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Isolation and Characterization of Acid Invertase cDNA Clone in Hot Pepper (*Capsicum annuum* L.) Fruits

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An acid invertase (EC 3.2.1.26.) cDNA clone, *CaAIV-18*, was isolated from the red pericarp cDNA library of the hot pepper (*Capsicum annuum* L.) fruit. The *CaAIV-18* clone has 2223 nucleotides and one open reading frame encoding 641 amino acid residues. Analysis of deduced amino acid sequences reveals that *CaAIV-18* has a 24-amino acid transmembrane anchor region in its N-terminal, implying acid invertase in hot pepper may be localized in the membrane and not in the cytosol. This clone showed high homology to tomato acid invertase, *Aiv-1*, in nucleotide and deduced amino acid sequences. In the Southern blot analysis, this clone proved to exist as single or low copy numbers on the genome of hot pepper. The clones had two well-conserved regions which appears in acid invertase of other plant species (*eg.* tomato, *Arabidopsis, etc.*) and yeasts. During fruit development, *CaAIV-18* was expressed preferentially in the ripe red stage.

Keywords: hot pepper, pericarp, cDNA library, acid invertase, fruit development

Fruits of higher plants are important subjects for plant breeding studies because of their value as a food source. Generally, fruit is an organ generated from the ovary wall which undergoes continuous cell division and differentiation after fertilization. Therefore, organogenesis of fruit is regarded to be parallel to zygotic embryogenesis. As a model system of study on fruit development, the tomato has been actively studied, especially in the field of fruit ripening (Della-Penna *et al.*, 1986; Giovannoni *et al.*, 1989). Plant growth regulators play important roles in fruit development. Whereas cytokinin or gibberellic acid induces cell division or cell expansion at the early stage of development, ethylene is crucial at the ripening stage (Gillaspy *et al.*, 1993). In the ripening stage of fruit, various biochemical phenomena and active metabolism leads to accumulation of secondary metabolites and various forms of carbon assimilates (Yelle *et al.*, 1991). Considering the importance of fruit as a food source, it is very important to know how atmospheric CO_2 is fixed, transported and sunk in fruit, because this fruit storage material is used as food. Carbon fixed in the leaf or epidermis of the fruit is transported as sucrose and stored in storage organs as polysaccharides, sucrose, or hexoses. According to the plant species, the major form of stored sugar is unique to that species. Whereas wild tomato stores the carbon assimilate as sucrose, domesticated tomato stores it as hexoses (Yelle *et al.*, 1991). Hot pepper is also known to be a hexose accumulator. Therefore, invertase (EC 3.2.1.26.) as well as sucrose synthase or sucrose phosphate synthase are suggested to be important research subjects.

Invertase is a hydrolase which hydrolyzes sucrose into glucose and fructose, and has either alkaline or acidic pH optima. Alkaline invertase has been characterized in only a few plant species, and its cDNA has not been cloned, yet (Sturm, 1996). Acid invertase has been studied extensively, and it seems to have functions in osmoregulation (Wyse *et al.*, 1986), gravitropism (Wu *et al.*, 1993), sucrose partitioning (Eschrich, 1989) and the response against wounding (Sturm and Chrispeels, 1990). Biochemical characterization of acid invertase has already been executed in bell pepper (Michaud *et al.*, 1993). In bell pepper fruits, the activity of acid invertase changes dramatically according to the age of the fruit (Hubbard and Pharr, 1992) as compared to sucrose synthase and sucrose phosphate synthase. This implies that acid invertase may play important roles in the developmental processes of pepper fruit. In this research, we isolated

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and characterized cDNA clone of acid invertase to investigate its role during the fruit development of hot pepper (*Capsicum annuum* L.).

MATERIALS AND METHODS

Plant Materials

F1 hybrid hot pepper plants (*Capsicum annuum* L.) were grown in greenhouse at 25±5°C under natural light. Fruits of various developmental stages were harvested and used for the experiment. Young-green (YG), mature-green (MG) and ripe-red (RR) fruits were used as a source of mRNA for the construction of the cDNA library.

