

PCR-based Single-strand Conformation Polymorphism (SSCP) Analysis to Clone Nine Aquaporin Genes in Cucumber

Jiahua Xie,¹ Todd C. Wehner,² and Mark A. Conkling³

Departments of Horticultural Science and Genetics, North Carolina State University, Raleigh, NC 27695

ADDITIONAL INDEX WORDS. *Cucumis sativus*, gene cloning, PCR-SSCP

ABSTRACT. Combining the use of PCR and single-strand conformation polymorphisms (SSCP), nine sequences from the cucumber genome were successfully identified and cloned that encoded two well-conserved asparagine-proline-alanine (NPA) domain homologues to aquaporin genes. The sensitivity and detection efficiency of SSCP and restriction enzyme analysis for detecting DNA sequence variation were evaluated using similar-sized DNA fragments. The SSCP analysis was more sensitive and efficient for discriminating different clones than restriction enzyme analysis, although some sequence variation inside similar-sized DNA fragments could be identified by restriction analysis. Consideration of the results of SSCP analysis with DNA sequence information indicated that one or two base pair changes in the amplified regions could be detected. Moreover, the SSCP analysis results of genomic DNA PCR products that were amplified by degenerate primers can provide rough information about the number of member genes. If the SSCP bands of a cloned fragment (such as CRB7) did not have the corresponding bands from genomic DNA PCR products, that fragment might be a misamplified product. The PCR-based SSCP method with degenerate oligonucleotide primers should facilitate the cloning of member genes.

The polymerase chain reaction (PCR) using degenerate oligonucleotide primers has made it possible to clone genes more quickly and easily (Takumi and Lodish, 1994; You and Scholl, 1998). With the accumulation of gene sequences, it becomes easy to design the degenerate primers for cloning new members of a gene family from related species. However, for members of a gene family amplified using degenerate primers, the PCR products for these genes can be similar in length. Thus, the different genes will be hidden when they are run on an agarose gel. Direct DNA sequencing would be the best method for distinguishing different DNA fragments, and sequencing as many clones as possible may permit the identification of some or all members of a gene family, but it is time-consuming and expensive.

After cloning, different restriction enzymes that cut DNA infrequently are used to identify the new homologue, especially if using enzymes with recognition sites of 6 to 8 bp (Preston, 1993). The problem is that restriction analysis for small (300 to 500 bp) DNA bands requires that the bands have a recognition site close to the middle. DNA bands without enzyme recognition sites, or with two outside recognition sites may not appear in the analysis. Therefore, a more sensitive method is needed for detection of DNA fragments of similar length to identify the member genes during cloning.

Single-strand conformation polymorphisms (SSCP) are based on electrophoretic detection of conformational changes in single-stranded DNA molecules resulting from point mutations or other forms of small nucleotide changes. It was originally developed

for rapid analysis of mutations (Orita et al., 1989). Single-strand conformation polymorphisms has been used extensively in human genetics to detect mutations within genes, and was able to detect single-point mutations (Hayashi, 1992). Single-strand conformation polymorphisms analysis was considered as a sensitive, inexpensive, and rapid method for detecting sequence variation (Sekiya, 1993; Slabaugh et al., 1997). This technique was also employed to characterize expressed products between two highly related member genes (Hedley et al., 1994), analyze the difference among members of a gene family (Hagiwara et al., 1998; Slabaugh et al., 1997), and produce SSCP markers for gene mapping (Slabaugh et al., 1997). Recently, the usefulness of this approach has been extended through the use of end-labeled primers and PCR amplification, with a small amount of genomic DNA or RNA being analyzed (Peterson et al., 1995; Slabaugh et al., 1997).

Aquaporins (AQPs) are a family of intrinsic membrane proteins with many homologous genes that function as water-selective channels in the plasma membranes of many water-transporting tissues (Agre et al., 1993). We needed to identify a root-specific aquaporin gene in cucumber for our root-knot nematode resistance work. Using PCR-based SSCP analysis, nine sequences have been successfully cloned from the cucumber genome that encoded two well-conserved NPA domains homologous to the aquaporin gene.

