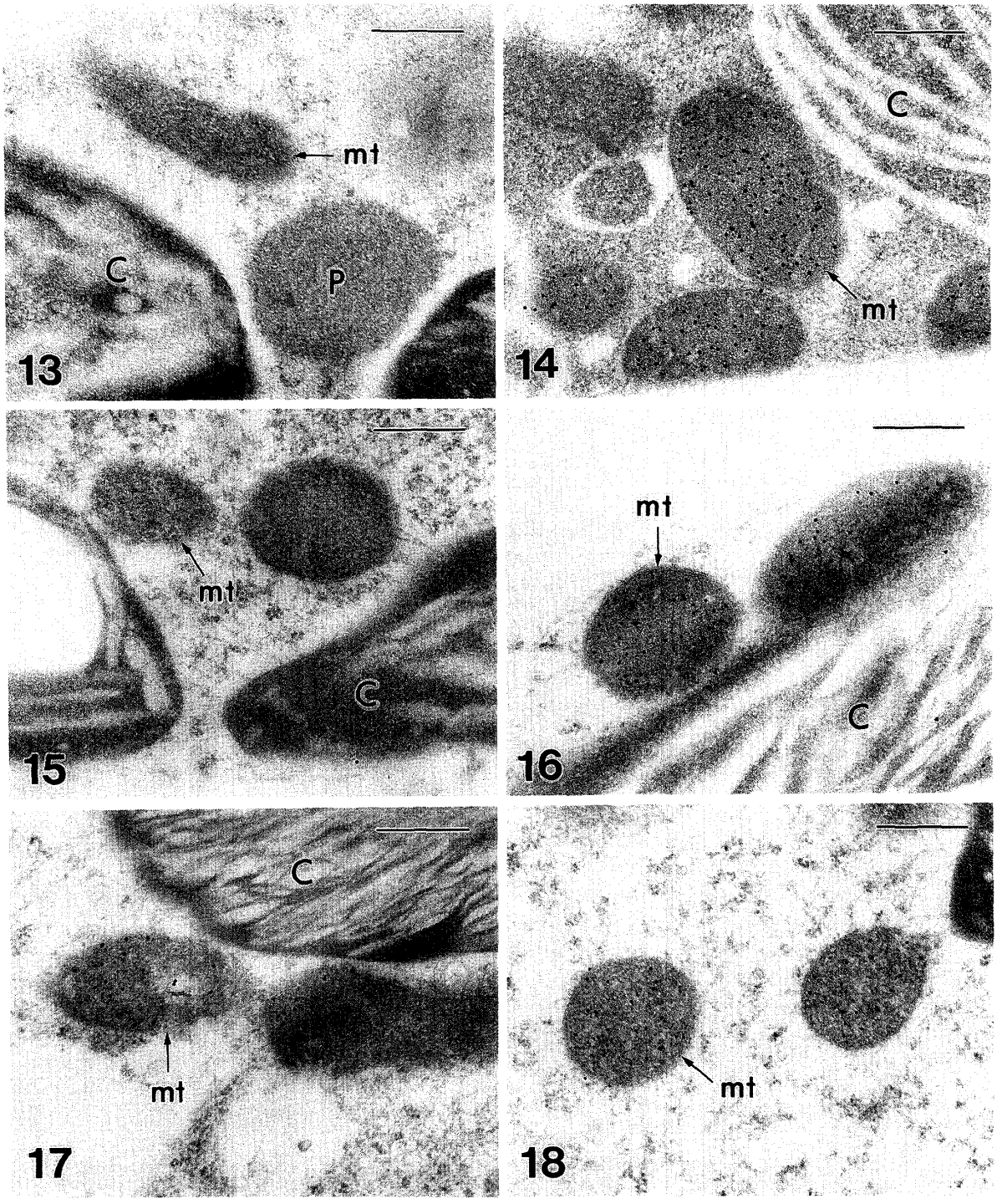


Fig. 9. Bundle sheath cells and a palisade mesophyll cell. The cell walls of the bundle sheath cells lack suberized lamellae. Fig. 10. A palisade mesophyll cell. Fig. 11. A spongy mesophyll cell. Fig. 12. An abaxial epidermal cell containing chloroplasts and mitochondria. Bar in Fig. 8=4 μm. Bars in Figs. 9-12=1 μm. C, Chloroplast ; mt, mitochondrion ; P, peroxisome ; Pd, plasmodesma ; s, starch grain ; T, tracheary element. Other abbreviations as in Figs. 1-7.