Construction of the cDNA Library

Total RNA was isolated from the pericarp tissues of YG, MG and RR fruits of hot pepper plants as described by Nagy *et al.* (1988). Two grams of pericarps were ground in mortars with liquid nitrogen to a fine powder. After transfer into 50 mL centrifuge tubes, 10 mL of RNA extraction buffer [5 M guanidium thiocyanate, 25 mM sodium citrate (pH 7.0), 0.5% (w/v) sarkosyl, 2 mM EDTA, and 5% (w/v) β-mercaptoethanol] was added and then incubated at 50°C for 10 min. RNA extracts were centrifuged at 15,000 rpm for 20 min at 20°C. CsCl powder was added to the supernatant for the final concentration of 0.1 mg/mL, and dissolved completely. Insoluble debris and undissolved CsCl were removed by centrifugation at 15,000 rpm for 20 min at 20°C. Eight to ten mL of supernatant containing RNA extract was loaded onto 3-5 mL of CsCl cushion (5.7 M CsCl, 0.1 M EDTA), and then ultracentrifuged at 35,000 rpm for 18 h at 20°C. RNA pellets were dissolved in 400 μL of 7 M urea/2% sarkosyl solution and extracted as an aqueous layer with 1 volume of phenol:chloroform (1:1) mixture and 1 volume of chloroform subsequently. RNAs in the aqueous layer were precipitated at -70°C for 1 h, after mixing with 0.1 volume of 3 M sodium acetate (pH 5.2) and 2.5 volumes of absolute ethanol. RNAs were precipitated by centrifugation at 12,000 rpm for 10 min at 4°C, washed with 70% ethanol and dissolved in DEPC-treated deionized distilled water.

Poly(A) RNAs were purified with an oligo d(T) push column and used in the synthesis of the cDNA libraries. cDNA libraries were constructed using a Stratagene cDNA synthesis kit on *Eco*RI/*Xho*I site of Uni-ZAP™ XR vector according to the manufacturer's instructions.

Screening of the cDNA Library

In the first screening, the cDNA library of YG fruit was used. A total of 5×10^5 plaques was plated at a density of 5×10^4 plaques per 15 cm petridish, and transferred to Hybond-N$^+$ filters (Amersham) and screened using a tomato acid invertase cDNA, *Aiv*-1 (Ohyama *et al.*, 1992) as a probe in the first screening. In the second screening, after obtaining a hot pepper acid invertase partial cDNA clone, *CaAIV-1*, a cDNA library of RR fruit was used and *CaAIV-1* used as a probe. The probe was labeled with [α-^{32}P] dCTP (3000 Ci/mM, Amersham) using the Prime-a-Gene system (Promega). The filters were prehybridized for about 4 h at 58°C in 6X SSC, 5X Denhardt's solution, 0.5% SDS and 0.1 mg/mL salmon sperm DNA. Hybridization was performed for 16 h at 58°C in prehybridization solution with radiolabeled probes. The filters were washed once in 2X SSC, 0.1% SDS at 58°C for 15 min, twice in 1X SSC, 0.1% SDS at 58°C for 15 min, once in 0.2X SSC, 0.1% SDS at 58°C for 15 min, and finally in 0.2X SSC at 58°C for 15 min.

DNA Sequencing and Analysis

cDNA inserts of the isolated phage clones were recovered by *in vivo* excision from the Uni-ZAP™ XR vector following the manufacturer's instructions. Double-stranded DNA was used as the template for DNA sequence analysis following the manufacturer's instructions (USB Sequenase ver. 2.0 Kit). Nucleotide and amino acid sequence analyses were performed using DNASIS and PROSIS programs (Hitachi). Nucleotide and amino acid sequence homology comparisons were performed using the BLAST version 1.4 application programs of the Experimental GENINFO(R) BLAST Network Service (Altschul *et al.*, 1990). Multiple alignments of amino acid sequences were performed using the CLUSTALW program served by GenomeNet world wide web server (http://www.genome.ad.jp/). Specific secondary structures and identities of transmembrane anchors were searched through the SOSUI system (http://www.tuat.ac.jp/~mitaku/adv_sosui/).

