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Environmental and hormonal regulation of gene expression of C_4 photosynthetic enzymes in the amphibious sedge *Eleocharis vivipara*☆

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Abstract

Eleocharis vivipara, an amphibious leafless sedge, develops C_4 traits with Kranz anatomy under terrestrial conditions and C_3 traits with non-Kranz anatomy under submerged conditions. To determine the molecular basis of these responses, we isolated a full-length cDNA for phospho*enol*pyruvate carboxylase (PEPC) from culms, the photosynthetic organs of the terrestrial form, and investigated the expression of this gene and two previously isolated isogenes for pyruvate,Pi dikinase (PPDK). The gene for PEPC, designated *pep1*, was 3303 bp long with a single open reading frame of 2904 nucleodides. Phylogenetic analysis indicated that the PEP1 protein is located between a cluster of C_4-form PEPCs of C_4 grasses and another large cluster including C_3- and CAM-form PEPCs of dicots and monocots and C_4-form PEPCs of the C_4 *Flaveria*. The *pep1* and the two PPDK isogenes, which encode chloroplastic and cytosolic PPDK, respectively, were expressed more strongly in culms of the terrestrial form than in those of the submerged form. The transcript levels were strictly modulated, even within a single plant growing both underwater and above the water surface. Exogenous application of abscisic acid (ABA) to the submerged form induced the enhanced expression of these genes in mature culms with non-Kranz anatomy, as well as in newly developing culms with Kranz anatomy, indicating the expression of C_4 genes without coordinate tissue differentiation.

Keywords: Abscisic acid; Amphibious plant; C_3 and C_4 photosynthesis; *Eleocharis vivipara*; Phosphoenolpyruvate carboxylase gene; Pyruvate,Pi dikinase isogenes

1. Introduction

In leaves of C_3 plants, photosynthetic metabolism functions in a single cell, the mesophyll cell. In leaves of C_4 plants, however, strict compartmentation of a number of photosynthetic enzymes and coordination of biochemical functions between two types of cell, the mesophyll cell and the bundle-sheath cell, are essential [1]. Therefore, it appears that much genetic modification is involved during evolution from the C_3 to the C_4 mode.

A dramatic change in the photosynthetic mechanism accompanying anatomical modification has been found in a freshwater amphibious leafless sedge, *Eleocharis vivipara* Link [2,3]. This plant expresses C_4 characteristics under terrestrial conditions and C_3 characteristics under submerged aquatic conditions. The terrestrial form exhibits well-developed Kranz-type anatomy and C_4 biochemical characteristics in the culm (the photosynthetic organ), whereas the submerged form shows non-Kranz anatomy and C_3 biochemical characteristics [2,4]. Unique patterns of cellular localization and differences in accumulation levels of photosynthetic enzymes, together with the structural modification, are the main factors responsible for the differential expression of photosynthetic modes between the two growth forms [4–6].

☆ The nucleotide sequence data reported here will appear in the DDBJ, EMBL and GenBank Nucleotide Sequence Databases under the accession number AB085948.

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In *E. vivipara* the change from C_4 to C_3 mode occurs gradually when terrestrial plants are immersed in water. On the other hand, the change from C_3 to C_4 mode occurs comparatively quickly when the submerged plants are exposed to air. Whereas the culms of submerged plants die as a result of rapid desiccation, the development of new culms with both Kranz anatomy and C_4 biochemical traits is initiated within several weeks [3]. It seems that osmotic stress triggers the anatomical and biochemical events in the C_3 to C_4 change. Recently, we discovered that the addition of abscisic acid (ABA), a plant stress hormone, to the water can induce the formation of new culms with Kranz anatomy and C_4-like biochemical traits in entirely submerged plants of *E. vivipara* [7].

This unique amphibious plant is an intriguing system in which to study the molecular mechanisms responsible for C_3 and C_4 differentiation in response to changes in environmental conditions [3,8]. Two cDNAs for the C_4 enzyme pyruvate,Pi dikinase (PPDK), designated *ppdk1* and *ppdk2*, have been isolated from the photosynthetic tissues of *E. vivipara* [8]. The *ppdk1* and *ppdk2* genes encode a chloroplastic and a cytosolic PPDK, respectively, and the transcript levels of these isogenes are skillfully regulated in the two growth forms, reflecting the difference in photosynthetic mode [8].

Here, we further attempted to isolate a cDNA for another key C_4 enzyme, phospho*enol*pyruvate carboxylase (PEPC; E.C. 4.1.1.31). PEPC catalyzes the irreversible β-carboxylation of phospho*enol*pyruvate in the presence of HCO^{3-} to yield oxaloacetate and Pi [9]. In C_4 plants this enzyme is responsible for primary carboxylation and is located in the cytosol of mesophyll cells. PEPC also plays anaplerotic roles in non-photosynthetic systems [9]. PEPC is encoded by multigene families in plants; and several isogenes, which encode the C_3-like housekeeping and root forms as well as the C_4 photosynthetic form, have been characterized [10–12]. In the facultative CAM plant *Mesembryanthemum crystallinum*, several isogenes for PEPC are also found, and a CAM-specific gene selectively increases the transcript level in response to salt stress during CAM induction [13]. It would be interesting to know how genes encoding photosynthetic enzymes are regulated in *E. vivipara* by environmental factors and hormones such as ABA. It is also important to characterize the structure of these genes in this unique amphibious C_3/C_4 sedge in comparison with those of ordinary C_3 and C_4 plants.

