Environmental Regulation of C$_3$ and C$_4$ Differentiation in the Amphibious Sedge *Eleocharis vivipara*[1]

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The biochemical and physiological characteristics of C$_4$ photosynthesis have been researched and clarified in detail. We now know that the differentiation of two cell types, mesophyll cells (MC) and bundle sheath cells (BSC), is required for efficient C$_4$ photosynthesis. Thus, the leaves of C$_4$ plants have more complicated structural and functional features than those of C$_3$ plants (Hatch, 1999; Kanai and Edwards, 1999). Current studies are focused on elucidating the regulatory mechanisms of genetic and developmental events in C$_4$ photosynthesis, but most of them are as yet poorly understood (Dengler and Nelson, 1999; Sheen, 1999). There is much indirect evidence that C$_4$ plants have evolved in parallel from C$_3$ plants among diverse taxonomic groups (Kellogg, 1999). Some C$_3$-C$_4$ intermediate plants, such as *Flaveria* spp., provide a suitable system for studying the possible process of evolution of C$_4$ plants (Ku et al., 1991; Westhoff et al., 1997). It is becoming clear that there is diversity not only in the structural and biochemical features, but also in the genetic and developmental aspects of C$_4$ photosynthesis (Dengler and Nelson, 1999; Sheen, 1999; Edwards et al., 2001).

This article reviews the available data on the differentiation of photosynthetic characteristics in some amphibious species of *Eleocharis* in the Cyperaceae, with particular reference to the sedge *Eleocharis vivipara. E. vivipara* has a unique nature that expresses C$_4$ characteristics under terrestrial conditions and C$_3$ characteristics under submerged aquatic conditions (Ueno et al., 1988). This characteristic is unknown in other C$_4$ plants, and this plant provides an excellent opportunity to investigate the development of C$_4$ photosynthesis in response to environmental factors. The amphibious species of *Eleocharis* are also useful for increasing our understanding of the ecological and adaptive aspects of C$_4$ plants.

C$_3$ AND C$_4$ PHOTOSYNTHESIS

In the C$_3$ pathway, CO$_2$ is fixed by Rubisco and is synthesized into carbohydrate. This metabolic pathway operates only in the MC. Leaves of C$_4$ plants display Kranz anatomy, in which vascular bundles are surrounded by an outer layer of MC and an inner layer of BSC (Dengler and Nelson, 1999). In the C$_4$ pathway, CO$_2$ is fixed initially by phospho*enol*pyruvate carboxylase (PEPC), which is localized in the MC, forming C$_4$ acids (malate and Asp). The C$_4$ acids are transported to the BSC, where they are decarboxylated by C$_4$-acid-decarboxylating enzymes. The released CO$_2$ is incorporated in the C$_3$ cycle by the operation of Rubisco. In the process of decarboxylation of the C$_4$ acids, pyruvate is also formed. This C$_3$ compound is returned to the MC and used for regeneration of phospho*enol*pyruvate by pyruvate, Pi dikinase (PPDK). The operation of the C$_4$ cycle results in the increased concentration of CO$_2$ at the active site of Rubisco in the BSC and suppression of the oxygenase reaction of Rubisco. As a consequence, C$_4$ photosynthesis is more efficient than C$_3$ photosynthesis under some environmental conditions (Hatch, 1999; Kanai and Edwards, 1999).

In general, most plants use one of these photosynthetic modes: Leaves of rice and maize fix carbon through the C$_3$ pathway and the C$_4$ pathway, respectively, although the reproductive organs may use different photosynthetic pathways (Imaizumi et al., 1990; Langdale and Nelson, 1991). Environmental factors influence the expression of photosynthetic machinery in some plants. Well-known examples occur in plants with Crassulacean acid metabolism (CAM), such as common ice plant (*Mesembryanthemum crystallinum*), and in some submerged aquatic plants, such as *Hydrilla verticillata*. In the former, a switch from C$_3$ to CAM mode is induced by NaCl stress (Winter and Smith, 1996), whereas in the latter a change from C$_3$ to C$_4$ metabolism occurs if CO$_2$ is limited in the water (Bowes and Salvucci, 1989; Reiskind et al., 1997). In these facultative CAM plants and aquatic plants, the photosynthetic carbon metabolism operates in a single cell, and no differentiation of two cell types is required. In contrast, C$_4$ photosynthesis requires structural differentiation and biochemical specialization of photosynthetic cells. Therefore, con-

version between C$_3$ and C$_4$ modes may not be as easy for plants as those between other modes.