Southern Blot Analysis

Genomic DNA was prepared from hot pepper (*Capsicum annuum* L.) leaves according to the protocol

of Shure *et al.* (1983). Thirteen μg samples of DNA were digested with *Eco*RI, *Eco*RV, *Hin*dIII and *Xba*I, subjected to electrophoresis in 0.8 % agarose gel and then blotted onto Hybond-N^+ filters (Amersham). The full-length *CaAIV-18* was labeled with [α-^{32}P]dCTP (3000 Ci/mM, Amersham) by random priming (Promega) and used as a probe. The filter was prehybridized, hybridized and washed with the same method used in the screening of the cDNA except for temperature, 62°C.

Northern Blot Analysis

Total RNA was extracted from the ovary type fruit and pericarps of fruits at four different developmental stages, YG, MG, TR (turning-red) and RR, respectively, according to the protocol of Chomczynski and Sacci (1987). The *Sph*I-*Hin*dIII fragment of *CaAIV-18* was used as a probe and labeled with [α-^{32}P] dCTP (3000 Ci/mM, Amersham) by random priming (Promega). RNA was electrophoresed on 1% agarose gel containing 2.2 M formaldehyde, blotted onto Nytran Plus membranes (Schleicher and Schuell) in 20 x SSC, and hybridized with the radio-labeled probe.

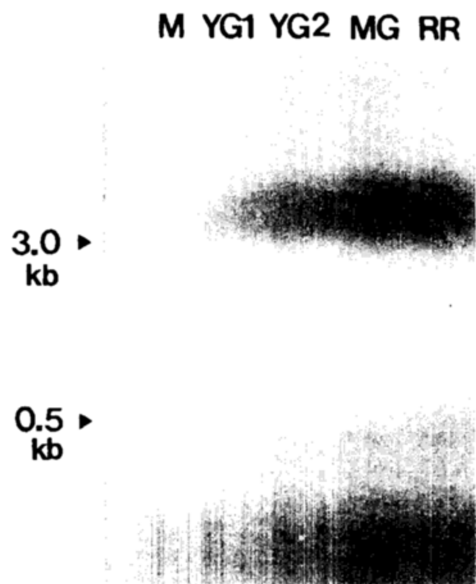

Fig. 1. Size distribution of inserts in the three cDNA libraries. YG1 and YG2 represent PCR products from YG (young-green fruit) cDNA library using T3 and T7 primers. YG1 used 5 μL of YG cDNA library as a template, and YG2 used 10 μL of same library. MG and RR represent PCR products from MG (mature-green) and RR (ripe-red) cDNA library, respectively. Both of PCR was conducted with T3 and T7 primers using 5 μL of each cDNA library. Molecular weight marker (M) indicates 1 kb DNA ladder.

RESULTS AND DISCUSSION

Construction of the cDNA Library

The initial titers of the cDNA libraries were 2.2×10^6 pfu/mL for YG, 6.0×10^5 pfu/mL for MG and 4.0×10^5 pfu/mL for the RR stage. After amplification and stabilization according to the manufacturer's guide, cDNA libraries were amplified by several 10^4 factors. The size distributions of the libraries ranged to the length of 3 kb and were considered to cover most of the full-sized cDNA clones (Fig. 1).