We isolated a full-length cDNA encoding PEPC from the terrestrial form of *E. vivipara* and characterized its structure. We also investigated a part of the environmental and hormonal regulation of this gene and the two previously isolated isogenes for PPDK.

2. Materials and methods

2.1. Plant materials and growth conditions

Plants were grown in a greenhouse at the National Institute of Agrobiological Sciences, Tsukuba, Japan. Shoots of the terrestrial form were transplanted into 500-ml pots that contained field soil and were grown in the greenhouse, which was maintained at 25–30 °C/20–25 °C (day/night temperature) under natural sunlight. After about 2 months, the culms were cut off at ground level, and the plants were transferred to a growth chamber, which was programmed at 25 °C during the light period (14 h) and at 20 °C during the dark period (10 h). Photon irradiance, provided by metal-halide lamps, was about 400 μmol m^{-2} s^{-1}. The submerged form was induced by submerging the terrestrial form in aquariums (50 cm deep) in the greenhouse for more than 3 months, as described previously [7]. The water in the aquariums was allowed to overflow by the addition of tap water at a slow rate to prevent the growth of epiphytes. The temperature of the water in the aquariums ranged from 20 to 26 °C. After removal of the culms, the submerged plants were transferred to water tanks (30 l capacity) that had been set up in the growth chamber and were grown for more than 1 month before the experiments. The water in the tanks was changed at weekly intervals.

For the ABA experiments, the culms of the submerged plants were cut off at ground level, and the plants were transferred to water tanks (30 l) set up in the growth chamber, which contained 5 μM ABA ([±]-*cis*, *trans*-isomers; Sigma), as described previously [7]. They were grown for 50 days before sampling. Newly formed culms were used for the experiments. The water was changed weekly with new ABA solution. For experiments on short-term ABA treatment, the submerged plants were transferred, without the culms being cut off, to water tanks that contained 5 μM ABA, and the mature culms were exposed to the ABA solution for up to 3 days.

For experiments on plants growing both underwater and above the water surface, culms were collected from plants grown in the aquariums.

2.2. Construction and screening of a cDNA library

Total RNA was prepared from the culms of the terrestrial form as described previously [14], and poly(A)$^+$ RNA was isolated with Oligotex-dT30 (Roche, Tokyo, Japan) according to the manufacturer's instructions. cDNAs were synthesized by reverse transcriptase from avian myeloma virus with random primers (Amersham International, Buckinghamshire, UK). A cDNA library was constructed in the λZAPII vector (Stratagene, La Jolla, CA) using adapters [15] at