Figs. 13-18. Immunogold localization of the P protein of glycine decarboxylase in leaves of *Flaveria anomala* (Figs. 13 and 14) and leaves of cassava cv. Rayong 3 grown under well-watered conditions (Figs. 15-18). Fig. 13. A mesophyll cell. Fig. 14. A bundle sheath cell. Fig. 15. Immunogold labeling of a palisade mesophyll cell with nonimmune serum. Fig. 16. A palisade mesophyll cell. Fig. 17. A spongy mesophyll cell. Fig. 18. A bundle sheath cell. Bars=0.4 μm. Abbreviations as in Figs. 8-12.

Table 1. Immunogold labeling of the P protein of glycine decarboxylase in the photosynthetic cells of leaves of cassava.

Cultivar	Growth condition	Cell fraction	No. of gold particles (μm^{-2})		
			PMC	SMC	BSC
Okinawa	Well-watered	Mit	208.0±9.2 (34)	150.0±6.0 (32)	158.9±11.5 (31)
		Cyt	5.6±0.8 (13)	2.8±0.4 (13)	5.8± 0.8 (14)
Izu	Well-watered	Mit	99.4±4.9 (38)	65.9±4.3 (40)	62.7± 4.2 (29)
		Cyt	3.0±0.4 (14)	3.3±0.4 (13)	4.0± 0.6 (16)
Rayong 3	Well-watered	Mit	116.6±4.9 (33)	84.4±4.0 (30)	75.9± 3.2 (40)
		Cyt	3.5±0.4 (12)	2.9±0.3 (11)	3.5± 0.4 (17)
Rayong 3	Water deficit	Mit	127.0±4.2 (27)	74.9±4.4 (29)	62.8± 3.7 (18)
		Cyt	3.8±0.7 (17)	1.7±0.3 (13)	1.9± 0.3 (9)

Numbers of gold particles per unit area (μm^2) are given as means ± SE. Numbers in parentheses show the number of profiles examined. PMC, Palisade mesophyll cells; SMC, spongy mesophyll cells; BSC, bundle sheath cells. Mit, mitochondria; Cyt, cytosol and organelles other than mitochondria.

Table 2. Chlorophyll contents and activities of PEP carboxylase in leaves of cassava and several control plants.

Species and cultivar	Chlorophyll (mg m^{-2})	PEP carboxylase (μmol mg^{-1}chl h^{-1})
Cassava		
Okinawa	497	31
Izu	286	—
Rayong 3	304	118
Controls		
Chenopodium album (C_3 species)	—	91
Oryza sativa cv. Nipponbare (C_3 species)	—	182
Flaveria anomala (C_3-C_4 intermediate)	—	275
Echinochloa crus-galli (C_4 species)	—	1,662

Values are means of results from 4 experiments for chlorophyll content and from 2 to 3 experiments for PEP carboxylase. —, not determined.

(data not shown). These data confirmed the differences in intercellular distribution of GDC among plants with different modes of photosynthesis that have already been established[10,14].

When sections of leaves of cassava were incubated with nonimmune serum, no specific labeling was recognized (Fig. 15). When they were incubated with the antiserum against GDC, labeling specific for GDC was observed on all the mitochondria of the PMCs (Fig. 16), the SMCs (Fig. 17) and the BSCs (Fig. 18). In the case of the cytosol and organelles other than mitochondria in these cells, no significant labeling was recognized (Figs. 16-18). The mitochondria in the cells in vascular bundles were hardly ever labeled with the antiserum against GDC. The mitochondria in the epidermal cells were also almost never labeled, even though these cells contained small chloroplasts, indicating that expression of GDC is associated with photosynthetically active cells[24].

The density of labeling of gold particles was examined for the mitochondria of the three types of photosynthetic cell in leaves of the three cultivars that had been grown under well-watered conditions (Table 1). In all cultivars, the density of labeling of mitochondria in the PMCs was always higher than in the SMCs and the BSCs. The relative densities of labeling of the PMCs, the SMCs and the BSCs were 1 : 0.72 : 0.76 for Okinawa (Fig. 19A), 1 : 0.66 : 0.63 for Izu (Fig. 19B) and 1 : 0.72 : 0.65 for Rayong 3 (Fig. 19C).