Isolation of an Acid Invertase Clone; *CaAIV-18*

To isolate cDNA clones encoding acid invertase from the hot pepper (*Capsicum annuum* L.) fruit cDNA library, we used the *Hin*dIII fragment of tomato *Aiv-1* cDNA clone (Ohyama *et al.*, 1992) as a probe. After a first screening with the YG fruit library, a partial cDNA clone, *CaAIV-1* was selected. Upon another screening with the RR fruit library, we used the *Kpn*I fragment of *CaAIV-1* (1823 bps) as a probe. As the result of the screening, four near full-length acid invertase cDNA clones, *CaAIV-3, CaAIV-4, CaAIV-18* and *CaAIV-23* were selected. *CaAIV-18* clone, the longest clone among them, was 2223 nucleotides and contained the 1923 nucleotide coding region and an 18 nucleotide poly(A) tail.

DNA Sequence Analysis

As results of sequence analysis and homology search, 5 clones selected from the cDNA library screening were identified as the same clones, except the sequences in the 3'-untranslated region. The longest clone, *CaAIV-18* was 2223 nucleotides in length containing 18 nucleotide poly (A) tails. The putative ATG initiation codons for *CaAIV-18* was located at position 9 with an open reading frame of 1929 nucleotides which encoded a polypeptide of 643 amino acids (Fig. 2). The calculated molecular weight was 7.14 kDa and pI was 5.66. Deduced amino acid sequence of *CaAIV-18* proved to have two conserved sequences of typical acid invertase, NDPNG (fructofuranosidase motif) and WECVD (catalytic site) moieties. And hot pepper acid invertase showed a high similarity with soluble acid invertases of other plant species, such as tomato (Ohyama *et al.*, 1992), carrot (Unger *et al.*, 1994) and potato (Zrenner *et al.*, 1996) in the amino acid