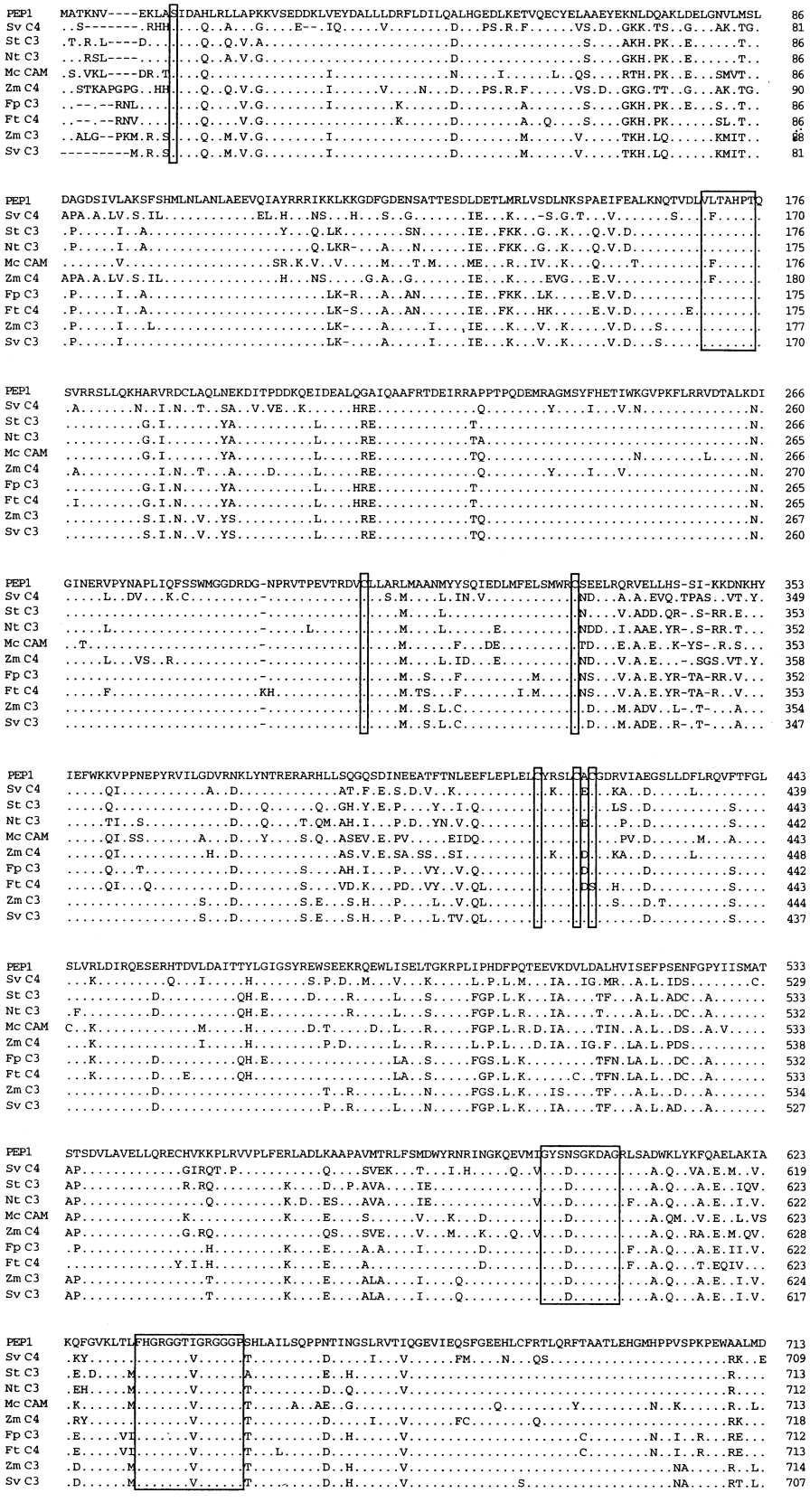

Fig. 1

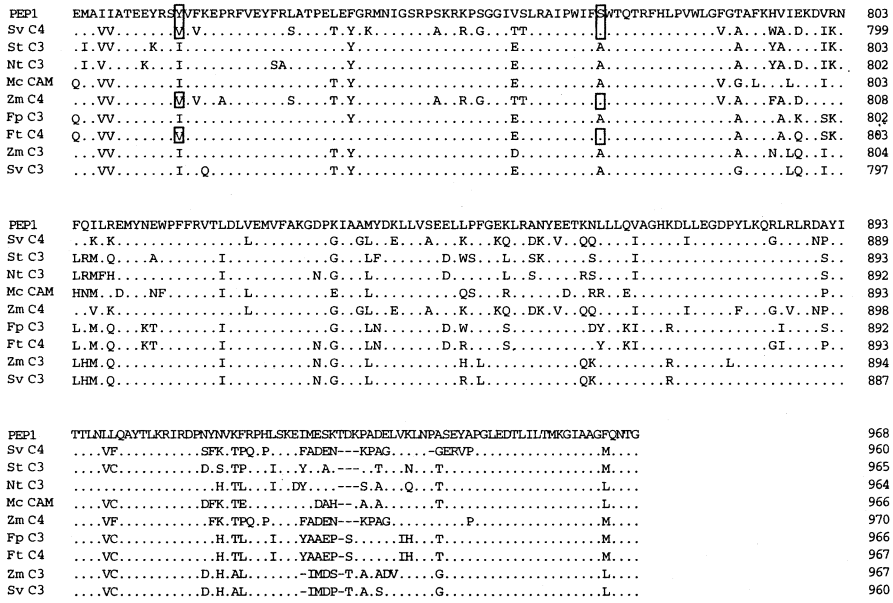

Fig. 1 (*Continued*). Comparison of the deduced amino acid sequence of PEPC from *Eleocharis vivipara* (PEP1) with the C_3-form PEPCs of *Solanum tuberosum* (St C3), *Nicotiana tabacum* (Nt C3) and *Flaveria pringlei* (Fp C3), the CAM-form PEPC of *Mesembryanthemum crystallinum* (Mc CAM), the C_4-form PEPC of *F. trinervia* (Ft C4), and the C_3- and C_4-form PEPCs of *Sorghum vulgare* (Sv C3 and Sv C4) and *Zea mays* (Zm C3 and Zm C4). Dots indicate amino acids identical to those of PEP1, and bars indicate gaps introduced for maximum alignment. The boxes indicate the location of amino acid residues that are suggested to be important for PEPC function (see text for details). Accession numbers of the amino acid sequences at the GenBank data library are: Sv C4, X63756; St C3, X67053; Nt C3, X59016; Mc CAM, X14587; Zm C4, X15239; Fp C3, X48966; Ft C4, X64143; Zm C3, X61489; Sv C3, X59925.

the termini of the cDNA instead of the *Eco*RI sites. The cDNA library was screened by plaque-hybridization with a ^{32}P-labeled DNA fragment as a probe, which was generated by reverse transcription (RT) and PCR from the RNA of *E. vivipara.*

2.3. Northern blot analysis

Total RNA was extracted from culms of the terrestrial and the submerged forms, as described previously [8]. Equal amounts (5 μg of total RNA) were denatured in a mixture of 56% formamide and 12% formaldehyde, and RNAs were separated on a 1% agarose gel that contained 1.6% formaldehyde. After transfer to nitrocellulose filters, the RNA bands were allowed to hybridize with ^{32}P-labeled PEPC cDNA probes (254 bp long from nucleotides [nt] 3013–3266) and PPDK cDNA probes described previously [8] at 60 °C in hybridization buffer that contained 1% SDS, 10% sodium dextran sulfate, and 1 M NaCl. The filters were then washed at 60 °C in 2 × SSC, 1% SDS.

