In 2003 typical phytosymptoms of witches’ broom and flower malformation were observed on staticle (*Limonium sinuatum*) plants grown at commer-
cial greenhouses in Busan, South Korea. The DNA extracted from the infected leaves was amplified using universal primer pair of P1/P6 derived from conserved 16S rDNA gene of Mollicutes giving the expected Polymerase chain reaction (PCR) product of 1.5 kb. In the nested PCR assays, the expected DNA fragment of 1.1 kb was amplified with the specific primer pair 16F1/ R1 that was designed on the basis of aster yellows (AY) phytosymptoms. The 1.1 kb PCR products were cloned and nucleotide sequences were determined. The sequences were identical to that of Onion yellows OY phytosymptom (GenBank accession no. D12569) isolated from Onion in Japan. Electron microscopy of thin sections of leaf veins showed phytosymptom bodies in the phloem. Staticle witches’ broom symptom occurred on staticle in commercial greenhouses in Korea was confirmed as infection of AY phytosymptom by transmission electron microscopy observation, and by determination of 16S rDNA gene sequences of phytosymptom.

**Keywords**: *Limonium sinuatum*, staticle, witches’ broom

Staticle (*Limonium sinuatum*) belongs to the family of the Plumbaginaceae. It is a floricultural crop grown for both fresh and dried flowers. Most of them are perennials and a very few are annuals. They are propagated by division of root. Staticle plants grown in South Korea occupied 70.5 ha in 2004 (Ministry of Agriculture and Forestry, 2004). In 2004, the sales of staticle in Korea totaled 3.4 million US dollars.

Phytosymptoms (previously called mycoplasma-like organisms) are phloem-limited plant pathogenic prokar-
yotes (Hopkins, 1977). They are known as the causal agents of yellowing, stunting and witches'-broom diseases in various plants.

Phytosymptom bodies were found in phloem cells of *L.*
were aligned using CLUSTAL Method of DNAStar software version 5.1 (Madison, USA).

**Electron microscopy.** Presence of phytoplasma was examined with the midribs of leaves. The segments were cut into small pieces by hand dissection and fixed in 2% Karnovsky's fixative solution, pH 7.2 for 4 h. Postfixation in 1% osmium tetroxide in cacodylate buffer, pH 7.2 for 2 h at 4°C. The material was dehydrated in concentration gradients of ethanol (50%, 75%, 90%, 95% and 100% for 30 min each step) and embedded in Spurr resin. Ultrathin sections were prepared with ultramicrotome (RMC-MTX), stained with 2% uranyl acetate and 0.08 M lead citrate buffer, pH 12.0. The grids were examined with a Carl Zeiss LEO 906 transmission electron microscope (Electron Microscopy Science, USA).

**Results**

**Detection of phytoplasma 16S rRNA gene from plants by PCR.** Using a universal primer set P1/P6, a 1.5 kb DNA fragment of the 16S rRNA gene was amplified from infected plants (Fig. 2). In the nested PCR assays, the expected DNA fragment of 1.1 kb was amplified with the specific primer pair R16F1/R1 that was designed on the basis of AY phytoplasma 16S rDNA sequences (Fig. 2).

**Sequence analyses.** The nucleotide sequences of the cloned 16S rRNA gene of the phytoplasma observed in statice have been deposited in the GenBank database under the accession no. of DQ192513. The nucleotide sequences were identical to those of phytoplasma from Ash trees witches' broom (GenBank accession no. AY566302) reported in Korea (unpublished). They were also identical to Onion yellows OY phytoplasma (GenBank accession no. D12569) isolated from onion in Japan (Namba et al., 1993). Meanwhile, it shared 99.1% and 93.9% homology with American aster yellows AAY (GenBank accession no. X68373) and Clover phyllody CPh (GenBank accession no. L33762), respectively (Table 1).

**Visualization of phytoplasma.** In the ultra-thin sections of

---

Fig. 1. Naturally phytoplasma-infected statice plants. (A) witches' broom symptom, (B) malformation of flowers with enlarged yellowish stem, (C) severely reduced flowers.

**Cloning of PCR products and nucleotide sequencing.** We cloned PCR amplified phytoplasma 16S rDNA, which had been amplified in nested PCR primed by R16F1/R1, using pGEM-T easy vector (Promega, USA) according to the manufacturer's instruction. The ligation mixture is used to transform competent cells of Escherichia coli JM 109. Recombinants were screened by blue and white screening method (Sambrook et al., 1989). Nucleotide sequences were determined using ABI Prism BigDye™ Terminator Cycle Sequencing Kit (Applied Biosystems, USA).