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60   ACA TTT CTC CCG GGT CAA CCC GAT TCC GGT CAC CGG AAG TCC ATT AAA GTT GTC TCC GTC   119
18    T   F   L   P   G   Q   P   D   S   G   H   R   K   S   I   K   V   V   S   V     37
120  ATT TTA CTC TCC TCT TTC TTT TTG CTT TAT TTA GCC GCC TTT GTT ATT CTC AAC AAC CAA   179
38    I   L   L   S   S   F   F   L   L   Y   L   A   A   F   V   I   L   N   N   Q     57
180  CCT CCC AAC TTG CAA AAT AAA TCT CCG TCG GCG TCG GAG ACT TTG ACG CCG GCG ACC CCG   239
58    P   P   N   L   Q   N   K   S   P   S   A   S   E   T   L   T   P   A   T   P     77
240  TCA AGA GGT GTT TCT CAG GGA GTG TCG GAG AAG ACT TTT AAG GAT GTT TCA GGT ACT AGT   299
78    S   R   G   V   S   Q   G   V   S   E   K   T   F   K   D   V   S   G   T   S     97
300  CAA GTT TCG TAC ACG TGG TCC AAT GCT ATG CTT AAC TGG CAA AGA ACT GCT TAT CAT TTT   359
98    Q   V   S   Y   T   W   S   N   A   M   L   N   W   Q   R   T   A   Y   H   F    117
360  CAA CCT CAA AAA AAT TGG ATG AAC GAT CCT AAT GGT CCA TTG TAC CAC AAG GGA TGG TAC   419
118   Q   P   Q   K   N   W   M   N   D   P   N   G   P   L   Y   H   K   G   W   Y    137
420  CAT CTA TTT TAT CAA TAC AAT CCA GAT TCT GCT ATT TGG GGA AAC ATC ACA TGG GCC CAT   479
138   H   L   F   Y   Q   Y   N   P   D   S   A   I   W   G   N   I   T   W   G   H    157
480  GCA GTA TCC ACT GAC TTG ATC CAC TGG CTT TAC TTG CCT TTT GCC ATG GTT CCT GAT CAA   539
158   A   V   S   T   D   L   I   H   W   L   Y   L   P   F   A   M   V   P   D   Q    177
540  TGG TAC GAT ATC AAC GGT GTT TGG ACT GGG TCC GCT ACC ATC CTA CCT GAC GGT CTG ATC   599
178   W   Y   D   I   N   G   V   W   T   G   S   A   T   I   L   P   D   G   L   I    197
600  ATG ATG CTC TAT ACT GGT GAT ACC GAT GAT TAT GTG CAG GTG CAA AAT CTT GCG TAC CCC   659
198   M   M   L   Y   T   G   D   T   D   D   Y   V   Q   V   Q   N   L   A   Y   P    217
660  GCC AAC TTA TCT GAT CCT CTC CTC CTT GAC TGG GTC AAA TAC CAG GGC AAC CCC GTC TTG   719
218   A   N   L   S   D   P   L   L   L   D   W   V   K   Y   Q   G   N   P   V   L    237
720  GTT CCC CCA CCC GGC ATT GGT GTC AAG GAC TTT AGA GAC CCG ACT ACT GCT TGG ACC GGA   779
238   V   P   P   P   G   I   G   V   K   D   F   R   D   P   T   T   A   W   T   G    257
780  CCC CAA AAT GGG CAA TGG CTG CTA ACC ATT GGG TCC AAG GTT GGT AAG ACC GGT ATT GCA   839
258   P   Q   N   G   Q   W   L   L   T   I   G   S   K   V   G   K   T   G   I   A    277
840  CTT GTT TAT GAA ACT TCC AAC TTT ACA AGT TTT AAG CTA TTG GAT GGA GTT TTG CAT GCG   899
278   L   V   Y   E   T   S   N   F   T   S   F   K   L   L   D   G   V   L   H   A    297
900  GTT CCG GGT ACG GGT ATG TGG GAG TGT GTG GAC TTT TAC CCG GTA TCC ACC TTA GAC GCA   959
298   V   P   G   T   G   M   W   E   C   V   D   F   Y   P   V   S   T   L   D   A    317
960  AAC GGG TTG GAC ACA TCA TAT AAC GGG CCG GGT ATA AAG CAC GTT CTA AAA GCA AGT TTA  1019
318   N   G   L   D   T   S   Y   N   G   P   G   I   K   H   V   L   K   A   S   L    337
1020 GAT GAC AAT AAG CAA GAT CAC TAT GTC ATT GGG ACA TAT GAC CCG GTA AAG AAC AAA TTT  1079
338   D   D   N   K   Q   D   H   Y   V   I   G   T   Y   D   P   V   K   N   K   F    357
1080 TCA CCC GAT AAT CCA GAT TTG GAT TGT GGA ATT GGG CTG AGG CTG GAC TAT GGG AGA TAT  1139
358   S   P   D   N   P   D   L   D   C   G   I   G   L   R   L   D   Y   G   R   Y    377