DISCOVERY OF C$_3$ AND C$_4$ DIFFERENTIATION IN *E. VIVIPARA*

Many C$_4$ species occur in the monocotyledonous family Cyperaceae, and most of them thrive in relatively wet habitats. Thus, the C$_4$ group of the Cyperaceae represents ecologically unusual C$_4$ plants (Ueno and Takeda, 1992). Several genera that include both C$_3$ and C$_4$ species, such as *Cyperus*, *Rhynchospora*, and *Eleocharis*, are found in this family (Takeda et al., 1980; Bruhl et al., 1987). At the New York Botanical Garden, I extensively screened the photosynthetic modes of *Eleocharis* spp. by examining herbarium specimens. This study revealed that most species in the genus are C$_3$ plants, but some species possess characteristics of C$_4$ and C$_3$-C$_4$ intermediate plants (Ueno et al., 1989). Furthermore, this study led to a more important discovery that the amphibious species *E. vivipara* exhibits Kranz anatomy in the photosynthetic tissues of the terrestrial form, but exhibits non-Kranz anatomy in those of the submerged form. Subsequent biochemical studies with fresh plants in Dr. Miyachi's laboratory demonstrated that *E. vivipara* can display C$_3$ or C$_4$ characteristics, depending on the environmental conditions (Ueno et al., 1988).

E. vivipara occurs in the margins of ponds, marshes, swamps, and wet ditches in Florida (Wunderlin, 1998). The plants grow continuously in conditions ranging from completely aerial to semi-aerial and semi-submerged, to completely submerged aquatic (Fig. 1A).

STRUCTURAL AND BIOCHEMICAL CHARACTERISTICS OF PHOTOSYNTHESIS IN *E. VIVIPARA*

In *E. vivipara*, the leaf blades are reduced, and the culms function as photosynthetic tissues, as seen in all other members of *Eleocharis* spp. Some amphibious plants exhibit heterophylly between aerial and aquatic leaves (Sculthorpe, 1967; Smith and Hake, 1992). This is the case in culms of *E. vivipara* (Fig. 1B). The terrestrial form has erect, hard culms. In contrast, the submerged form shows a hair-like morphology consisting of slender, soft culms, and reproduces new culms by proliferation from sterile spikelets at the apex of the culms.

The dimorphism is clearer in the anatomical features of the culms (Ueno et al., 1988; Ueno, 1996a). The culms of the terrestrial form show Kranz anatomy, but the structure is complex: There are three bundle sheaths—the outermost parenchymatous
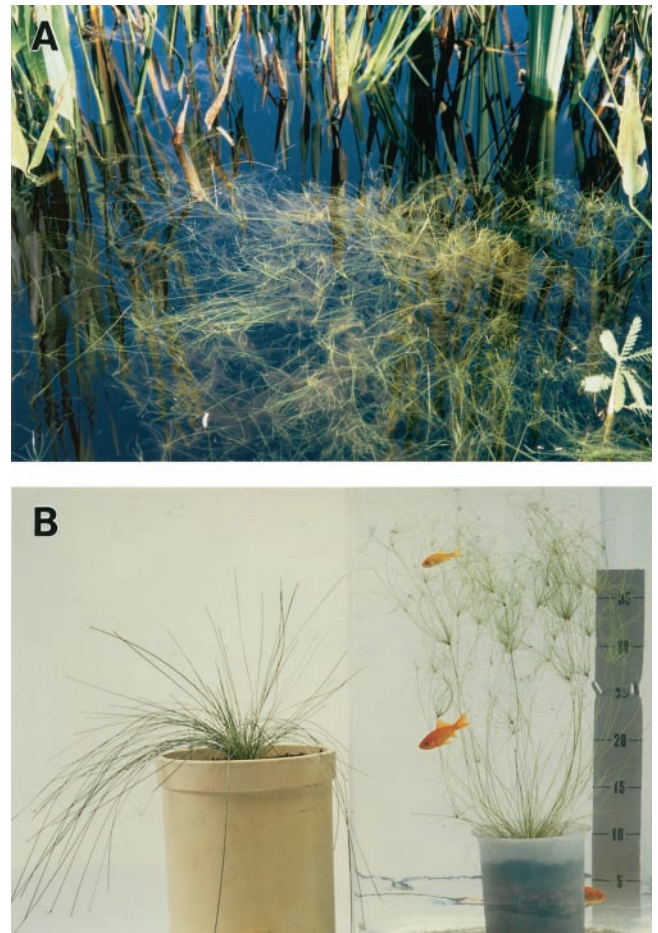

Figure 1. A, The submerged form of *E. vivipara* growing in a creek (about 50 cm deep). B, Gross morphologies of the terrestrial form (left) and the submerged form (right) of *E. vivipara*.

sheath, the middle mestome sheath, and the innermost organelle-rich sheath—together with the MC (Fig. 2A). The innermost BSC (Kranz cells) include many granal chloroplasts and large mitochondria. In contrast, the culms of the submerged form lack Kranz anatomy (Fig. 2B). The MC have a spherical shape, forming one or two layers inside the epidermis. The innermost BSC are small and include only a few small chloroplasts and mitochondria. As a result, the MC to BSC volume ratio is much higher in the submerged form than in the terrestrial form. The culms of the submerged form lack stomata, and the vascular bundles are reduced both in size and number compared with those in the terrestrial form.

