# Accumulation of Pathogenesis-related Type-5 Like Proteins in Phytoplasma-infected Garland Chrysanthemum *Chrysanthemum coronarium*

Bo-Xiong ZHONG* and Yan-Wei SHEN

*Department of Bioresource Science, College of Animal Science, Zhejiang University, Hangzhou 310029, China*

**Abstract**     Soluble proteins extracted from leaves, apical shoots, axillary shoots, and stems of garland chrysanthemum plants infected by onion yellows phytoplasma were analyzed by two-dimensional gel electrophoresis. Computerized matching analysis revealed that at least six soluble proteins were accumulated specifically in phytoplasma-infected garland chrysanthemum. N-terminal amino acids sequences of these soluble proteins, determined by Edman degradation, shared high sequence similarities with those of pathogenesis-related type-5 (PR-5) proteins such as tobacco thaumatin-like protein. Accumulation of these six proteins was also found in garland chrysanthemum plants infected by other phytoplasmas. These results demonstrate that phytoplasmal infection induces the accumulation of PR-5 like proteins in garland chrysanthemum plants.

**Key words**     phytoplasma; garland chrysanthemum *Chrysanthemum coronarium*; pathogenesis-related proteins; thaumatin; osmotin

Phytoplasmas, a group of cell wall-less, phloem-limited and uncultivable plant-pathogenic prokaryotes, which compose a genus in the class Mollicutes [1–3], are associated with diseases in several hundred species of plants [4]. In Japan, where phytoplasma was first observed then named as MLOs [5], a large number of phytoplasmal diseases in herbaceous crops such as vegetables, flowering plants, and medicinal herbs have occurred in various places. Furthermore most of these associated phytoplasmas are transmitted by either *Macrosteles striifrons* Anufriev or *Scleroracus flavopictus* Ishihara [6]. One group of phytoplasmas transmitted by *M. striifrons* (MS-phytoplasma) was classified as a member of the aster yellows group (16SrI), while the other group of phytoplasmas transmitted by *S. flavopictus* (SF-phytoplasma) was classified as a member of the X-disease group (16SrIII) on the basis of sequence and/or restriction analysis of 16S rDNA [7,8]. The host ranges of these two phytoplasmas are very wide and overlapped with each other. *Chrysanthemum coronarium* (garland chrysanthemum), one of the common leafy vegetables, is highly susceptible to both phytoplasmas and is an attractive source of food for both species of leafhopper vectors.

Pathogenesis-related (PR) proteins [9] which accumulate in plants attacked by pathogens or treated with an induce such as salicylate [10,11] or plant hormones [12] were first observed in tobacco varieties resistant to tobacco mosaic virus (TMV) and exhibited hypersensitive reaction with necrosis lesions against TMV infection [13, 14]. Since then, many and various kinds of PR proteins had been identified in many species of plants [9,15–18]. Van Loon et al proposed the classification of PR proteins into eleven families according to the similarity of amino acid sequences, serological relationships, and/or biological functions [19]. PR proteins related to phytoplasmal infection, however, have not been reported until now, whereas various plant pathogens including virus, viroids, bacteria, and fungi had been proved to induce PR protein accumulation in plants [20–25].

In this research, we report our analysis of several soluble proteins, considered PR-5 like proteins, accumulated specifically in garland chrysanthemum infected with phytoplasma.

## Materials and Methods

### Phytoplasma isolates

Phytoplasma isolates, onion yellows (OY) [26], spinach yellows (SY) [27], statice witches' broom 1 (SW1) [28], statice witches' broom 2 (SW2) [28], lettuce yellows 1 (LY1) [29] and lettuce yellows 2 (LY2) [29] were originally collected from naturally diseased plants in various places. These six isolates of MS-phytoplasma were similar in the biological characteristics, but LY2 on garland chrysanthemum was distinguished from others in symptoms [29]. Gentian witches' broom (GW), an isolate of SF-phytoplasma [30], was maintained and proliferated in garland chrysanthemum plants using viruliferous insects, which had fed on infected plants.

### Soluble protein extraction

Garland chrysanthemum seedlings were inoculated with viruliferous ("infected") or non-viruliferous insects ("pseudo-inoculated"). Thirty or 45 days after inoculation, young leaves, apical shoots, axillary shoots, and stems of infected or pseudo-inoculated garland chrysanthemum plants were excised for extracting the soluble proteins according to the method of Zhong *et al.* [31]. One gram of fresh plant tissue was homogenized with a mortar and a pestle on ice with 5 ml of chilled phosphate buffer (pH 8.0) containing 37 mM $K_2HPO_4$, 1.7 mM $KH_2PO_4$, and 400 mM NaC1. The homogenate was centrifuged at 12,000 $g$ for 10 min. 100% trichloroacetic acid was added to the supernatant to achieve a final concentration of 10%, and the mixture was kept at 4 °C for 20 min. After centrifugation at 12,000 $g$ for 10 min, the resulting precipitate was suspended in 1 ml of lysis buffer, sonicated for 2 min, adjusted to pH 7.0 with 2 M NaOH, and stored at –20 °C until use.