1140 TAT GCA TCA AAG ACA TTT TAT GAC CCA AAG AAA CAA AGA AGA GTA CTT TGG GGA TGG ATT  1199
378   Y   A   S   K   T   F   Y   D   P   K   K   Q   R   R   V   L   W   G   W   I    397
1200 GGG GAA ACT GAC AGT GAA TCT GCT GAC CTC CAA AAG GGA TGG GCA TCT GTA CAG AGT ATT  1259
398   G   E   T   D   S   E   S   A   D   L   Q   K   G   W   A   S   V   Q   S   I    417
1260 CCA AGG ACA GTG CTT TTT GAC AAG AAG ACA GGG ACA CAT CTA CTT CAG TGG CCA GTT GCA  1319
418   P   R   T   V   L   F   D   K   K   T   G   T   H   L   L   Q   W   P   V   A    437
1320 GAA ATT GAA AGC TTA AGA TCA GGT GAT CCT AAA GTG AAG GAG GTC AAT CTT CAA CCA GGC  1379
438   E   I   E   S   L   R   S   G   D   P   K   V   K   E   V   N   L   Q   P   G    457
1380 TCA ATT GAG TTA CTC CAT GTT GAC TCA GCT GCA CAG TTT GAT ATA GAA GCG TCA TTT GAA  1439
458   S   I   E   L   L   H   V   D   S   A   A   Q   F   D   I   E   A   S   F   E    477
1440 GTG GAC AGA GTC ACG CTC GAG GGA ATA ATT GAA GCA GAT GTA GGT TAC AAC TGC TCT ACT  1499
478   V   D   R   V   T   L   E   G   I   I   E   A   D   V   G   Y   N   C   S   T    497
1500 AGT GGA GGT GCT GCT AGC AGA GGC ATT TTG GGA CCA TTT GGT GTT GTT GTA ATT GCT GAT  1559
498   S   G   G   A   A   S   R   G   I   L   G   P   F   G   V   V   V   I   A   D    517
1560 CAA ACA TTG TCC GAG TTA ACT CCA GTT TAC TTC TAC ATT TCT AGA GGC GCT GAT GGC CGA  1619
518   Q   T   L   S   E   L   T   P   V   Y   F   Y   I   S   R   G   A   D   G   R    537
1620 GCA GAG GCT CAC TTC TGT GCT GAT CAA ACT AGA TCC TCA GAG GCT CCT GGA GTT GCT AAG  1679
538   A   E   A   H   F   C   A   D   Q   T   R   S   S   E   A   P   G   V   A   K    557
1680 CAA GTT TAT GGT AGT TCA GTA CCA GTG TTG GAT GGT GAA AAA CAT AGG ATG AGA TTA TTG  1739
558   Q   V   Y   G   S   S   V   P   V   L   D   G   E   K   H   R   M   R   L   L    577
1740 GTG GAC CAC TCA ATT GTG GAG AGC TTT GCT CAA GGA GGA AGA ACA GTG ATA ACA TCG CGA  1799
578   V   D   H   S   I   V   E   S   F   A   Q   G   G   R   T   V   I   T   S   R    597
1800 ATT TAT CCA ACA AAG GCA GTG AAT GGA GCA GCA CGG CTC TTC GTT TTC AAC AAT GCT ACA  1859
598   I   Y   P   T   K   A   V   N   G   A   A   R   L   F   V   F   N   N   A   T    617
1860 GGG GCT ATT GTG ACT GCC TCC CTC AAG ATT TGG TCA CTT GAG TCG GCT GAT ATT CCA TCC  1919
618   G   A   I   V   T   A   S   L   K   I   W   S   L   E   S   A   D   I   P   S    637
1920 TTC CCA CTG CAA AAG TTG TGA TTT ATA TCT TCT TCA TTC TTT TCT CAT TTG AAG GTT ATT  1979
638   F   P   L   Q   K   L   *                                                          643
1980 CCA CCG TCC CAT CAA GAA ATC CAA GAT AGA GAG TAC CGT AGA GTA TGT AGA TTT ATT TCC  2039
2040 CAT TTT AGT GGA GTA TTT TTC TTC TGG ACT GCT ATT GTT GCC CAG CAG TGT CAG CGT CGG  2099
2100 CGG ATT ACC ATA TGA TAT GGG TTC TGT GAA CCC AAT AGC TCG GAT TCT GTA AAA GTA AGA  2159
2160 AAG TTC AGC AAA TAT GTA CAA ATA ATT TAT TGG CTT CTT AGT TCT TAA AAA AAA AAA AAA  2219
2220 AAA A                                                                             2223
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Fig. 2. The nucleotide and deduced amino-acid sequences of *CaAIV-18* cDNA. Underlines indicate the putative membrane anchor or transit peptide region, the β-fructofuranosidase motif (NDPNG), the catalytic domain (WECVD), sequentially.