**Phylogenetic analysis.** The 16S rRNA gene sequences

---

Fig. 2. Amplification of a 16S rDNA sequence using universal primer pair P1/P6 (A) and AY specific primer pair R16F1/R1 (B). (A) lane 1, healthy statice; lane 2-4, diseased statice; (B) all lanes, diseased statice. Lane M, 1 kb DNA ladder.
<table>
<thead>
<tr>
<th></th>
<th>Ash WB</th>
<th>OY</th>
<th>AAY</th>
<th>SWB</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPh</td>
<td>93.9</td>
<td>92.9</td>
<td>93.0</td>
<td>93.9</td>
</tr>
<tr>
<td>Ash WB</td>
<td>100.0</td>
<td>99.1</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>OY</td>
<td>99.1</td>
<td>100.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAY</td>
<td>99.1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Sequence homology percent of the 16S rDNA of statice witches’ broom with other phytoplasmas.

\*Ash WB: Ash witches’ broom (AY566302); OY: Onion yellows (D12569); AAY: American aster yellows (X68373); SWB: Statice witches’ broom (DQ192513); CPh: clover phyllody (L33762).

Fig. 3. Electron micrograph of phloem cells of phytoplasma-infected statice leaf showing pleomorphic phytoplasma structures. Bar indicates 200 nm.

the leaf midribs phytoplasma-like structures were observed (Fig. 3). The structures had round or pleomorphic shapes, with a diameter of 130-300 nm and were limited to phloem cells. Fine fibrils were observed inside of the phytoplasma bodies.

Discussion

In 2003, typical phytoplasma infection symptom of witches’ broom on statice cultivars of ‘Yellow’, ‘Lavender’, ‘Gold’ and ‘Purple’ were abundantly occurred in commercial greenhouses in Busan, South Korea. The witches’ broom symptom of statice had been found in Gangwon alpine areas (Hahn et al., 1998), and in Namwon, Jeolla Province (Lee, 2004), South Korea. The present study was undertaken to identify the causal agent of witches’ broom in statice plants in Korea. The results were based on electron-microscopic examination and nucleotide sequence analysis of 16S rRNA gene.

Phytoplasma disease were reported in statice in Poland (Kamińska et al., 1996), Japan (Wakibe and Guo, 1998) and USA (Baker et al., 1983). Phytoplasma caused leaf and flower discoloration and malformation, shoot dieback and seed sterility (Kamińska et al., 1999), and witches’ broom with yellowing (Wakibe and Guo, 1998) in statice. Symptoms of witches’ broom and flower malformation revealed in phytoplasma-affected statice in this study were similar to those described in above reports.

The nucleotide sequences determined in present study were identical to those of phytoplasma from Ash trees witches’ broom (GenBank accession no. AY566302) reported in Korea. They were also identical to Onion yellows OY phytoplasma (16SrI-B) (GenBank accession no. D12569) isolated from onion in Japan (Namba et al., 1993). The Onion yellows OY phytoplasma was isolated from field-collected onions. *Macrosteles striifrons* transmitted phytoplasma disease in statice in Japan, and the same vector also transmitted phytoplasma diseases to tomato, onion and turnip (Wakibe et al., 1996). We inferred from the report that phytoplasma pathogen infecting statice might be the same one with that infected onion, resulting in same nucleotide sequence.

Sequence similarity of Statice witches’ broom with phytoplasma grouped in AY indicated symptom of witches’ broom in statice plants was caused by phytoplasma infection involved in AY was group according to classification of Lee et al. (1998). In Poland, they grouped the causal phytoplasma of statice as AY, subgroup B by restriction fragment length polymorphism (RFLP) analysis of PCR products of 16S rDNA (Kamińska et al., 1999). Nucleotide sequence analysis of phytoplasma found in statice has never been conducted. This study proved the witches’ broom symptom in statice was caused by phytoplasma disease by determination of nucleotide sequences of 16S rRNA gene.

The witches’ broom symptom on statice occurred in Korea was proved as infection of AY phytoplasma by transmission electron microscopy observation, and by determination of 16S rRNA gene sequences of phytoplasma. This study provided the first evidence of association of AY phytoplasma with statice witches’ broom in Korea.

References