3. Results

3.1. Isolation and characterization of a cDNA for PEPC

A cDNA library was constructed from RNA extracted from culms of the terrestrial form. To obtain a probe for PEPC genes, a DNA fragment was amplified by RT-PCR from RNA that had been isolated from the culms of the terrestrial form of *E. vivipara* as a template, with degenerate primers that corresponded to highly homologous regions in the nucleotide sequences of the PEPC genes of *Zea mays* [16] and *Flaveria trinervia* [17]. The DNA fragment was 839 bp long and corresponded to the sequence from nt 1985 to 2824 of *Z. mays* and nt 1936–2770 of *F. trinervia.* The cDNA library from the terrestrial culms was screened by plaque-hybridization with the probe. Ten positive clones with insert sizes of ca. 3.2 kb (2 clones) and ca. 3.0 kb (8 clones) were obtained and partly sequenced from both ends. All ten clones were identical in overlapping regions, indicating that the ten cDNAs isolated are coded by a single gene. Whereas the longer clones encoded the complete PEPC, the shorter clones encoded a PEPC lacking the amino terminus of the enzyme. The 3303-bp-long insert had a major open reading frame (ORF) of 2904 bp, 105 bp of a 5′ leader sequence, and a 3′ untranslated region of 294 bp (including a poly(A) tail of 37 bp). We designated this full-length cDNA *pep1*.

3.2. Protein structure of PEP1

The open reading frame of pep1 encoded a polypeptide, designated PEP1, of 968 amino acids with a predicted molecular mass of 110 kDa. The deduced amino acid sequence was aligned with those of PEPCs of

other plants (Fig. 1). It revealed a high degree of similarity (81–83%) to the C_3-form PEPCs. A reduced level of similarity was found in the C_4-form PEPCs of the C_4 monocots sorghum and maize (73 and 76%, respectively). PEP1 showed homologies of 79 and 81% with the salt-induced CAM-form PEPC of *Mesembryanthemum crystallinum* and the C_4-form PEPC of a C_4 dicot, *F. trinervia*, respectively.

PEP1 contains serine-11 in the N-terminus (Fig. 1), which is the putative phosphorylation motif for serine kinase [18]. Other sequence motifs proposed to be essential for oxaloacetate formation (VLTAHPT, 169–175) [19], the active site (GYSDSGKDAG, 595–604) [17,20], and the substrate-binding site (FHGRGGTVGRGGGP, 633–646) [21] are also highly conserved in PEP1, although in PEP1 aspartic acid-598 and valine-640 are substituted by asparagine and isoleucine, respectively. PEP1 also contained cysteine residues (residues 304, 331, 414, 419, and 421), which are considered to be involved in subunit interaction to maintain the tetrameric structure [22] or redox regulation [23]. It was previously suggested that there were C_4-specific amino acid residues for valine-731 and serine-780 in the maize C_4-form PEPC, since other C_4-form PEPCs also contained these residues but C_3- and CAM-form PEPCs lacked them [17]. PEP1 also contained serine (residue 775), as do other C_4-form PEPCs, but it contained tyrosine (residue 726) instead of the valine.

3.3. Phylogenetic analysis of PEP1

Analysis of the phylogeny of PEPCs showed that PEP1 is localized between a cluster of the C_4-form PEPCs of C_4 monocots (maize and sorghum) and another large cluster of PEPCs of other seed plants (Fig. 2). The latter cluster includes the C_3- and CAM-form PEPCs of dicots and monocots and the C_4-form PEPCs of C_4 dicots (*Flaveria* spp.).