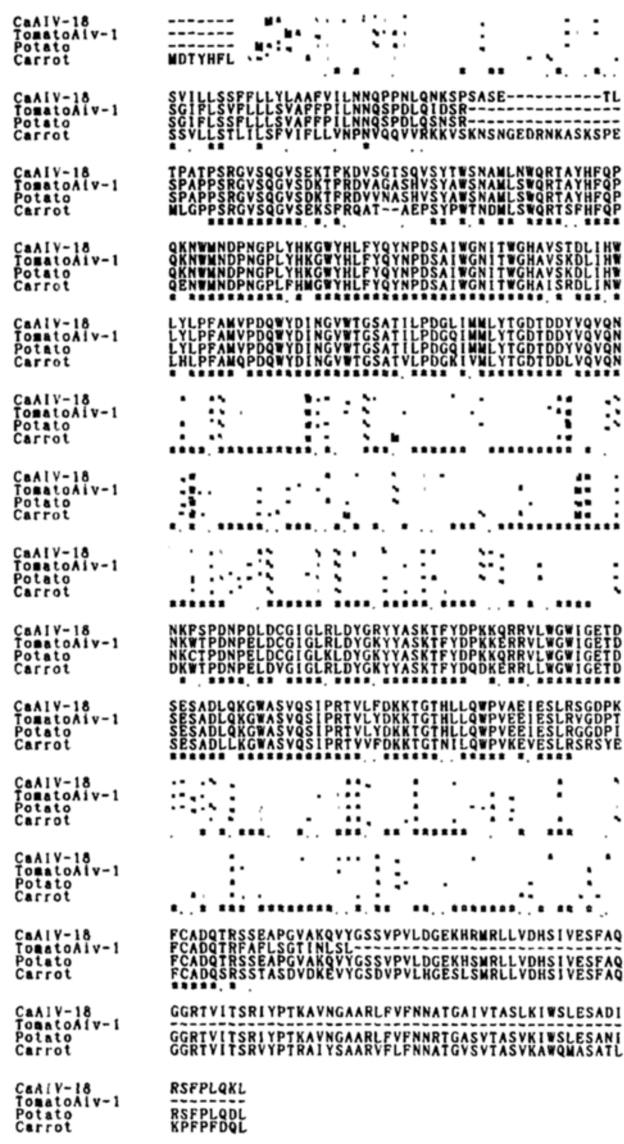

Fig. 3. Multiple alignment of four soluble acid invertases. Asterisk indicates the identical amino acid sequences; "----" was added to maximize alignment.

sequences (Fig. 3). CaAIV-18 as well as other genes or cDNA's encoding acid invertase of plant species have disputable primary structures in the vicinity of their 5' regions. In the analysis of this region with the SOSUI system and hydropathy profile, the 24-25 amino acid region of the N-terminal is suspected to be a kind of membrane anchor because of its high hydrophobicity. If true, acid invertase must be a membrane protein targetted into the plasma membrane or membrane of a certain cell organelle. Or, the hydrophobic region can be transit peptide introducing the mature peptide into a cell organelle, *eg*. vacuole.

Genomic Southern Analysis of the *CaAIV-18* Clone

To determine the number of genes encoding acid invertase in hot pepper, genomic DNA blot analysis was performed. As shown in Fig. 4, one or two hybridizing fragments were detected. Considering that the *Eco*RV, *Hin*dIII and *Xba*I recognition sites are in the *CaAIV-18* clone, this gene is considered to be located as single or low copies in the hot pepper