The ^{14}C pulse-^{12}C chase experiment indicates that the terrestrial form shows a pattern of ^{14}C labeling characteristic of C$_4$ plants, forming Asp and malate as initial photosynthetic products. However, the submerged form shows a C$_3$ pattern of labeling, forming C$_3$ compounds as initial products (Ueno et al., 1988). The terrestrial form has high activities of C$_4$ enzymes, such as PEPC, PPDK, and NAD-malic enzyme (ME), whereas in the submerged form these activities are
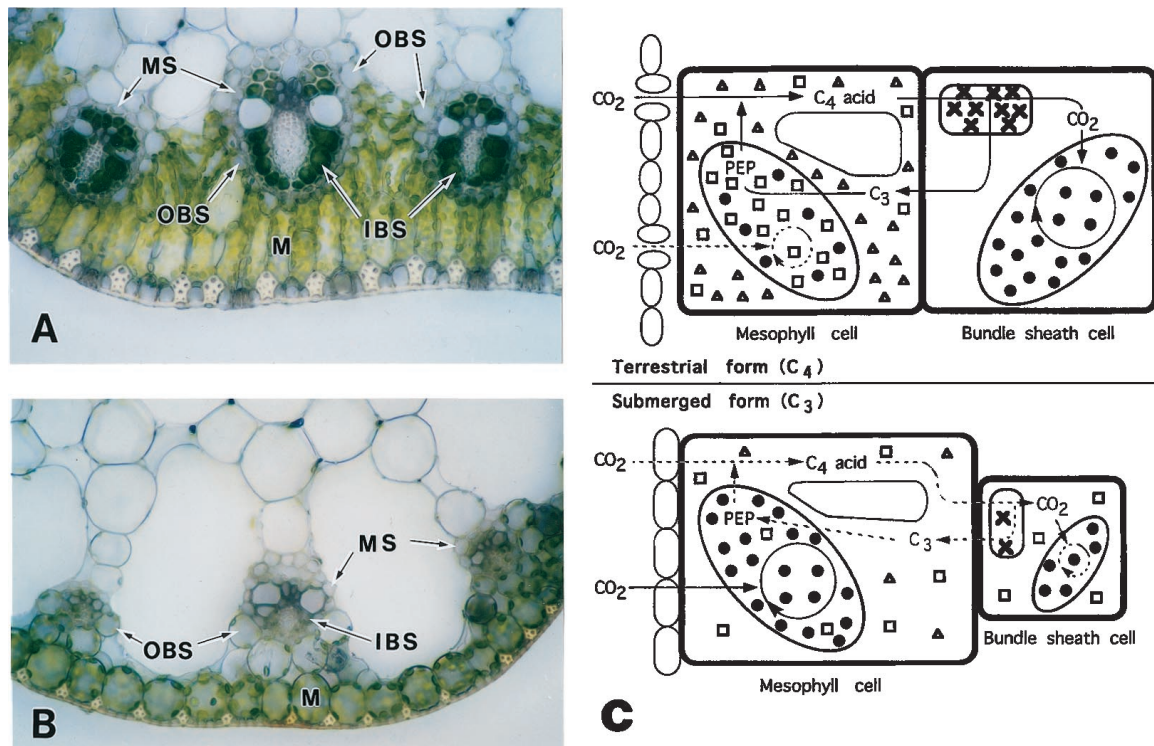
Figure 2. Structural and biochemical features of the terrestrial and submerged forms of *E. vivipara*. A, Culm anatomy of the terrestrial form (Kranz type). B, Culm anatomy of the submerged form (non-Kranz type). M, MC; OBS, outermost BSC; MS, mestome sheath cells; and IBS, innermost BSC (Kranz cells). C, Cellular localization of C_3 and C_4 enzymes and proposed scheme for photosynthetic carbon metabolism in the terrestrial and submerged forms. Only the MC and the innermost BSC (Kranz cells) are shown. The outermost BSC and the middle mestome sheath cells are omitted, because the former are functionally equivalent to the MC and the latter lack photosynthetic enzymes. Solid and dashed lines represent the proposed major and minor fluxes, respectively, of photosynthetic intermediates. The circles show the C_3 pathway. ●, Rubisco; △, PEPC; □, PPDK; ×, NAD-ME; C_3, C_3 compound.

low. Rubisco activity in the submerged form is almost the same as, or higher than, that in the terrestrial form (Ueno et al., 1988; Ueno, 1998a). These trends in enzymatic activity have also been confirmed by the amounts of these enzyme proteins present after immunoblotting (Ueno, 1996b).