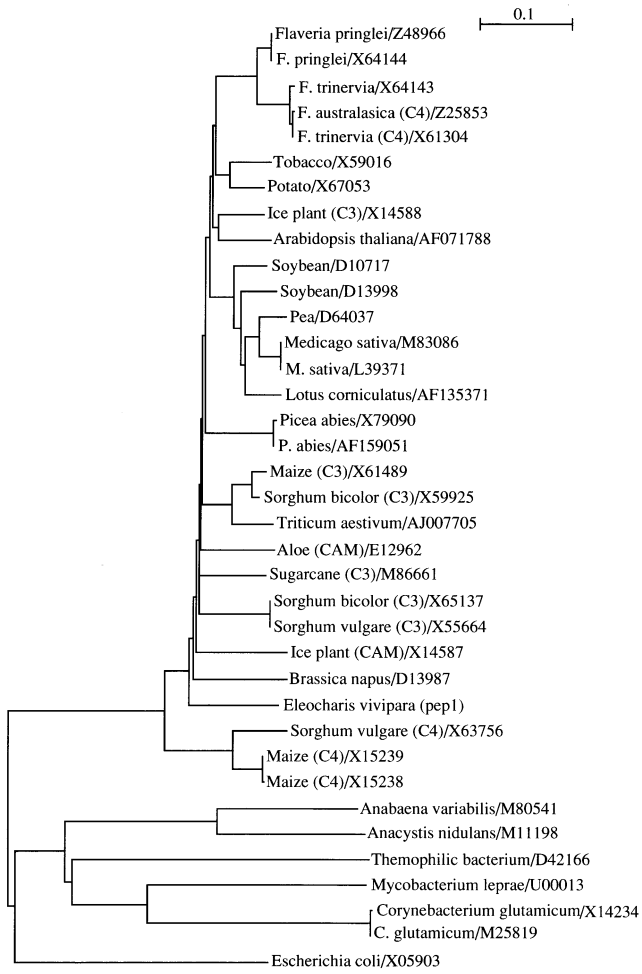

Fig. 2. Phylogenetic tree of amino acid sequences of PEPCs. Amino acid sequences of PEPC used here are taken from the GenBank data library. The phylogenetic tree was constructed by using Clustal X [24].

3.4. Expression of pep1 and PPDK isogenes under different growth conditions

To investigate the expression of *pep1* and the PPDK isogenes *ppdk1* and *ppdk2* in the terrestrial and submerged forms of *E. vivipara*, we performed Northern blot analysis with RNA from the culms (Fig. 3). The levels of transcripts for these genes were far more abundant in culms of the terrestrial form than in those of the submerged form (Fig. 3). To examine the light response of *pep1* we analyzed the levels of transcripts for *pep1* produced in response to a light-to-dark transition. Terrestrial plants grown under light conditions were transferred to darkness for up to 3 days, and the levels of transcripts for *pep1* in the culms were chased (Fig. 4). The level of *pep1* transcript was reduced during the period of dark treatment, indicating that the expression of *pep1* is regulated by light.

When the submerged form of *E. vivipara* was grown under water for a long period, the tips of some culms sometimes reached the water surface. Under such conditions, the underwater culms produced aerial secondary culms, together with secondary culms floating at the water surface, by prolification (Fig. 5A). The aerial secondary culms possessed Kranz anatomy (Fig. 5B), although the underwater culms had non-Kranz anatomy (Fig. 5D). The floating secondary culms showed various intermediate anatomies (Fig. 5C). As a consequence, a single plant could grow both underwater and above the water surface and have culms with differing anatomical traits. To examine whether the expression levels of *pep1*, *ppdk1* and *ppdk2* differed among these culms, we performed Northern blot analysis with RNA isolated from them (Fig. 6). The level of *pep1* transcript was most abundant in the above-water secondary culms and least abundant in the underwater culms. In the secondary culms floating at the water surface the level was

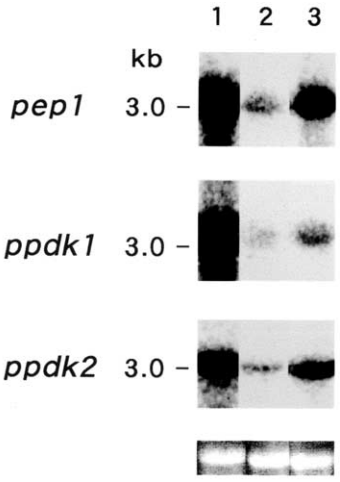

Fig. 3. Northern blot analysis of transcript levels of *pep1, ppdk1*, and *ppdk2* in the culms of the terrestrial (1) and submerged (2) forms and in the ABA-induced culms (3). To produce ABA-induced culms, the submerged plants were grown under water that contained 5 μM ABA. Newly developed culms, which had Kranz anatomy, were used for the analysis. Equivalence of RNA loading among lanes of agarose gels was demonstrated by ethidium bromide staining of RNA on the gel.

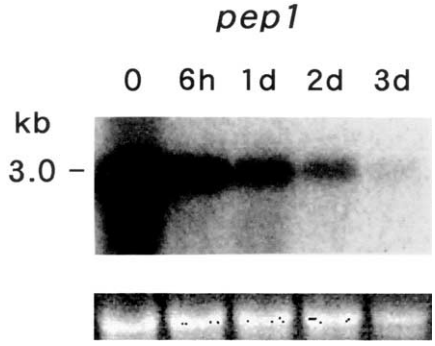

Fig. 4. Northern blot analysis of transcript levels of *pep1* in light-to-dark transition. Terrestrial plants grown in the light were transferred to darkness for up to 3 days. Staining with ethidium bromide is also shown.

intermediate (Fig. 6). A similar trend was also found in the levels of transcripts of *ppdk1* and *ppdk2*, although the difference in the transcript level among the three types of culms was smaller in *ppdk2* than in *ppdk1* (Fig. 6). Therefore, the transcript levels of these genes were controlled by the water environment, even in a single plant growing both underwater and above the water surface.