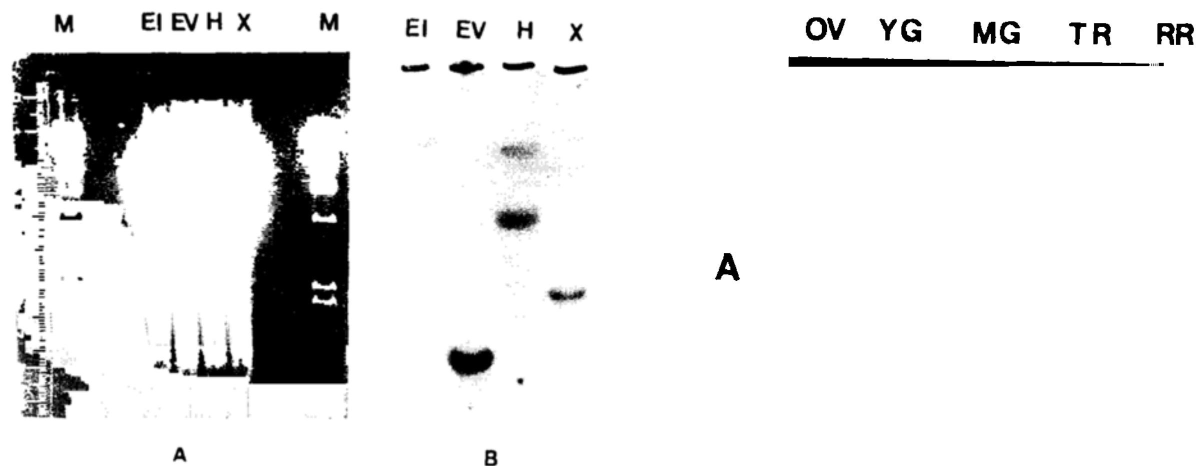

Fig. 4. DNA gel blot analysis of genomic DNA with *CaAIV-18* cDNA (*Eco*RI-*Apa*I cut, 2.3 kb) as a probe. A, thirteen μg of genomic DNA digested and electrophoresed in 0.8% agarose gel; B, corresponding autoradiography of A. M, λ/*Hind*III size marker; EI, *Eco*RI; EV, *Eco*RV; H, *Hind*III; X, *Xba*I digests, respectively.

OV YG MG TR RR

B

Fig. 5. Northern blot analysis of total RNA with the fragment of *CaAIV-18* cDNA (*Sph*I-*Hind*III cut) as a probe. A, twenty μg of total RNA electrophoresed in 1.0% agarose gel containing formaldehyde; B. corresponding autoradiography of A. OV, ovary type fruit; YG, young-green fruit; MG, mature-green fruit; TR, turning-red fruit; RR, ripe-red fruit.

genome.

Previous studies suggested that only one type of acid invertase might exist in bell pepper through enzyme analysis (Nielsen *et al.*, 1991; Michaud *et al.*, 1993). This fact deviates from many reports of many other plant species. *Arabidopsis*, tomato, and carrot were reported to have several isozymes of acid invertase (Schwebel-Dugue *et al.*, 1994; Ohyama *et al.*, 1992; Unger *et al.*, 1994). But, because we acquired only one or two hybridizing signals, the hot pepper genome does not seem to have any other type of acid invertase.

Expression Pattern of the *CaAIV-18* Clone

Some genes or cDNA clones expressed during fruit development of bell pepper were reported. Among them, ascorbate peroxidase, cysteine synthase, geranylgeranyl pyrophosphate synthase, phytoene synthase, and capsanthin-capsorubin synthase genes were closely related to fruit ripening. Most of them show low level of expression in young fruit, but in RR fruit, they show very strong expression. In our experiment, acid invertase in hot pepper fruit showed a very interesting expression pattern (Fig. 5). In ovary type fruit, the primordium of the fruit, it was not expressed at all and then slightly expressed in YG fruit. But in MG fruit, it showed lower level of expression than in YG fruit, and finally in RR fruit, it was expressed maximally. This result suggests the role of acid invertase during the development of pepper fruit. Though it cannot play a role in fruit initiation, acid invertase may provide hexoses to pericarp tissue as an energy source for vegetative growth of the fruit in early stages and for the synthesis of ripening-related metabolites, *eg.* capsanthin and isoprenoids, *via* a glycolytic pathway.

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