To understand the photosynthetic pathways operating in the two growth forms, it is important to determine in which cells and compartments the photosynthetic enzymes are localized (Fig. 2C). Immunogold localization studies reveal that in the terrestrial form PEPC is localized in the cytosol of both the MC and the outermost BSC (Fig. 3A), and NAD-ME is found in the mitochondria of the innermost BSC. In the submerged form, these enzymes occur in the same sites as in the terrestrial form, but at low levels (Fig. 3B; Ueno, 1996b, 1998a). PPDK is also distributed in the chloroplasts of the MC and the outermost BSC, and at higher levels in the terrestrial form than in the submerged form. In addition, cytosolic PPDK is found in the photosynthetic cells of both growth forms, which is unusual in C_4 leaves (Ueno, 1996b). In both the terrestrial and submerged forms, Rubisco is distributed in the chloroplasts of the MC, the outermost BSC, and the innermost BSC (Ueno, 1996b).

This cellular distribution of Rubisco is unknown in typical C_4 plants. In C_4 species of the Cyperaceae, such as *Fimbristylis* and *Cyperus*, which also show unusual Kranz anatomy, Rubisco is restricted to only the innermost BSC (Ueno, 1998b).

From these studies, it is evident that the NAD-ME-dependent C_4 pathway is operative in the terrestrial form (Fig. 2C). However, the culms of the terrestrial form of *E. vivipara* show slightly more negative $\delta^{13}C$ values than those of other *Eleocharis* spp. with Kranz anatomy (Ueno et al., 1988, 1989). An inhibitor of PEPC, 3,3-dichloro-2-(dihydroxyphosphinoylmethyl)-propenoate (Jenkins, 1989), does not completely suppress photosynthesis in the terrestrial form, despite the fact that it completely inhibits photosynthesis of a typical C_4 species of *Fimbristylis* (O. Ueno and K. Ishimaru, unpublished data). Thus, some CO_2 may be fixed by Rubisco (probably through the C_3 cycle) present in the MC, even though most CO_2 is fixed through the C_4 pathway. Such features are reminiscent of those of C_4-like plants of *Flaveria* spp. (Cheng et al., 1989), but they may not be identical, because in *E. vivipara* PEPC and NAD-ME are compartmentalized between the MC and the BSC. In the submerged form the MC are the main photosynthetic tissues,
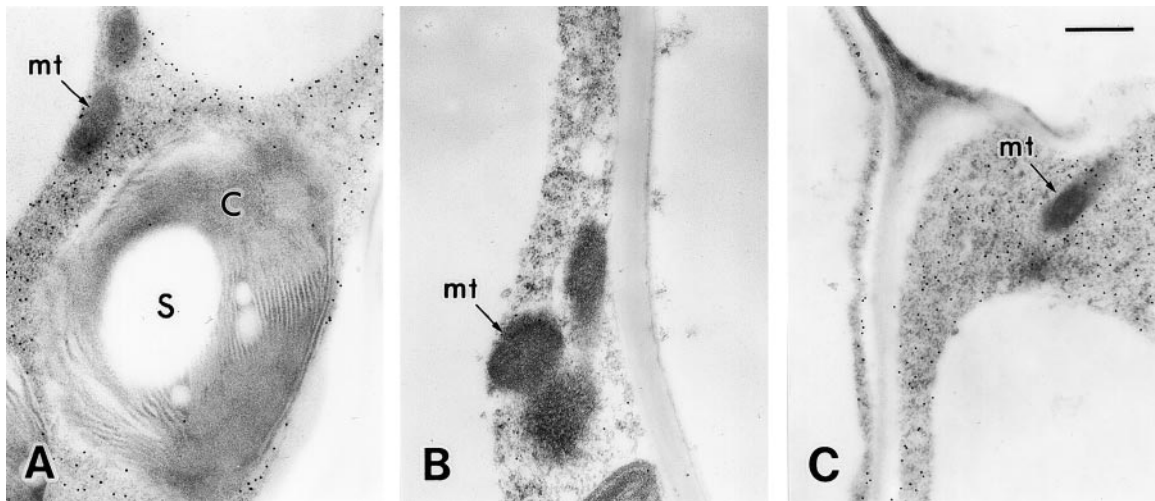
Figure 3. Immunogold localization of PEPC in photosynthetic cells of *E. vivipara* (Ueno, 1998a). A, Outer BSC of the terrestrial form. B, Outer BSC of the submerged form. C, Outer BSC and MC (left margin) of ABA-induced tissue. Scale bar = 0.5 μm. C, Chloroplast; S, starch grain; mt, mitochondrion.