Materials and Methods

DNA PREPARATION. Seedlings of *Cucumis sativus* L. 'Sumter', a monoecious inbred pickling cucumber, were grown in the greenhouse. Leaves of four-week-old seedlings were harvested for DNA isolation. Total DNA was prepared by grinding frozen leaf tissue and suspending the powder in CTAB buffer containing 50 mM EDTA, 10 mM Tris-HCl (pH 8.0), 1% CTAB, 0.7 M NaCl, and 0.2% β -mercaptoethanol. DNA was precipitated by ice-cold 100% iso-propanol, and then washed with 76% ethanol with 0.2 M NaOAc and 76% ethanol with 10 mM NH₄OAc, respectively.

SYNTHESIS OF OLIGONUCLEOTIDE PRIMERS. Multiple alignments

Received for publication 1 May 2002. Accepted for publication 2 Sept. 2002. The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service (NCARS), Raleigh, NC 27695-7643 of the products named, or criticism of similar ones not mentioned. This work was supported by a grant from Pickle Packers International. The authors gratefully acknowledge the technical assistance of J.P. Fellers, Y. Li, G.N. Acedo, and W.L. Liu.

¹Postdoctoral research associate. Currently researcher, Vector Tobacco Ltd., 700 Main Street, Durham, NC 27701.

²Professor and corresponding author; email Todd_Wehner@NCSU.edu.

³Professor. Currently vice president, Vector Tobacco Ltd., 700 Main Street, Durham, NC 27701.

of the most highly conserved portions of 52 major intrinsic protein (MIP) family proteins showed that there were the two well-conserved NPA motifs in their amino acid sequence (Park and Saier, 1996). Two degenerate primers were designed based on amino acid sequence of the two well-conserved NPA motifs. Twenty base pair forward primer Cu1 5' [5'-GG(A/T/C/G)-CAC-ATC-AA(C/T)-CC(A/T/C/G)-GC(A/T/C/G)-GT-3'], based on the amino acid sequence GHINPAV, and 20 bp reverse primer Cu2 3' [5'-ACT-GG(A/T/C/G)-ATC-AA(C/T)-CCA-GC(A/T/C/G)-AG-3'], based upon the amino acid sequence TGINPAR, were synthesized (Genosys Biotechnologies Inc, The Woodlands, Texas).

END LABELING OF OLIGONUCLEOTIDE PRIMERS. The 5 µg of each primer Cu1 and Cu2 were end-labeled with γ -³²P dATP. Twenty microliters of reaction solution contained 1 µL T4 polynucleotide kinase (PNK) (Boehringer Mannheim, Indianapolis, Ind.), 5 µL (\approx 50 µci) γ -³²P dATP, 2 µL 10× PNK buffer, and 7 µL distilled water. The solution was incubated at 37 °C for 30 min. Inactivation of T4 PNK was by heating at 65 °C for 10 min. Volume was brought to 100 µL with 1× TE (pH 7.6). Unincorporated dATP was removed using a G-50 Sephadex column.

POLYMERASE CHAIN REACTION AND CLONING. Genomic DNA was used for amplification with degenerate primers Cu1 and Cu2. Each 25 µL reaction contained 100 ng of genomic DNA template, 1.25 units of Taq DNA polymerase (Sigma, St Louis, Mo.), 150 ng of each of two primers Cu1 and Cu2, 10 µmol dNTPs and PCR buffer (Sigma) to a final concentration of 10 mM Tris-HCl, 1.5 mM MgCl₂, 50 mM KCl with a pH of 8.3. Polymerase chain reactions were initiated by a denaturation step at 94 °C for 2 min, then the reaction was subjected to 30 cycles of 94 °C for 1 min, 58 °C for 1 min, and 72 °C for 1 min with a final elongation step of 10 min at 72 °C. Amplification products were resolved by electrophoresis on a 0.8% agarose gel with ethidium bromide in TAE buffer and visualized under UV illumination.