Rayong 3 plants were grown under a water

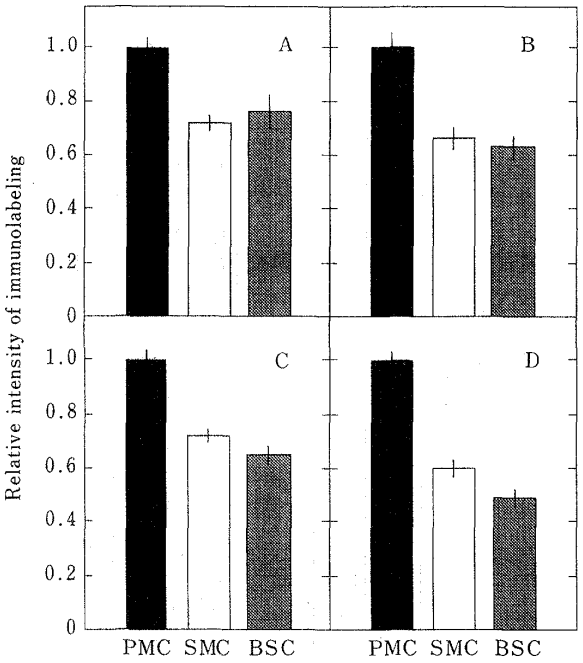


Fig. 19. Relative intensities of immunolabeling with gold particles of the P protein of glycine decarboxylase in the mitochondria of different photosynthetic cells of leaves of cassava. Based on the data from Table 1. A, cv. Okinawa (well-watered) ; B, cv. Izu (well-watered) ; C, cv. Rayong 3 (well-watered) ; D, cv. Rayong 3 (water deficit). PMC, Palisade mesophyll cells ; SMC, spongy mesophyll cells ; BSC, bundle sheath cells. Standard errors are indicated at the top of bars (n=18 to 40).

deficit, and newly expanded leaves were then examined immunocytochemically. The leaf water potential of the plants declined from −0.60 to −0.87 MPa during treatment. Such small decline in leaf water potential of cassava plants subjected to water stress was also reported by Palta[19]. Under the conditions, the water-stressed plants are known to cause reduction in leaf conductance and net-photosynthesis[19]. The pattern of intercellular distribution of GDC in the leaves of the plants grown under water dificit was similar to that of plants grown under well-watered conditions, which was described above. However, differences in the density of labeling between the mitochondria of the PMCs and those of the SMCs and those of the BSCs in the water-stressed plants were clearer than those in the well-watered plants (Table 1). The ratio of the labeling densities in the former plants was 1 : 0.60 : 0.49 (Fig. 19D).

4. Activities of PEP carboxylase

The activity of a key C_4 enzyme, PEP carboxylase, was assayed in extracts of leaves of cassava grown under well-watered conditions (Table 2). The measured activities were within the range of those of the control C_3 species, and they were lower than those of the C_3-C_4 intermediate, *F. anomala*, and the C_4 species, *E. crus-galli*. Considerable variations in activities and chlorophyll contents were also found among the cultivars (Table 2).

Discussion

1. Is cassava a C_3-C_4 intermediate or a C_3 plant?

Mahon et al. reported that the CO_2 compensation point (68 $\mu l/l$) of leaves of cassava was typical of a C_3 plant[12]. However, El-Sharkawy et al. reported that cassava has traits intermediate between those of C_3 and C_4 plants with regard to the CO_2 compensation point (25 to 29 $\mu l/l$), the post illumination CO_2 burst and initial products of photosynthesis, and suggested the hypothesis that cassava might be a C_3-C_4 intermediate plant[4,7,8]. By contrast, Edwards et al. found no evidence to support the hypothesis[6]. The conflicting results might be due to differences in growth conditions[9]. In spite of their use of cassava plants that included some of the same cultivars as those examined by El-Sharkawy et al., as well as the same growth conditions of plants, Angelor et al. also failed to find any biochemical and physiological traits of C_3-C_4 intermediate plants[1]. These data suggest that the conflicting results cannot be explained by differences in growth conditions, and they do not support the hypothesis that cassava might change aspects of photosynthetic metabolism in response to different growth conditions. Our data on the activities of PEP carboxylase support the possibility that cassava is a C_3 plant.

In leaves of C_3-C_4 intermediate plants, the MCs are arranged more or less radially around the vascular bundles, and the BSCs contain large numbers of organelles, such as chloroplasts and mitochondria[3,5,21]. In terms of the metabolism in C_3-C_4 intermediate plants, the concentration of mitochondria in the BSCs is associated with the confined expression of GDC, as mentioned below. In leaves of cassava, the BSCs contained considerable numbers of chloroplasts but not of

mitochondria, and the MCs were not arranged radially. From these structural features, it is concluded that leaves of cassava have a C_3 type of anatomy, rather than C_3-C_4 intermediate anatomy.

It is prerequisite that, in leaves of C_3-C_4 intermediate plants, GDC be confined to the mitochondria of the BSCs. Since release of CO_2 from photorespiration occurs predominantly in the BSCs as a result of such localization of GDC, considerable amounts of CO_2 are probably captured by ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) before CO_2 is released from leaves. As a result, C_3-C_4 intermediate plants exhibit reduced rates of apparent photorespiration, as compared to C_3 plants[10,21]. Some intermediate plants also exploit a C_4 cycle with such an ingenious mechanism[5]. The present study showed unambiguously that, in leaves of cassava, GDC accumulates at significant levels in all mitochondria of the MCs, as well as of the BSCs, although a cell-specific difference in the density of labeling was detected and the magnitude of the difference was intensified under a water deficit. These data indicate that examined cassava plants have a C_3 pattern of cellular localization of GDC, and they support the claim that cassava is a C_3 plant.