because of the reduction of the BSC. In the MC of the submerged form, both PEPC and Rubisco are present, but the level of PEPC is low relative to that of Rubisco. Thus, it is suggested that CO_2 in water is fixed mainly by Rubisco and the C₃ cycle in the chloroplasts of the MC (Fig. 2C; Ueno, 1996b). Under water conditions of low carbon, the proportion of ^{14}C incorporated into C₄ compounds is higher than under conditions of high carbon. However, the turnover of ^{14}C in C₄ compounds is very slow (Ueno, 1998a). Therefore, it is thought that even though the ME-dependent C₄ cycle also operates in the submerged form, the contribution to total carbon flux is not large. It is concluded that cellular regulation of photosynthetic enzyme accumulation, the unusual localization of Rubisco, and the ana-

Figure 4. Environmental and hormonal regulation of the conversion of structural and biochemical traits involved in photosynthetic mechanisms in *E. vivipara*.

tomical differentiation of photosynthetic tissues are the main factors responsible for the expression of C₃ and C₄ characteristics in *E. vivipara* (Ueno, 1996a, 1996b).

PLASTIC EXPRESSION OF C₃ AND C₄ CHARACTERISTICS IN *E. VIVIPARA* IN FLUCTUATING ENVIRONMENTS

Transfer experiments demonstrate that the two growth forms of *E. vivipara* can change reversibly into one another. When submerged plants are exposed to air, the culms wither from the rapid drying. However, plants produce new culms, which possess both Kranz anatomy and the C₄ biochemical traits. When terrestrial plants are immersed in water, the plants develop new culms with intermediate characteristics and finally with C₃ characteristics. It takes several weeks for the switch from the submerged to the terrestrial form and several months for the reverse change (Ueno et al., 1988). When the submerged form is growing underwater, the tips of the culms often reach the water surface. The plants then develop aerial culms at these tips, together with culms floating at the water surface. The aerial culms possess both Kranz anatomy and C₄ biochemical traits, despite the fact that the underwater culms have non-Kranz anatomy and C₃ characteristics. The floating culms show intermediate anatomies between Kranz and non-Kranz types. Therefore, it appears that different photosynthetic modes operate within a single plant and between joined tissues in contrasting environments. Therefore, plants growing in habitats with fluctuating water levels may possess various culms with different anatomical and biochemical features. It seems that the environmental response of *E. vivipara* represents a very plastic expression of C₃ and C₄
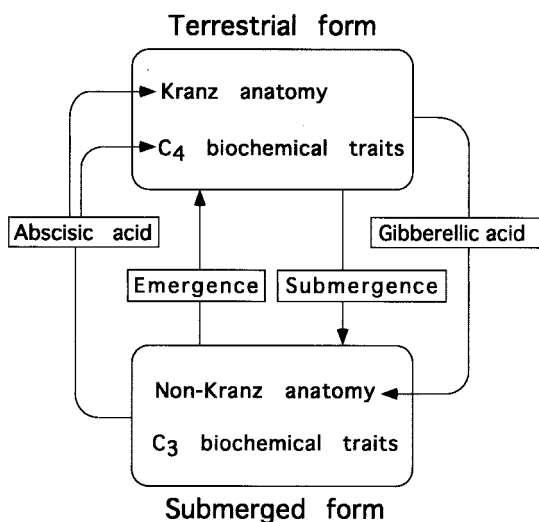
characteristics accompanied by tissue differentiation within a single plant.

HORMONAL REGULATION OF C$_3$ AND C$_4$ DIFFERENTIATION IN *E. VIVIPARA*

The heterophylly in aquatic plants is controlled by various environmental and hormonal factors (Smith and Hake, 1992). Although abscisic acid (ABA) is considered to be a stress hormone in plants, there is evidence that it is involved in the determination of leaf identity in some heterophyllic aquatic plants (Goliber and Feldman, 1989). It is thought that when plant shoots emerge from underwater into the air, the concomitant osmotic stress and higher light levels induce ABA production, which leads to the development of aerial leaves (Goliber and Feldman, 1989). In fact, when submerged *E. vivipara* are grown in an aqueous solution of ABA, they begin to develop new culms with Kranz anatomy (Ueno, 1998a). The ABA-induced tissues have several times more C$_4$ enzyme activity than have tissues of untreated submerged plants and accumulate large amounts of C$_4$ enzymes at the appropriate cellular sites (Fig. 3C). They exhibit a C$_4$-like pattern of ^{14}C fixation under aqueous conditions of low carbon, indicating enhanced C$_4$ capacity in the tissues (Ueno, 1998a). These facts imply that in *E. vivipara* ABA acts as a trigger for the regulatory cascade of complex developmental processes that lead to the formation of Kranz anatomy and C$_4$ biochemical characteristics (Fig. 4).

