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Detection of Viroid-like RNA Molecules in Korean Peonies (*Paeonia lactiflora*)

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한국산 작약(*Paeonia lactiflora*)으로부터 바이로이드 유사 RNA 분자의 검출

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ABSTRACT : Viroid-like RNA molecules were detected from the low molecular weight RNAs isolated from the Korean peonies which showed typical viroid symptoms of epinasty and dwarfing. Low molecular weight RNAs including viroid RNA molecules were purified by the Qiagen anion exchange minicolumns. Viroid-like RNA molecules showed a single viroid specific band in the native polyacrylamide gel. They were separated into two bands in the denaturing gel conditions. The band of circular form of viroid-like RNAs was crossed over the horizontal band of the linear form of viroid-like RNA molecules in 0~8 M urea gradient gel under the denaturing conditions of 37°C. The two circular forms of viroid-like RNA molecules were detected in the reverse polyacrylamide gel electrophoresis. The viroid-like RNA molecules purified from the peonies were supposed to be unidentified viroid RNA molecules.

***Key words* : viroid-like RNA molecules, reverse directional polyacrylamide gel electrophoresis (PAGE), urea-gradient PAGE.**

Viroids are the smallest plant pathogens which have been known so far. They consist of uncapsidated, single-stranded, circular RNA molecules with 240~480 nucleotides. They exist as highly base-paired, rod-like structures. Viroids can be mechanically transmitted and cause pathogenicity in many crop plants by the vegetative propagation. Since the rediscovery of viroid RNA molecules about a quarter century ago (2, 6), over twenty viroids excluding variants have been characterized at the molecular level (3).

Peonies (*Paeonia lactiflora* var. *hortensis* MAK) can be propagated by seeds and roots, but the divided roots are more effective for the cultivation. Over several thousand years, peonies have been propagated by dividing the roots. Any causal pathogenic agent including virus and viroid has not been identified in peonies until now, but we have found dwarf symptoms from the cultivated peonies. Dwarf is one of the typical symptoms of plant virus disease. From the dwarfed peonies we could not find any specific plant pathogens of fungi, bacteria and virus by the investigation with optical microscope and even TEM (transmission electron microscope). For the detection of viroid RNA molecules, we examined the low molecular weight RNA samples purified from the dwarfed peonies by the biochemical and molecular biological detection methods.

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MATERIALS AND METHODS

Peony is a perennial plant, but it defoliates early in summer. The fresh young leaves of peonies should be collected during early in spring for the effective purification of plant nucleic acids and they could be stored in a deep-freezer for the further experiments.

Pure nucleic acids excluding polysaccharides and polyphenols can hardly be extracted from plant samples. Chromatographic separation procedures can be applied as a superior purification method of nucleic acids. We have purified low molecular weight plant RNAs including viroid RNA molecules by the Qiagen anion-exchange minicolumns which are able to bind specific nucleic acids at different salt concentrations. After phenol-chloroform treatments, the partially purified extracts of peonies would be applied into Qiagen column chromatography.

In order to isolate viroid RNA molecules from peonies, fresh or deep-frozen young leaves were homogenized in liquid nitrogen with STE (100 mM NaCl, 10 mM Tris, 1 mM EDTA, pH 8.0) buffer containing the end concentration of 0.2% bentonite and 2% SDS. The homogenate was treated with phenol-chloroform (1 : 1). Aqueous phase was added with 3 M sodium acetate and isopropanol after treatment of chloroform. RNA pellets were washed carefully with 70% ethanol containing 200 mM sodium acetate. RNA samples were dissolved with QV1 (300 mM NaCl, 50 mM MOPS, 2 M urea, pH 7.0) and applied to Qiagen columns. According to the procedures QV2 (600 mM NaCl, 50 mM MOPS, 2 M urea, 15% ethanol, pH 7.5) and QV3 (1.0 M NaCl, 50 mM MOPS, 15% ethanol, pH 7.5) were applied to wash and elute through the Qiagen columns, respectively. RNAs including viroid molecules were recovered and applied into further experiments for the biochemical and molecular biological characterization.

The 0~8 M urea gradient and reverse directional polyacrylamide gel electrophoresis were conducted according to the methods described previously (4, 5). After electrophoresis the gels were stained with silver nitrate for higher resolution (7).

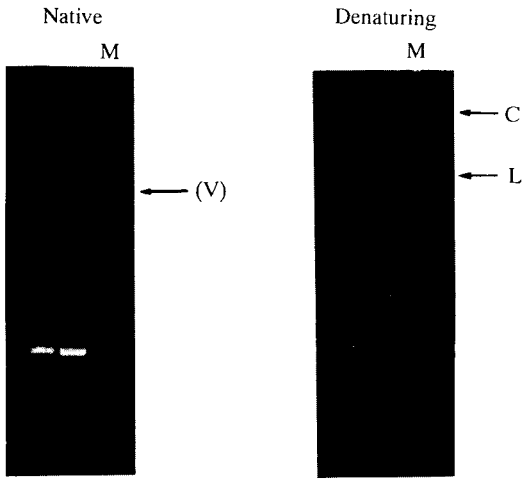

Fig. 1. Polyacrylamide gel electrophoresis of the low molecular weight RNAs extracted from the dwarfed peonies under native and denaturing conditions. Viroid-like RNA (V) molecules can be separated into circular (C) and linear (L) forms. Size marker (M) is pBR 322 digested with *Hinf*I.