3.5. Expression of pep1 and PPDK isogenes in response to ABA

When the submerged form was grown in a solution that contained ABA, the plants developed new culms with both Kranz anatomy and C_4-like biochemical traits [7]. We confirmed that enhanced expression of *pep1*, *ppdk1*, and *ppdk2* occurs in these culms (see Fig. 3). The levels of transcript in the culms that were newly formed in 5 μM ABA solution were higher for all three genes than those in culms of the submerged form grown in water without ABA, but lower than those in culms of the terrestrial form (Fig. 3). In the ABA-induced culms as well as in culms of the submerged form, the level of transcript of *ppdk2* was higher than that of *ppdk1*.

When mature culms formed before ABA treatment in the submerged form of the plant were exposed to ABA solution, they gradually browned and finally died [7]. To investigate whether ABA induces the expression of *pep1* and *ppdk1* in mature culms of the submerged form with non-Kranz anatomy, we transferred plants of the submerged form to 5 μM ABA solution, and chased the levels of transcript for up to 3 days by Northern blot analysis (Fig. 7). The levels of transcript of *pep1* and *ppdk1* increased with time, peaking on the second day after ABA treatment. These results show that exogenously applied ABA also induces expression of these genes even in mature photosynthetic tissues, without coordinate differentiation of Kranz anatomy.

4. Discussion

As in PEPCs of other plants, PEP1 conserved the amino acid residues that are suggested to be involved in the function of PEPC [18–20]. The C_4-specific amino acid residues are still unknown [25], although two possible C_4-specific residues have been suggested [17]. In our study, too, PEP1 contained one of the proposed C_4-specific residues, but not other residue. *E. vivipara* belongs to the Cyperaceae, a monocot family with C_4 members, like the Poaceae. Homology analysis showed that PEP1 has a higher similarity to the C_3-form PEPCs than to the C_4-form PEPCs of C_4 grasses. Phylogenetic analysis showed that PEP1 is located between the cluster of the C_4-form PEPCs in C_4 grasses and the cluster that includes the C_3- and CAM-form PEPCs in dicots and monocots and the C_4-form PEPCs in C_4 dicots. Whereas this position of PEP1 may reflect the taxonomic distinction between the Cyperaceae and the Poaceae, we should also consider that the terrestrial form of *E. vivipara* possesses C_4-like characteristics rather than complete C_4 traits, because of the accumulation of ribulose-1,5-bisphosphate carboxylase/oxygenase in the mesophyll cells as well as in the Kranz (bundle-sheath) cells [3,5]. The PEPC of the terrestrial form in *E. vivipara* has kinetic properties that differ from those of typical C_4-PEPC and are somewhat intermediate between C_3 and C_4 [3]. A comparative study with PEPC from typical C_4 species of the Cyperaceae may help us to understand the phylogenetic relationship of PEP1.

Previous studies have revealed that the terrestrial form of *E. vivipara* accumulates higher amounts of PEPC protein and has higher levels of PEPC activity