ABA has been reported to induce CAM in some facultative CAM plants (Chu et al., 1990; McElwain et al., 1992; Edwards et al., 1996; Taybi and Cushman, 1999). However, no stimulatory effects of ABA on C$_4$ enzymes have been observed in maize (Sugiharto et al., 1992), C$_4$ species of *Flaveria*, or the obligate CAM species *Kalanchoë daigremontiana* (Chu et al., 1990). It would be interesting to examine whether a similar signaling system that leads to changes in photosynthetic metabolism in response to environmental stimuli and that is mediated by ABA might have evolved simultaneously in facultative CAM species and *E. vivipara* (Ueno, 1998a). The induction of PEPC and NADP-ME by ABA has also been reported in a submerged aquatic plant, *Egeria densa*, which expresses C$_4$ metabolism under high temperature and light conditions, which limit CO$_2$ availability (Casati et al., 2000).

There is no evidence that a single gene is capable of setting in motion the entire C$_4$ machinery. Thus, C$_4$ photosynthesis appears to be a combination of independently inherited characteristics (Brown and Bouton, 1993). Our understanding of the molecular basis of the control of C$_4$ differentiation is still limited. However, transcriptional regulators suggesting a specific role in the differentiation of cell types of maize leaves have recently been reported (Hall et al., 1998; Rossini et al., 2001). When gibberellic acid is exogenously applied to terrestrial *E. vivipara* plants, the plants develop new tissues, without stomata, that are similar to the non-Kranz type tissues of submerged plants. Nevertheless, the tissues show a high accumulation of C$_4$ enzymes (Fig. 4; O. Ueno and M. Kai, unpublished data). This fact suggests that in *E. vivipara*, the structural and biochemical characteristics of C$_4$ photosynthesis are not always differentiated in a coordinated manner, implying that separate signaling systems are responsible for the individual differentiation of structural and biochemical characteristics. Similar results have also been observed in differentiation of these characteristics during the transition from the terrestrial to the submerged form (Uchino et al., 1998).

EXPRESSION OF C$_3$ AND C$_4$ PHOTOSYNTHETIC GENES IN *E. VIVIPARA*

It is intriguing to address how C$_3$ and C$_4$ differentiation is regulated within *E. vivipara* at the molecular level. At present, this molecular basis remains to be uncovered, but the isolation and expression analysis of several genes encoding C$_3$ and C$_4$ enzymes have been performed (Agarie et al., 1997a, 1997b; Baba et al., 1997; Uchino et al., 1998). It is thought that the difference in levels of C$_3$ and C$_4$ enzymes between the two growth forms of *E. vivipara* is regulated largely at a transcriptional level of the corresponding genes (Agarie et al., 1997a, 1997b; Baba et al., 1997; Uchino et al., 1998). This is clearly observed in plants growing under semi-submerged and semi-aerial conditions; the underwater culms show lower expression of genes for PEPC and PPDK, whereas the aerial culms show enhanced expression of the genes. The culms floating at the water surface reveal intermediate expression of the genes (Agarie et al., 1997b). The intercellular patterns of expression of genes for PEPC, PPDK, and the large and small subunits of Rubisco correspond well with the patterns of accumulation of the enzyme proteins (Baba et al., 1997; Uchino et al., 1998).

The kinetic properties of PEPC in *E. vivipara* differ between the terrestrial and submerged forms. The K_m for phospho*enol*pyruvate of the terrestrial form's PEPC is intermediate between those of typical C$_3$ and C$_4$ plants, whereas that of the submerged form's PEPC is C$_3$-like (O. Ueno, unpublished data). It is possible that several isogenes for PEPC are expressed in *E. vivipara*, as is the case in other plants (Cushman et al., 1989; Kawamura et al., 1992; Westhoff et al., 1997), and that the expression patterns of respective isogenes differ between the terrestrial and submerged forms. Recently, a gene for PEPC, which probably encodes a PEPC expressed most strongly in the terrestrial form, has been isolated (Agarie et al., 1997b). Homology research shows that this PEPC is located between a cluster of C$_4$-form PEPCs from C$_4$ grasses and a cluster consisting of C$_3$-form PEPCs

from C_3 and C_4 species and a CAM-form PEPC from the facultative CAM species *M. crystallinum*.