RESULTS

The RNA molecules purified from peony leaves were electrophoresed in 6% polyacrylamide gel under native and denaturing conditions. In order to confirm the viroid RNA molecules from unknown samples, we have investigated the separation patterns of the circular and linear forms under native and denaturing conditions. Low molecular weight RNA molecules including viroid-like RNA molecules purified from the dwarfed peonies showed single viroid specific band in the 6% native polyacrylamide gel. It was separated into two bands in the denaturing polyacrylamide gel (Fig. 1) and in the 0~8 M urea gradient gel (Fig. 2) under the denaturing conditions of 37°C. The band of the circular form of viroid-like RNAs were crossed over the horizontal band of the linear form of viroid-like RNA molecules in the urea gradient gel as shown in Fig. 2.

The circular viroid RNA molecules can be detected as a single band in the reverse polyacrylamide gel of the second gel electrophoresis under the denaturing conditions. Fig. 3 showed the viroid specific bands in the lane of RNAs purified from the peony ES 32, 33 and 42. An additional weak band has been detected in

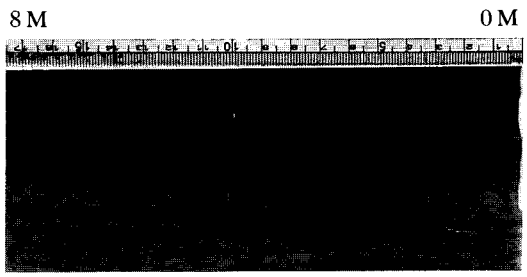

Fig. 2. Electrophoretic migration patterns of the low molecular weight RNAs extracted from the dwarfed peonies in the 0~8 M urea gradient 6% polyacrylamide gel at 37°C.

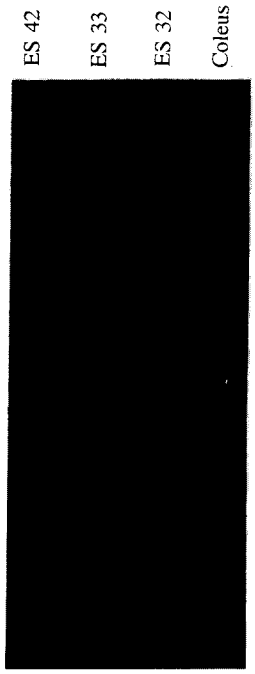

Fig. 3. Electrophoretic migration patterns of the circular viroid RNA forms in the reverse polyacrylamide gel electrophoresis under denaturing conditions of 8 M urea and 37°C. Single and two bands of viroid-like RNAs were detected in different RNA samples purified from the dwarfed peonies. Standard viroid RNAs were Coleus viroid 1, 2 and 3.

the lane of ES 42. The standard viroids applied in the reveres gel electrophoresis were Coleus viroid 1, 2 and 3 which consist of 250, 302 and 362 nucleotides, respectively. The circular form of viroid-like RNA molecules purified from different peonies could be expected about 280 nucleotides long, and the additional viroid-like RNAs could be estimated 310 nucleotides long approximately.

DISCUSSION

Viroid RNA molecules showed different mobilities in the native and denaturing polyacrylamide gel electrophoresis. Viroid molecules exist as a single band under native conditions, but they are separated into circular and linear forms under the denaturing conditions of polyacrylamide gel containing 8 M urea (8, 10). The separation of viroid molecules into two bands is caused by the denatured structure of circular forms, which migrate slower than linear forms in the denaturing polyacrylamide gel conditions.

The variable electrophoretic mobility of circular forms crossed over the constant mobility of linear viroid molecules, and consequently the band of circular forms drew an oblique line across the horizontal band of linear forms in the urea gradient gel (4, 9). The electrophoretic mobilities of the circular and linear viroid RNA molecules were changed drastically under denaturing 0~8 M urea gradient gel conditions as shown in Fig. 2. As the results shown in Figs. 1 and 2, the RNA molecules purified from peonies with dwarf symptom could be an unidentified viroid RNA molecules.

Fig. 3 shows the circular form of the viroid-like RNA molecules in the reverse gel electrophoresis. One additional band of the circular form of viroid-like RNA molecules was detected from the peony of ES 42. Comparing with the standard viroids of Coleus viroid 1, 2 and 3 (11, 12), the two viroid-like RNA molecules could be estimated about 280 and 310 nucleotides long, respectively. As a result of these experiments, the purified RNAs of peonies with dwarf symptoms could be supposed unidentified viroid-like RNA molecules. The peonies with dwarf and epinasty symptoms could be infected with a certain other known viroid molecules or unidentified viroid-like RNA molecules at least.

So far we have got some pathological and genetic informations including mechanical transmission, host range and lethal infectivity. The experiments for the detection of the viroid-like RNA molecules from the dwarfed peonies have not been investigated completely. Recent experiments of viroid RNA molecules are concerning to the biochemical and molecular biological techniques of reverse transcription, polymerase chain reaction, cDNA cloning and nucleotide sequence analysis (1). Viroid cDNA clones have been also widely applied to the experiments for viroid replication and pathogenicity. The viroid-like RNA molecules purified from the dwarfed peonies are under the experiments for the cDNA cloning and the genomic analysis.

요 약

잘록 증상과 순말림 증세를 보이는 작약으로부터 바이로이드 유사 RNA 분자를 확인하였다. 바이로이드 유사 RNA 분자를 포함하는 저분자량의 식물성 RNA 분자는 Qiagen으로 분리하여 특성을 조사하였다. 바이로이드 유사 RNA 분자는 비변성 전기영동에서는 하나의 band로 나타나지만, 변성 상태에서는 두

개의 band로 분리되었다. 변성 상태에서 진행된 0~8 M의 요소기울기 전기영동에서 고리 모양의 분자와 선 모양의 분자가 이동성의 차이에 의해 교차하는 것을 확인하였다. 또한 2차 변성 전기영동에서 고리 모양의 바이로이드 분자로 보이는 두 개의 band를 확인하였다. 따라서 이 RNA 분자는 바이로이드와 매우 유사한 분자로 여겨진다.

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