Short communication

Analysis of Distribution and Diversity of Patatin Promoters in *Solanum* Species by Using the Polymerase Chain Reaction Technique

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Distribution and diversity of patatin promoters in *Solanum* species were analyzed by using the Polymerase Chain Reaction (PCR) technique. The promoter region (–580 to +11) of the B33 gene, one of the class I patatin genes, was cloned for use as a probe for Southern blotting. All the lines used in this experiment, even the no-tuber-bearing wild species, exhibited a homologous sequence to the class I patatin promoter region which controls the tuber-specific expression. The fragment pattern of amplified DNA varied with the lines.

KEY WORDS: potato, *Solanum*, *Lycopersicon peruvianum*, patatin promoter, polymerase chain reaction

Introduction

Class I patatin protein is normally present only in tubers, and class I patatin promoter controls the tuber-specific expression.

We attempted to reveal the existence of the class I patatin promoter region in the no-tuber-bearing potato wild species, to examine the relationship between the bearing of tubers and the existence of the promoter region, and analyzed the diversity of this region in potato cultivars, dihaploids and wild species by using the PCR technique.

Patatin genes are divided into class I and class II genes, and each class of genes builds up a multigene family. There are 10 to 15 patatin genes per haploid genome in potato cultivars (BEVAN *et al.* 1986; TWELL and OOMS 1988). BEVAN *et al.* (1986) observed that even tomato cultivars contained the sequences homologous to the patatin-coding region by hybridization with a patatin cDNA probe. However when a cDNA probe was used, the 5'flanking region could not be revealed. Although, tomato also contains sequences highly homologous to the class I potato patatin promoter region (PARK 1990), this region has not been documented in potato wild species.

Materials and Methods

Plant materials used in this experiment consisted of 18 potato cultivars, 23 dihaploids, 5 wild species belonging to the *Solanum* genus (including 3 no-tuber-bearing ones) and 1 wild species of the *Lycopersicon* genus (no-tuber-bearing). DNA was isolated from these plants according to the procedure reported by HONDA and HIRAI (1990). Two oligonucleotides corresponding to the –519 to –497 and –12 to +11 regions of the B33 gene were used as

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primers representing the class I patatin gene common sequence. B33 is one of the class I patatin genes in which the upstream region has been found to control the tuber-specific expression (ROCHA-SOSA *et al.* 1989). The B33 promoter region (−580 to +11) was amplified by PCR from the potato cultivar Norin-1, then cloned into plasmid pUC19. The DNA sequencing analysis showed that the cloned DNA corresponded to the B33 promoter region. The DNA was used as a probe for Southern blotting to identify the homologous sequences synthesized in another PCR experiment.

A 500ng aliquot of DNA was subjected to PCR in a total volume of 100 μ l with 2 units of *Tth* DNA polymerase (TOYOBO), 1 $\times$ *Tth* buffer (TOYOBO), 15pmol of each primer and 10nmol of each dATP, dGTP, dCTP, dTTP. DNA was pre-heated at 94°C for 2 minutes for pre-warming followed by 30 cycles of step 1: denaturation at 94°C for 1 minute, step 2: annealing at 60°C for 2 minutes, step 3: polymerization at 72°C for 3 minutes, then post-

Fig. 1. Electrophoretic pattern of PCR products.

Fragment pattern and density vary with the lines.

In lane 20, DNA was not amplified while the 1.7kb fragment was amplified in another experiment.

Foggy bands below the 550bp fragment correspond to the primers used in PCR.

M. size marker; λ DNA digested with *Hind*III C. control for Southern blot; cloned B33 promoter region 1. *Solanum acaule* 2. *Solanum brevidens* (no-tuber-bearing) 3. *Solanum nigrum* (no-tuber-bearing) 4. *Solanum photeinocarpum* (no-tuber-bearing) 5. *Solanum bulbocastanum* 6. *Lycopersicon peruvianum* (no-tuber-bearing) 7. cultivar May Queen 8. cultivar Danshaku 9. cultivar Seto-yutaka 10 – 32. dihaploids

heated at 72°C for 10 minutes for complete polymerization.