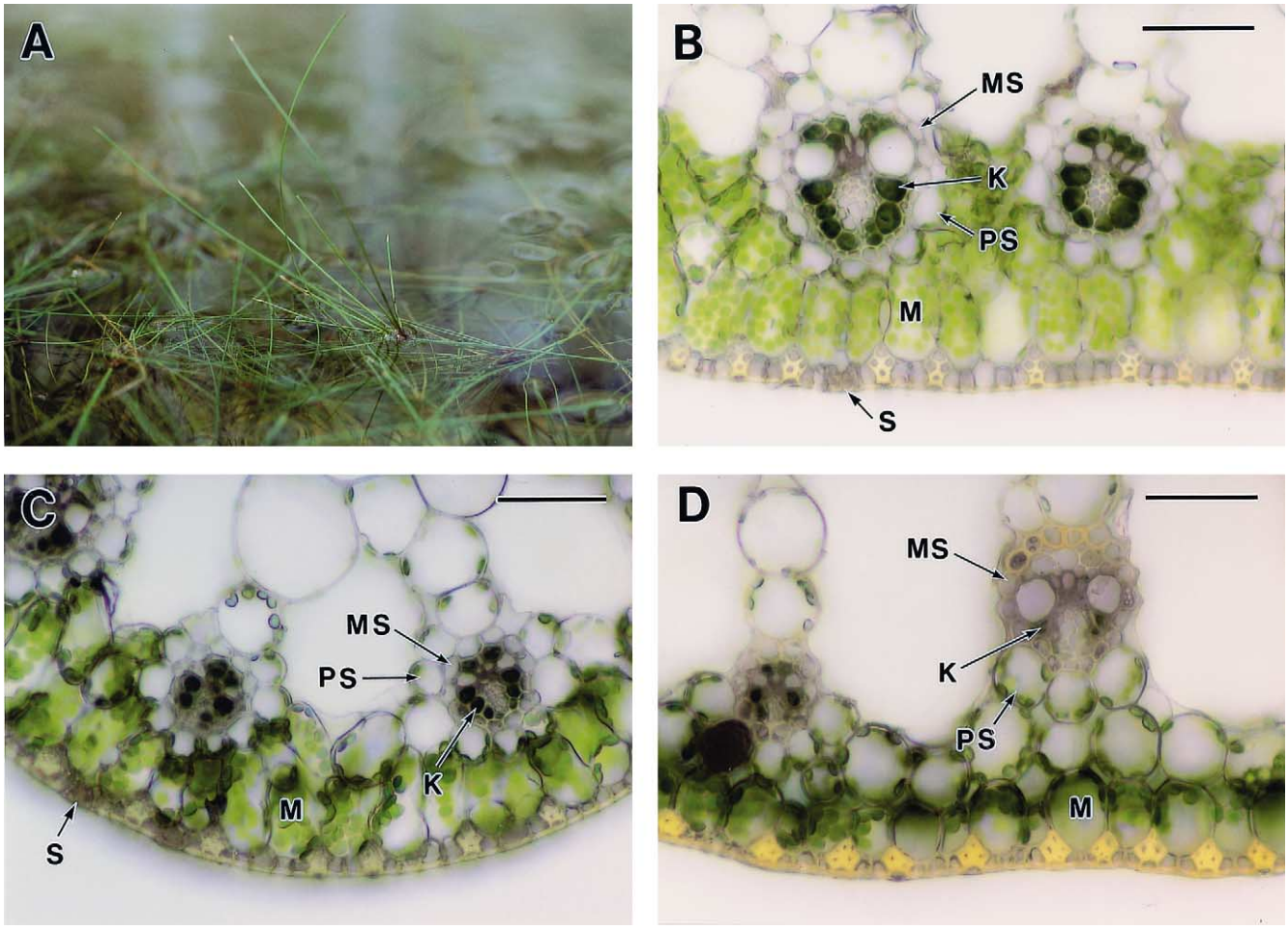

Fig. 5. An *E. vivipara* plant growing both underwater and above the water surface (A), and the anatomical structures of culms (B–D). (A) A plant growing in a water tank set up in a greenhouse. (B–D) Comparison of anatomical structures of culms growing under different conditions. (B) Above-water secondary culm with Kranz anatomy. There are semi-radially arranged mesophyll cells (M) and three bundle sheaths that consist of the outermost parenchyma sheath cells (PS), the middle mestome sheath cells (MS), and the innermost Kranz cells (K). The outermost parenchyma sheath cells are functionally equivalent to the mesophyll cells [5]. (C) Floating secondary culm with intermediate anatomy. (D) Underwater culm with non-Kranz anatomy. Note the well-developed mesophyll cells, relative to the reduced Kranz cells. Scales = 50 μm. S, stoma.

than the submerged form [2,5]. It seems certain that the transcript level of *pep1* is responsible for the regulation of accumulation of this enzyme in photosynthetic tissues. Our study revealed that the transcript level in culms of the terrestrial form was reduced in light-to-dark transition. It is known that in leaves of C_4 plants, light regulates the expression level of the PEPC gene [26,27]. These results indicate that *pep1* has similar behavior to the isogene for C_4 PEPC, although it holds high homology to the C_3-form PEPCs. A recent study of NADP-malic enzyme in *Flaveria* species showed that 72- and 62-kDa isoforms are predominantly accumulated in leaves of C_3 and C_4 species, respectively, whereas a 64-kDa isoform occurs in the leaves of C_3–C_4 intermediate and C_4-like species, together with two other isoforms [28]. It cannot be ruled out that PEP1 may be neither a typical C_3 form nor a typical C_4 form of PEPC. We need to isolate other isogenes for PEPC from *E. vivipra* and to compare the expression patterns of these genes.

The results of Northern blot analysis suggest that the difference in the level of PEPC transcript, as well as that for PPDK [8], is one of the key factors responsible for the expression of different modes of photosynthesis between the terrestrial and submerged forms. Under field conditions, *E. vivipara* grows on the edges of creeks and ponds along a gradation from terrestrial to completely submerged conditions [2,3]. This amphibious sedge can also grow under semi-aerial and semi-submerged conditions, forming photosynthetic tissues adaptive to each respective environment. Northern blot analysis with RNA isolated from various culms of plants growing under such conditions showed that the transcript levels of *pep1* and the PPDK genes were strictly controlled even in a single plant by water environments surrounding the respective culms. It seems

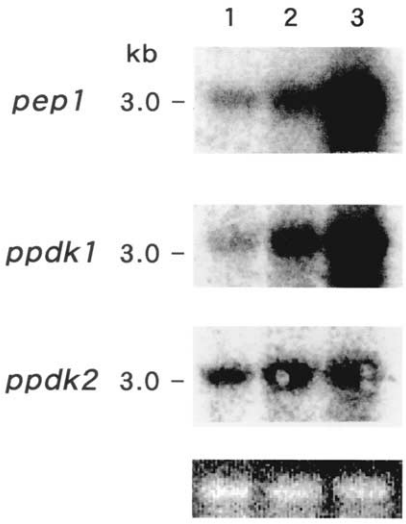

Fig. 6. Northern blot analysis of transcript levels of *pep1*, *ppdk1*, and *ppdk2* in a plant growing both underwater and above the water surface. 1, underwater culms; 2, floating secondary culms; 3, above-water secondary culms. Staining with ethidium bromide is also shown.