Although a gene for chloroplastic PPDK is strongly expressed in the leaves of C_4 plants (Sheen, 1999), a gene for cytosolic PPDK and a gene for chloroplastic PPDK are simultaneously expressed in the culms of *E. vivipara* (Agarie et al., 1997a). Both genes are more strongly expressed in the terrestrial form than in the submerged form. In the terrestrial form, the gene for chloroplastic PPDK is more highly expressed than the other gene, whereas in the submerged form, the reverse trend is found. In general, cytosolic PPDK seems to be involved in functions other than photosynthesis (Moons et al., 1998). However, it has recently been reported that in some CAM plants, both chloroplastic and cytosolic PPDKs are accumulated in the leaves (Kondo et al., 2000). In the facultative CAM plant *Kalanchoë blossfeldiana*, coordinated accumulation of both PPDKs is observed during enhanced CAM expression, suggesting that they are involved in CAM function (Kondo et al., 2001). At present, it is not known whether the unusual pattern of PPDK expression in *E. vivipara* is related to its unique C_3/C_4 property.

The ABA-induced culms of the submerged plants exhibit high expression of the genes for PEPC and PPDK. When culms formed before ABA treatment of submerged plants (which lack Kranz anatomy) are exposed to ABA solution, they also exhibit high expression of the genes for PEPC and PPDK (Agarie et al., 1997b). Thus, it seems again that expression of these genes can occur without coordinated differentiation of Kranz anatomy.

VARIATION IN EXPRESSION OF PHOTOSYNTHETIC MODES IN AMPHIBIOUS SPECIES OF *ELEOCHARIS*

In the genus *Eleocharis*, two other amphibious species, *Eleocharis baldwinii* and *Eleocharis retroflexa* subsp. *chaetaria*, show C_4 characteristics in their terrestrial forms (Ueno et al., 1989). The responses of these species to aquatic environments differ from that of *E. vivipara* (Table I; Uchino et al., 1995; Ueno et al., 1998).

The terrestrial forms of *E. baldwinii* and *E. retroflexa* possess structural and biochemical characteristics of the NAD-ME-type C_4 plant (Table I). The culms also exhibit unusual Kranz anatomy in which the mestome sheath is interposed between the MC and the BSC. The chloroplasts of the MC, as well as those of the BSC, accumulate Rubisco, but less than in *E. vivipara* (Ueno, 2000). Consistent with the enzyme distribution, the $\delta^{13}C$ values of the terrestrial forms of *E. baldwinii* and *E. retroflexa* are less negative than those of *E. vivipara*, although the values of the three species are within C_4 range (Ueno et al., 1989). The activities of the C_4 enzymes in the terrestrial forms of all three amphibious species are high, ranging in the following order: *E. vivipara* less than *E. baldwinii* less than *E. retroflexa*. The inhibitory effects of 3,3-dichloro-2-(dihydroxyphosphinoylmethyl)-propenoate on photosynthesis also show the same trend. Thus, the terrestrial forms of *E. baldwinii* and *E. retroflexa* also differ from typical C_4 plants, and there is a gradient in the degree of expression of C_4 characteristics among the terrestrial forms of the three species.

When *E. baldwinii* is growing under submerged conditions, it develops culms with traits intermediate between C_3 and C_4 biochemistry (Uchino et al., 1995). The BSC become small, and the MC develop well. In the MC, the amount of Rubisco becomes higher than that in the terrestrial form, and the amount of PEPC becomes lower (Ueno, 2000). Despite a decrease in NAD-ME activity, NADP-ME activity is maintained or slightly increased in the submerged form. There is evidence that a Kranz-less C_4 metabolism is operative in some submerged aquatic plants such as *Hydrilla* and *Egeria* spp. (Bowes and Salvucci, 1989; Reiskind et al., 1997; Casati et al., 2000). A possible unicellular C_4 metabolism has recently been reported in a marine diatom, *Thalassiosira weissflogii* (Reinfelder et al., 2000). In *Hydrilla* spp. and *Egeria* spp., enhanced expression of NADP-ME is observed during induction of C_4 metabolism (Magnin et al., 1997; Casati et al., 2000). It would be interesting to examine whether the submerged form of *E. baldwinii* fixes some CO_2 through a similar C_4 metabolism. In contrast, *E. retroflexa* essentially maintains C_4 characteristics even underwater; the culms of the submerged form have