Fresh PCR products derived from genomic DNA with primers Cu1 and Cu2 that produced a single band in agarose gel electrophoresis were cloned into pCR2.1 vector with the Original TA Cloning Kit (Invitrogen Corp., Carlsbad, Calif.) using the manufacturer's recommended protocol. After ligation, INVa⁺ *Escherichia coli* cells (Invitrogen Corp.) were transformed with the plasmid, and colonies containing plasmid with insert were selected on plates with LB kanamycin medium containing 50 µg·mL⁻¹ kanamycin and X-Gal. Forty clones were amplified by PCR with primers Cu1 and Cu2 to check ligation results. For colony PCR reaction, the bacterial colony was picked by a toothpick and put into the reaction tube as template DNA. The reaction condition is same as mentioned above for genomic DNA. The PCR products were electrophoresed on a 0.8% agarose gel to visualize ligation products.

RESTRICTION ANALYSIS. Polymerase chain reaction products derived from 39 inserted clones were digested by four restriction enzymes *Alu* I, *Rsa* I, *Sau*3 AI, and *Hae* III (GIBCO/BRL, Grand Island, N.Y.) with 4 bp recognition sites (Table 1) to distinguish different clones, and 5 µL of PCR products were digested with 5 unit restriction enzyme, respectively. The digested products were subjected to electrophoresis on a 0.8% agarose gel.

SSCP ANALYSIS. The protocol for SSCP analysis was based on the methods described by Lee et al. (1992) with some modifications. To carry out SSCP gel electrophoresis, 6% polyacrylamide gels (49 acrylamide : 1 bisacrylamide) were made up in 0.5×TBE buffer containing 5% glycerol. Bacterial colonies or 100 ng genomic DNA were used as template DNA for the labeling PCR.

The PCR reaction with two labeled primers Cu1 and Cu2 was set as follows: 25 µL volume containing 37.5 ng labeled Cu1 and Cu2 primers, same amount of dNTPs, Taq polymerase and PCR buffer as mentioned above for genomic PCR. Reaction conditions were the same as above. Heat-denatured PCR samples (1 µL) mixed with 2 µL sequence stop solution containing formamide (United States Biochemical, Cleveland, Ohio) were applied to the gel and electrophoresis was carried out at a constant power of 2 W for 20 hours. The gel was kept at constant room temperature. At the end of electrophoresis, the gel was transferred onto a sheet of Whatman 3 MM paper and vacuum dried at 80 °C for 1.5 h. Autoradiography was carried out overnight at -80 °C using Kodak diagnostic film (Rochester, N.Y.).

SEQUENCE AND ANALYSIS. The high quality plasmid DNA of each group of clones were prepared using the QIAprep miniprep kit (QIAGEN, Valencia, Calif.) using the manufacturer's recommended protocol. The clones were sequenced as ds DNA using Perkin-Elmer ABI prism 377 sequenator with analysis version 2.1.1. The alignment with other reported aquaporin genes was performed with the FASTA (Pearson and Miller, 1992) or Blast (Altschul et al., 1990) programs (GenBank).

Results and Discussion

PCR PRODUCTS AND CLONING. Total DNA of cucumber was amplified by PCR using two degenerate primers Cu1 and Cu2. After PCR products were resolved on a 0.8% agarose gel, only one band of \approx 500 bp was observed, while no band was observed in the control having no template DNA. Compared with the two well-conserved NPA motif genomic sequences for aquaporin genes cloned from tobacco and *Arabidopsis thaliana* (Höfte et al., 1992; Yamamoto et al., 1991), this band is shorter than those of the tobacco aquaporin gene *TobRB7* (\approx 660 bp including a 284 bp intron) (Yamamoto et al., 1991) as well as that of the *Arabidopsis* aquaporin gene α -*TIP* (\approx 580 bp with one 120 bp intron) (Höfte et al., 1992). It is similar in size to the other *Arabidopsis* aquaporin gene γ -*TIP* (\approx 450 bp with a 91 bp intron) (Höfte et al., 1992).

The fresh PCR products were cloned into the pCR2.1 vector. Forty white colonies (designated as C1-1 to C1-40) were taken at random to do PCR with Cu1 and Cu2 primers to test the insertion. The results indicated that 39 out of 40 clones (all except C1-39) had a PCR product of \approx 500 bp.