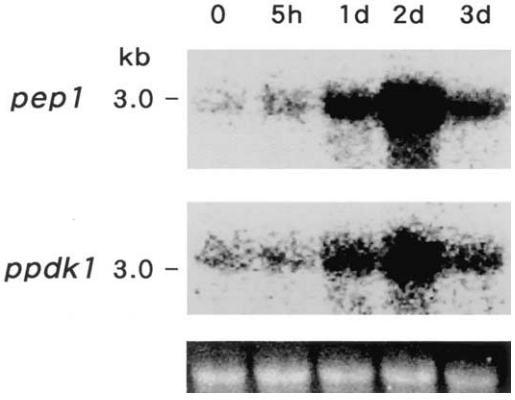

Fig. 7. Northern blot analysis of ABA response of *pep1* and *ppdk1* in mature culms of the submerged form. The mature submerged culms, which had non-Kranz anatomy, were exposed to water that contained 5 μM ABA, and the changes in transcript levels were chased for up to 3 days. Staining with ethidium bromide is also shown.

that this molecular control of the C_4 photosynthetic genes is known only in this amphibious sedge, which experiences fluctuations of water levels under natural conditions. A preliminary immunocytochemical study confirmed that the above-water secondary culms accumulate large amounts of PEPC and PPDK in the mesophyll cells and the outermost parenchyma sheath cells, whereas the underwater culms accumulate only small amounts of the enzyme proteins in these cells. The amounts of PEPC and PPDK in the floating secondary culms were intermediate between those in the above-water and the underwater culms (Ueno, O., unpublished data).

ABA is well known as a stress-induced hormone, and the levels of ABA in plants increase rapidly during water stress [29]. In some facultative CAM plants, ABA induces a transition from the C_3 mode to the CAM mode [30–32]. On the other hand, ABA is involved in the determination of leaf identity in some heterophyllic aquatic plants [33–35]. ABA induces the formation of new culms with Kranz anatomy and C_4-like biochemical traits underwater in the submerged form of *E. vivipara* [7]. These culms show increases in the accumulation and activity of key C_4 enzymes, such as PEPC, PPDK, and NAD-malic anzyme, and the operation of C_4-like carbon metabolism [7]. Our study revealed that the transcript levels of *pep1*, *ppdk1*, and *ppdk2* are enhanced in the culms induced by ABA, as compared with those of the submerged form grown in water without ABA. This result indicates that the increases of PEPC and PPDK in ABA-induced culms are regulated largely at a transcriptional level. An analytical study of endogenous ABA levels in the heterophyllous aquatic plant *Hippuris vulgaris* has indicated that ABA levels rise in response to osmotic stress encountered when a submerged shoot grows up through the water–air interface [33]. It would be possible that in *E. vivipara*, too, endogenous ABA levels are controlled by a similar mechanism, and increased levels of ABA are involved in the induction of both C_4 biochemical and anatomical characteristics.

Our short-term experiment on ABA treatment revealed that the transcript levels of *pep1* and *ppdk1* increased quickly even in mature culms of the submerged form, which had non-Kranz anatomy. Thus, this result indicates that ABA can induce the expression of these genes without coordinate development of Kranz anatomy. This also suggests that the anatomical and biochemical components of the C_4 traits are controlled independently, although the two components seem to be well coordinated in naturally growing plants.

Of two isogenes for PPDK, *ppdk1* encodes chloroplastic PPDK and is involved in C_4 photosynthesis. However, *ppdk2* encodes cytosolic PPDK, whose function is unknown [8]. Correspondingly, the expression of *ppdk1* is light-regulated, but that of *ppdk2* is not affected by light/dark transition [8]. Both isogenes are strongly expressed in the culms of the terrestrial form, compared with those of the submerged form. One may expect that ABA would stimulate the expression of *ppdk1* more strongly than that of *ppdk2*. However, this is not the case: it triggered stronger expression of *ppdk2* than of *ppdk1* (Fig. 3). In the culms of the submerged form the transcript level of *ppdk2* was higher than that of *ppdk1*, whereas in those of the terrestrial form a contrary trend was found [8] (Fig. 3). Therefore, the ABA response of the two isogenes appears to be a combination of the patterns in both the terrestrial and submerged forms. In rice roots, a cytosolic PPDK was induced by ABA, polyethylene glycol, and submergence, implying its involvement in a metabolic response to

water deficit and low-oxygen stress [36]. The function of cytosolic PPDK in *E. vivipara* remains to be determined.

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