Table I. *Comparison of photosynthetic characteristics in three amphibious species of Eleocharis*

Characteristics	E. vivipara		E. baldwinii		E. retroflexa	
	Terrestrial	Submerged	Terrestrial	Submerged	Terrestrial	Submerged
Culm anatomy	Kranz	Non-Kranz	Kranz	Non-Kranz-like	Kranz	Kranz-like
Organelles in bundle sheath cells	Prominent	Few	Prominent	Present	Prominent	Prominent[a]
^{14}C pulse-^{12}C chase pattern	C_4	C_3	C_4	Intermediate	C_4	C_4
C_4 enzymes	High[b]	Low	High[b]	Intermediate	High[b]	High
$\delta^{13}C$ values[c]	C_4	–	C_4	–	C_4	–

[a] There are fewer organelles than in the terrestrial form. [b] There is a gradient in the activities and amounts of C_4 enzymes among the terrestrial forms of the three species. See text for details. [c] Range of $\delta^{13}C$ values is shown only for the terrestrial forms. The $\delta^{13}C$ values of the terrestrial form of *E. vivipara* are slightly more negative than those of other terrestrial forms.

both Kranz-like anatomy and C_4 biochemical characteristics (Ueno et al., 1998). Therefore, its features resemble those of some aquatic C_4 grasses in the Orcuttieae (Keeley, 1998). Also, in the submerged form of *E. retroflexa*, the level of Rubisco in the MC is slightly higher than in the terrestrial form (O. Ueno, unpublished data).

We may well ask why there is variation in the growth-form-specific expression of photosynthetic modes among the amphibious species of *Eleocharis*. This might be partly explained by the difference in the degree of C_4 expression in the terrestrial forms. From taxonomic study of the photosynthetic modes of *Eleocharis* spp. it seems that C_4 photosynthesis evolved relatively recently in the genus, generating various intermediate stages (Ueno et al., 1989). According to this scenario, it appears that the terrestrial form of *E. vivipara* represents an evolutionary stage that is somewhat less advanced toward a full C_4 syndrome than the terrestrial forms of the other two species. This may facilitate the intriguingly plastic expression of photosynthetic modes seen in *E. vivipara* in contrasting environments.

CONCLUSIONS AND FUTURE PERSPECTIVES

There is ample evidence to suggest that C_4 photosynthesis evolved from C_3 photosynthesis in parallel among diverse taxonomic groups, thereby, generating extensive diversity in the structural, biochemical, and developmental aspects of C_4 photosynthesis among present-day species (Sheen, 1999; Freitag and Stichler, 2000; Edwards et al., 2001). These include some C_3-C_4 intermediate species of *Flaveria* that have been useful in elucidating the sequence of events during the evolution in the mode of photosynthesis (Ku et al., 1991). However, the *E. vivipara*, which has retained its ability to switch between C_3 and C_4 mode of photosynthesis depending upon environmental conditions, provides an attractive opportunity to investigate the developmental process from C_3 to C_4 photosynthesis in its totality.

We still do not understand the molecular mechanism(s) that brings about the switching between photosynthetic modes in *E. vivipara*. A comparative study of the signaling mechanisms involved in the expression of photosynthetic traits in *E. vivipara*, facultative CAM plants (Edwards et al., 1996; Taybi and Cushman, 1999), and some submerged aquatic plants (Magnin et al., 1997; Casati et al., 2000) might be of help in determining the universality and/or diversity of the underlying molecular mechanisms. One of the salient features of C_4 photosynthesis is the differentiation of MC and BSC. Although characterization of mutants from maize (Hall et al., 1998; Rossini et al., 2001) and Arabidopsis (Kinsman and Pyke, 1998) can provide some insight into the mechanism, analyses of plants such as *E. vivipara* and some C_4 dicots that develop cotyledons with a C_3 mode (Voznesenskaya

et al., 1999) may provide a better system to understand this process of cell differentiation.

Undoubtedly, the amphibious species of *Eleocharis* are worth studying if we are to expand our knowledge of the ecological and adaptive aspects of C_4 plants. We still do not know the adaptive significance of the alteration of the photosynthetic characteristics of *E. vivipara* from C_4 to C_3 and of *E. baldwinii* from C_4 to C_3-C_4 intermediate when these plants are grown underwater. Further research of the amphibious species of *Eleocharis*, *Hydrilla* (Reiskind et al., 1997), and aquatic C_4 grasses (Keeley, 1998) may contribute toward a deeper understanding not only of the diversity of photosynthetic metabolism in aquatic environments but also of evolutionary significance of C_4 photosynthesis.

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