RESTRICTION ENZYME ANALYSIS. Restriction analysis of PCR products showed that all four enzymes (*Alu* I, *Rsa* I, *Sau*3 AI, and *Hae* III) could be employed to detect differences among 39 samples (Table 1). When PCR products of 39 clones were digested by *Rsa* I, 35 clones had the same restriction band pattern.

Table 1. The polymorphism of clones identified by the restriction enzyme analysis.²

Enzyme	Tested clones (no.)	Clones with different restriction polymorphisms from each major group	Potential groups (no.)
<i>Rsa</i> I	39	C1-16 ^a , C1-20, C1-35 (C1-40)	4
<i>Alu</i> I	39	C1-16 ^a , C1-1, C1-35 (C1-40)	4
<i>Sau</i> 3AI	39	C1-16, C1-20, C1-35 (C1-40)	4
<i>Hae</i> III	39	C1-16, C1-20, C1-35 (C1-40)	4
Total	39	C1-1, C1-16, C1-20, C1-35 (C1-40)	5

²Samples inside parentheses had the same restriction polymorphism as samples outside parentheses.

³Undigested sample.

The band pattern of clone C1-20 was different from the others. Clones C1-35 and C1-40 were the same, but were different from the other clones. Clone C1-16 was undigested, possibly because there was no recognition site between the cloned regions. When *Alu* I was used, 35 clones (including C1-20) had the same restriction band pattern, and clones C1-35 and C1-40 were the same as each other but different from the others. Clone C1-1 was different from all of the others. Clone C1-16 was still undigested. For *Sau* 3AI and *Hae* III, 35 out of 39 clones had the same restriction band patterns, clone C1-16 was digested and different from the others. Clone C1-20 was different from the others. Clones C1-35 and C1-40 were the same as each other but different from the others.

We concluded that clones C1-1, C1-16, and C1-20 were different from each other and the other clones. Clones C1-35 and C1-40 were the same as each other but different from the others. The remaining 34 clones were the same. Results of the restriction analysis suggested that the 39 clones could be divided into five groups, and that four potentially homologous aquaporin gene members were identified by this analysis. Our results were similar to a previous report that more member genes could be obtained by digesting clones with restriction enzymes (Preston, 1993). Clone C1-13 from the majority group, along with four different clones (C1-16, C1-20, C1-35 and C1-1) were designated CRB1, CRB2, CRB3, CRB4 and CRB5 for further study.

SSCP ANALYSIS. We tested the ability of SSCP analysis to

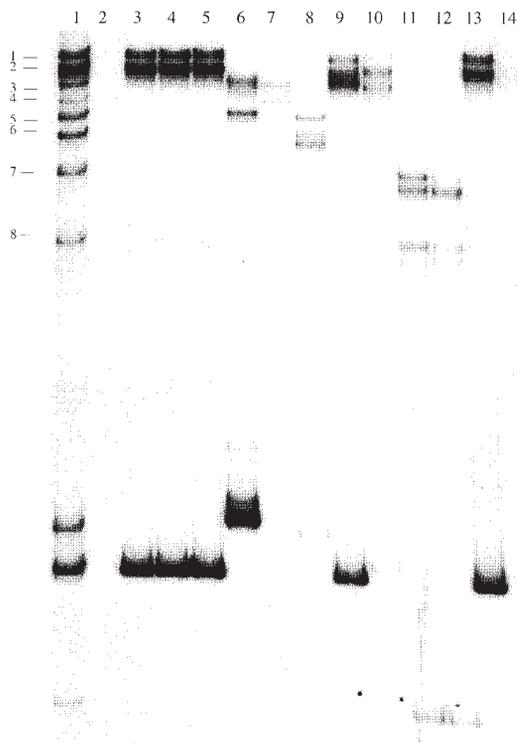


Fig. 1. SSCP bands of cucumber genomic DNA and different clone PCR products amplified by two degenerate primers Cu1 and Cu2. Lane 1 = genomic DNA; lane 2 = without template DNA as control; lane 3 = CRB1; lane 4 = C2-5; lane 5 = C2-2; lane 6 = CRB2; lane 7 = CRB3; lane 8 = CRB4; lane 9 = CRB6; lane 10 = CRB8; lane 11 = CRB9; lane 12 = CRB10; lane 13 = C2-1; lane 14 = CRB5.