El-Sharkawy et al. proposed the hypothesis that, in leaves of cassava, a C_4 cycle might operate in the PMCs while the C_3 pathway operates in the SMCs and the BSCs. The co-functioning of both mechanisms might then lead to the apparent expression of an intermediate mode of photosynthesis[7]. Therefore, we examined the cellular distributions of two key C_4 enzymes, PEP carboxylase and pyruvate, orthophosphate dikinase, using the same immunocytochemical technique as that described here. However, we found no significant labeling specific for these C_4 enzymes in the PMCs. This result reflects the low levels of activity of PEP carboxylase in leaves of cassava. Thus, we suggest that at least the cultivars of cassava examined are not C_3-C_4 intermediates and should be regarded as C_3 plants.

2. Difference in the extent of accumulation of GDC in mitochondria between the PMCs and the SMCs

The mitochondria in PMCs were labeled more strongly with the antiserum against GDC than those in the SMCs and the BSCs. In general, the amount of antigen (enzyme protein) on a section is reflected by the density of immunolabeling with gold particles[2]. In leaves of cassava, therefore, it seems likely that GDC accumulates more densely in the mitochondria of the PMCs than in those of the SMCs and the BSCs. To our knowledge, this is the first report that the extent of accumulation of GDC can vary cell-specifically in mesophyll tissue.

In leaves of cassava, the PMCs are packed tightly while the SMCs are arranged loosely with large intercellular spaces. Thus, when the total numbers of organelles in each type of mesophyll tissue are compared on the basis of leaf area, those in the palisade mesophyll are significantly higher than those in the spongy mesophyll. As a result, the difference in the amount of GDC between the two types of mesophyll becomes larger than that determined from a comparison of the density of labeling of mitochondria, suggesting that the palisade mesophyll might have considerably higher GDC activity and a much greater capacity for photorespiration than the spongy mesophyll. In leaves of spinach, the chloroplasts of the PMCs have higher rates of electron transport and CO_2-fixation and larger amounts of Rubisco, on the basis of chlorophyll content, than those of the SMCs[23]. The difference in levels of accumulation of GDC in leaves of cassava might also be associated with such biochemical differences between the types of mesophyll tissue. However, a preliminary study of leaves of spinach and soybean indicated that there is no clear difference in the density of labeling of GDC between the mitochondria of the PMCs and the SMCs (unpublished data).

The leaves of cassava are essentially hypostomatous, while those of spinach and soybean are amphistomatous. If the activity of GDC is higher in the palisade mesophyll than in the spongy mesophyll of cassava leaves, such activity might be advantageous for the recycling of the CO_2 formed by the decarboxylation of glycine because of the long path-length for diffusion of CO_2. This situation is reminiscent of the cellular sites of GDC in leaves of C_3-C_4 intermediate plants. However, many investigators have reported high rates of apparent photorespiration, typical of C_3 plants, in leaves of cassava, as discussed

above[1,6,12]. It has been suggested that intercellar gaseous diffusion might limit the photosynthetic carbon assimilation, especially in hypostomatous leaves[20,22], and the partial pressure of CO_2 should not be treated as uniform throughout the mesophyll[20]. If such is the case in the leaves of cassava, the CO_2 partial pressure in the palisade mesophyll might be lower than that in the spongy mesophyll.

It has been proposed that photorespiration and the Mehler-peroxidase pathway play roles in sustaining electron transport and in protection from photoinhibition[18]. Both processes promote non-assimilatory electron transport and stimulate the photon utilization during CO_2-limited photosynthesis in bright light[18]. They might become important when plants are subjected to stress, such as a water deficit or high temperature under high levels of solar radiation, because rates of electron transport associated with the photosynthetic carbon assimilation are reduced as a result of stomatal closure[11]. The same situation might also arise in thick leaves with a long path-length for CO_2 diffusion and in leaves with dense tissue[11]. It will be of interest to examine whether the intercellular differences in the accumulation of GDC in cassava leaves can be explained in such terms. In this regard, it may be worth noting that, in leaves that developed under water deficit, the difference in the relative density of labeling of GDC in mitochondria between the two types of cell became clearer than that in leaves that had developed under well-watered conditions (Fig. 19D *vs.* 19C). More direct biochemical studies are required if we are to understand the physiological significance of the intercellular distribution of GDC in leaves of cassava.

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