After electrophoresis, DNA was transferred to Hybond-N nylon membranes (Amersham) for Southern hybridization analysis. Southern blotting was performed almost as suggested by the manufacturer, using the DNA Labeling and Detection Kit Nonradioactive (Boehringer Mannheim).

Results and Discussion

Electrophoretic analysis showed the amplification of DNA in 46 lines (Fig. 1). While Southern hybridization analysis of the PCR products revealed the amplification of the 550bp fragment in 47 lines including *S. bulbocastanum*, which was not detected by electrophoresis (Fig. 2).

Thus, it was demonstrated that all the 47 lines used in this experiment harboured homologous sequences (550bp) to the class I patatin promoter region which controls the tuber-specific expression even in the four no-tuber-bearing wild species. This result suggests that the class I patatin promoter region is widely distributed at least in the *Solanum* genus and the *Lycopersicon* genus and that tuber-bearing occurred after the acquisition of the class I

Fig. 2. Hybridization of labeled B33 promoter region cloned from cultivar Norin-1 to PCR products.

Lane 5 (*S. bulbocastanum*) shows the amplification of the 550bp fragment which was not detected by electrophoresis.

C. control for Southern blot; cloned B33 promoter region 1. *Solanum acaule*
 2. *Solanum brevidens* (no-tuber-bearing) 3. *Solanum nigrum* (no-tuber-bearing)
 4. *Solanum photeinocarpum* (no-tuber-bearing) 5. *Solanum bulbocastanum* 6.
Lycopersicon peruvianum (no-tuber-bearing) 7. cultivar May Queen 8. cultivar
 Danshaku 9. cultivar Seto-yutaka 10 – 32. dihaploids

patatin promoter region. It is important to determine why these no-tuber-bearing wild species do not produce class I patatin protein, though they have a class I patatin promoter region and also a coding region of class I patatin (BEVAN *et al.* 1986).

The typical electrophoretic patterns and the amounts of amplified DNA varied with the lines and in most of the cultivars, the 1.0 kb and 550bp fragments were amplified mainly. Half of the dihaploid lines showed the same pattern as that of the cultivars but others showed the 1.7 kb fragment mainly.

These results suggest that the length of the fragments with patatin promoter homologous sequences is not uniform and that the specific band pattern of the dihaploids which harbour only half of the genome is revealed by small variations in the patatin promoters. One hypothesis is that these variations of length of the fragments were due to insertions, because several wild species did not show the 1.7kb fragment.

S. nigrum, *S. photeinocarpum*, *S. bulbocastanum* and *L. peruvianum* showed faint bands or lacked bands in the electrophoretic analysis. To verify the assumption that they may harbour a small number of class I patatin promoters, genomic Southern blotting analysis should be performed. Also the relationship between the copy number of class I patatin promoters and the absence of tubers or the amount of class I patatin protein should be investigated.

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Polymerase Chain Reaction 法による
バレイショパタチン遺伝子プロモーター領域の分布と変異の分析

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Polymerase Chain Reaction 法 (PCR 法) を用いて, 塊茎特異的に発現するバレイショ class I パタチン遺伝子のプロモーター領域の分布と変異について調べ, 塊茎を形成しない野生種にも同領域が存在することを明らかにした。

材料として, 栽培種 18, 2 倍性半数体 23, *Solanum* 属野生種 5 (内塊茎を形成しないもの 3), *Lycopersicon* 属野生種 1 (塊茎を形成しない) の計 47 系統を用いた。class I パタチン遺伝子の 1 つである B 33 のプロモーター領域 (−580 から +11) をクローニングし, サザンハイブリダイゼーションのプローブとした。

塊茎を形成しない野生種を含む 47 系統すべてに, class I パタチンプロモーター領域と相同性のある DNA 配列が存在することが示された。また, 系統によりパタチンプロモーター群の種類にばらつきのあることが示された。