Fig. 1. SSCP bands of cucumber genomic DNA and different clone PCR products amplified by two degenerate primers Cu1 and Cu2. Lane 1 = genomic DNA; lane 2 = without template DNA as control; lane 3 = CRB1; lane 4 = C2-5; lane 5 = C2-2; lane 6 = CRB2; lane 7 = CRB3; lane 8 = CRB4; lane 9 = CRB6; lane 10 = CRB8; lane 11 = CRB9; lane 12 = CRB10; lane 13 = C2-1; lane 14 = CRB5.

distinguish different clones. Seven clones CRB1 to CRB5 and two more samples (C1-5 and C1-8) from the remaining majority group were subjected to SSCP analysis. The results showed that SSCP analysis was highly sensitive in distinguishing differences among clones. The band patterns of the five clones CRB1 to CRB5 were different on the SSCP gel, while they could not be distinguished by normal agarose gel electrophoresis. The SSCP bands of CRB1, CRB2, CRB3, CRB4 and CRB5 were similar to lanes 3, 6, 7, 8, and 14 in Fig. 1. Clone CRB4 migrated faster than the other four, followed by Clone CRB3, and then CRB2. Three clones (C1-5, C1-8 and CRB1) from the remaining group characterized by restriction analysis produced monomorphic SSCP bands (data not shown). Thus, the results suggested that SSCP gel analysis could distinguish differences among clones.

Generally, two single-strand DNAs should produce only two conformation polymorphisms, and yield two SSCP bands. However, some electrophoretic profiles showed three or four instead of two bands. This might be due to the presence of two stable conformations for one of the bands (Hayashi, 1991), or to an excess of primers that might bind to the DNA strands and form a detectable subspecies (Cai and Tuitou, 1993). These types of artifacts can be avoided by removing the oligonucleotides from the PCR product (Fujita and Silver, 1994) or by diluting it (Rubio et al., 1996).

For testing the detection efficiency of SSCP analysis, 21 untested white clones derived from cloning of PCR products were selected and designated as C2-1 to C2-21 for analysis. The five different clones CRB1 to CRB5 that had been confirmed by the restriction enzyme and SSCP analysis were analyzed again to determine the reproducibility of SSCP bands. Meanwhile, PCR products of cucumber genomic DNA that were amplified by two degenerate primers (Cu1 and Cu2) were analyzed together with the 21 new clones.

The results indicated that the SSCP band patterns of five different groups were repeatable. The five different clones had different band patterns as observed before. Our work further confirmed the previous report that the resulting SSCP pattern was highly reproducible when conditions were held constant (Fan et al., 1993). Among the 21 newly tested clones, there were 8 SSCP band patterns. Clone C2-21 was different from the other 20 clones, but it had the same pattern as clone CRB4 that already has been observed. Clones C2-3, C2-12, and C2-19 were the same as each other and the same as clone CRB3. The band patterns of clones C2-4, C2-6, C2-14, C2-16, and C2-18 were different from each other, and different from those of the previously observed 5 clones. The remaining 12 clones were the same as each other and the same as the previously observed majority group (data not shown). Clones C2-4, C2-6, C2-14, C2-16, and C2-18 were designated as CRB6, CRB7, CRB8, CRB9, and CRB10. Band patterns of CRB6, CRB8, CRB9, and CRB10 were similar to lanes 9 through 12 of Fig. 1. The CRB9 and CRB10 clones migrated rapidly on the SSCP gel, and they appeared to have small fragments. Clone CRB7 migrated slowly on the gel, and there were two separate bands on top of the SSCP gel (data not shown). Only the band patterns of CRB2 and CRB5 were not observed in the new sample population of 21 clones. From the above observation, 8 different clones, including 5 new clones, were obtained in one SSCP gel containing 21 clone samples, suggesting that SSCP analysis can distinguish among clones with great sensitivity.

Only one band of ≈ 500 bp was observed when the PCR products of cucumber genomic DNA using degenerate primers

were electrophoresed in the agarose gel. However, 8 bands were separated when they were analyzed by an SSCP gel, and all 8 had corresponding bands from different clones. Also, one or all SSCP bands of 10 different clones except CRB7 had corresponding bands from genomic PCR products. It appears that all of the different fragments of genomic PCR products that were amplified by the two degenerate primers have been subcloned.

The advantage of SSCP analysis with genomic PCR products is that it can provide relevant information on the number of member genes that are amplified by degenerate primers. Theoretically, genomic PCR products that were amplified by the degenerate primers should contain many different fragments representing different member genes. These different fragments have a different single strand conformation and will produce polymorphism on the SSCP gel. Thus, while different clones were analyzed by SSCP, the band pattern of different clones should have corresponding bands from genomic DNA SSCP. By comparing SSCP band patterns of genomic PCR products with SSCP band patterns of clones, we can get a rough identification of the uncloned members. In our study, all 8 bands from genomic DNA SSCP had corresponding bands from cloned fragments. If some SSCP bands of genomic DNA PCR products do not have corresponding bands from cloned fragments, this indicates that some members may not yet have been cloned. To get these uncloned members, additional clones should be analyzed with SSCP until the corresponding band is cloned. Alternatively, the uncloned band from SSCP gel can be cut, reamplified and cloned, as suggested by Slabaugh et al. (1997).

To demonstrate the results of the SSCP analysis, cucumber genomic DNA and the 9 clones representing 9 groups (except CRB7, which had an unexpectedly large size) were re-amplified with two end-labeled degenerate primers Cu1 and Cu2 and rerun on the SSCP gel. Three additional clones (C2-1, C2-2, and C2-5) from the largest group were added on lanes 13, 5 and 4 to control two sides of the gel. The representative results of SSCP analysis of 12 partial aquaporin member genes and genomic DNA PCR products are shown in Fig. 1. The SSCP band patterns of each sample were similar to those in the previous tests. The SSCP results of genomic DNA PCR products had 8 bands in the SSCP gel. The SSCP band patterns of CRB1, CRB2, CRB3, CRB4, CRB5, CRB6, CRB8, CRB9 and CRB10 were different. The CRB9 and CRB10 clones migrated rapidly on the gel. Four clones (CRB1, C2-1, C2-2, and C2-5) from the same group had the same SSCP band patterns.

SEQUENCE RESULTS AND ANALYSIS. All ten different clones CRB1 to CRB10 were subjected to sequencing. The fragment of CRB7 was large and atypical, and since there was no relevant band from genomic DNA PCR products, we decided to sequence it for comparison. All ten clones were sequenced as ds-DNA using a Perkin-Elmer ABI Prism 377 sequencer. They were subjected to BLAST search with and without primers. Search results revealed that nine of the ten clones were homologues of aquaporin genes (data not shown); only CRB7 was a homologue of the 16S ribosomal RNA gene. CRB7 may have been misamplified and cloned. Among nine analyzed fragments that were amplified by degenerate oligonucleotide primers, four of



Fig. 2. The nucleotide sequence alignment of nine cucumber aquaporin homologue fragments amplified by two degenerate primers Cu1 and Cu2. Identical nucleotides are highlighted with black boxes; the nucleotides that are missing are indicated by the "-" symbol.

them (CRB1, CRB5, CRB6, and CRB8) had the same molecular size (493 bp), three of them (CRB2, CRB3, and CRB4) had smaller molecular sizes (478, 479, and 466 bp), and CRB9 and CRB10 were the same size (383 bp), but smaller than the other seven.

The nine DNA sequences were aligned as showed in Fig. 2 after spreading the sequences in the missing nucleotides. Comparing the nucleotide sequences, the nine clones were different from each other in sequence, even though some of them had the same molecular size. Some small differences between two sequences were observed. There were only 2, 2, 5, 4, 5, 7, and 1 bp differences respectively in sequences without primer regions, and 5, 5, 9, 8, 8, 12, and 6 bp differences respectively in sequences with primer regions between the pairs CRB1/CRB5, CRB1/CRB6, CRB1/CRB8, CRB5/CRB6, CRB5/CRB8, CRB6/CRB8, and CRB9/CRB10.

Sequencing results showed that the fragments that were amplified by degenerate primers based on a highly conserved amino acid sequence had the same or similar molecular size. Most clones in our experiment belonged to the majority group according to SSCP analysis. They were the same as CRB1 with 493 bp molecular sizes. Moreover, three (CRB5, CRB6, and CRB8) out of the nine groups had the same molecular size as CRB1, and the other three groups (CRB2, CRB3, and CRB4) had a size similar to CRB1. Only CRB9 and CRB10 were 110 bp shorter than that of the majority group (CRB1). This means that most initial PCR products of genomic DNA amplified by degenerate primers Cu1 and Cu2 had the same or similar molecular size. When the PCR products were run on an agarose gel, they could not be separated from each other owing to their similar molecular size or because they were hidden by majority products, as in the case of CRB9 and CRB10.

From nucleotide sequences along with SSCP analysis, we have found that SSCP analysis can distinguish 6 to 17 bp differences in sequences with primer ends even if sequences have the similar or same size. However, they would not be distinguishable from each other using agarose gel or restriction enzyme analysis because of similar molecular size and only small differences in base pair number.

By comparing the nine genomic DNA clone sequences with the cucumber aquaporin cDNA sequence (data not shown) amplified by RT-PCR with two degenerate primers Cu1 and Cu2, we found that seven fragments (CRB1, CRB2, CRB3, CRB4, CRB5, CRB6, and CRB8) had an intron between two well-conserved NPA motif regions, and they share identical intron positions localized in connecting loop C. Most characterized plant plasma membrane intrinsic protein members also share that intron position (Johanson et al., 2001). The CRB9 and CRB10 clones were intronless in this region. The CRB10 and CRB9 clones do not have any intron, but have same 124 putative amino acids in the NPA to NPA repeated region with the other 7 fragments. The CRB9 and CRB10 clones are highly homologous to CRB4 and CRB2, which possess one intron in the NPA repeated region. This suggests that CRB9 and CRB10 evolved with loss of introns. Intron deletion has been reported to occur often during evolution, and may be associated with genome slimming (Petrov et al. 1996).

It is important to clone and characterize many member genes in a family to provide maximum information for the understanding of their function and evolutionary relationship with each family member. Therefore, an efficient procedure for cloning member genes will be useful for the study of gene families.

However, the question arises as to whether the fragments with 1 to 7 bp difference in sequences without primer end regions and 6 to 17 bp differences in sequences with primer ends that were cloned by the PCR-based SSCP analysis are really different gene fragments or just the results of misamplification or sequencing error of the same gene.

A precise comparison of the nucleotides for each pair of gene fragments indicated that they were really different sequences. For example, one pair of gene fragments differing at one base pair was localized to the 39th nucleotide position between CRB9 (thymine 'T') and CRB10 (cytosine 'C'). The nucleotide of the 39th position of CRB4 had its 'T' at the same position as that of CRB9. The CRB1, CRB2, CRB3, CRB5, CRB6, and CRB8 clones had their 'C' at the same position as that of CRB10. The remaining pair of gene fragments, with two to seven base pairs different, was confirmed by finding identical nucleotides from other homologous genes at that position. According to sequence information of nine fragments, nine different pairs of primers were designed to re-amplify each specific fragment from the cucumber genome. Results showed that all pairs of primers could produce predicted PCR bands with cucumber genomic DNAs. Owing to functional importance, a large number of aquaporin genes have been cloned and characterized in animals, plants and microorganisms. Members of the aquaporin family are highly conserved from bacteria to mammals all of which have similar sequences (Johanson et al., 2001). Plant aquaporin gene families with many members and one or several base pairs difference between some members were also well documented in *Arabidopsis* (Johanson et al., 2001) and maize (*Zea mays* Bonaf.) (Chaumont et al., 2001). Nine fragments might be real different gene fragments. These nine fragments allow us to identify a root specific aquaporin gene and study phylogenetic relationships of cucumber aquaporin family.

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