### Two-dimensional gel electrophoresis

Two-dimensional gel electrophoresis (2D-E) with isoelectric focusing (IEF) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were carried out essentially as described by O'Farrell [32] with slight modifications [33]. Proteins were first separated by IEF in the tube gel containing 2% ampholine (the ratio of pH 5–8 vs. pH 3–10 is 1:1). IEF was run at 200 V for l h at first, then at 400 V for 16 h, and finally at 800 V for l h with disc gel unit (Marysol Industry Co., Ltd., Tokyo, Japan). Following the first-dimension electrophoresis, each tube gel was equilibrated in SDS-PAGE sample buffer for 30 min, then placed on top of 15% SDS-PAGE gel (with 5% stacking gel). SDS-PAGE was run at 25 mA per gel for approximately 4 h with dual vertical slab gel unit (Nihon Eido Co., Ltd., Tokyo, Japan).

### 2D-E gel image analysis

After 2D-E, the gels were stained with silver. 2D-E gel images were captured with a flatbed scanner and analyzed automatically with ImageMaster 2D elite software (Amersham Pharmacia Biotech Uppsala, Sweden). The isoelectric point and molecular weight of each protein was determined using isoelectric focusing and molecular weight calibration kit (Amersham Pharmacia Biotech Uppsala, Sweden).

### N-terminal sequence analysis

Following separation by 2D-E, proteins were electroblotted onto PVDF membranes using semidry transfer unit (Nihon Eido Co., Ltd., Tokyo, Japan), and briefly stained with Coomassie Brilliant Blue R250 (CBB). Objective protein spots were then excised and applied for determination of their N-terminal amino acid sequences by automated Edman degradation with the 492 gas phase protein sequencer (PE Biosystems, Norwalk, CT). Amino acid sequence similarity was searched on the SWISS-PROT and PIR databases through the National Center for Biotechnology Information using BLAST program.

## Results

### The soluble proteins identified from garland chrysanthemum infected with phytoplasma

The OY-infected and pseudo-inoculated garland chrysanthemum plants did not change significantly in the appearance after 30 days inoculation, however, an early symptom about yellowing on newly emerged leaves was observed on OY-infected plants [Fig. 1(A)]. Few marked differences were found in both 2D-E separation patterns of soluble proteins extracted from newborn leaves [Fig. 2 (A,C)]. After 45 days inoculation, appearances of the two were considerably different. Typical and severe symptoms of stunting, yellowing and proliferation were observed on OY-infected plants, whereas only natural yellowing by leaf senescence were observed on pseudo-inoculated plants [Fig. l(B)]. Specific accumulations of several soluble proteins were observed in OY-infected but not in pseudo-inoculated plant leaves by the comparison of 2D-E separation pattern [Fig. 2(B,D)]. Especially, six kinds of these
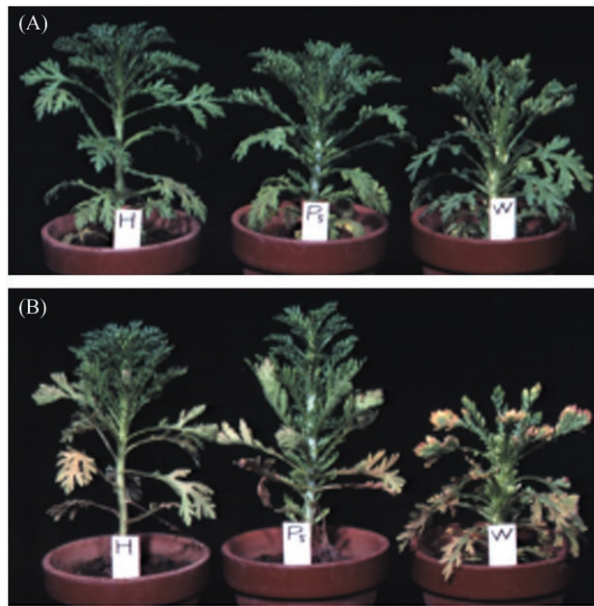
**Fig. 1    The development of the Garland chrysanthemum infected by the phytoplasma**
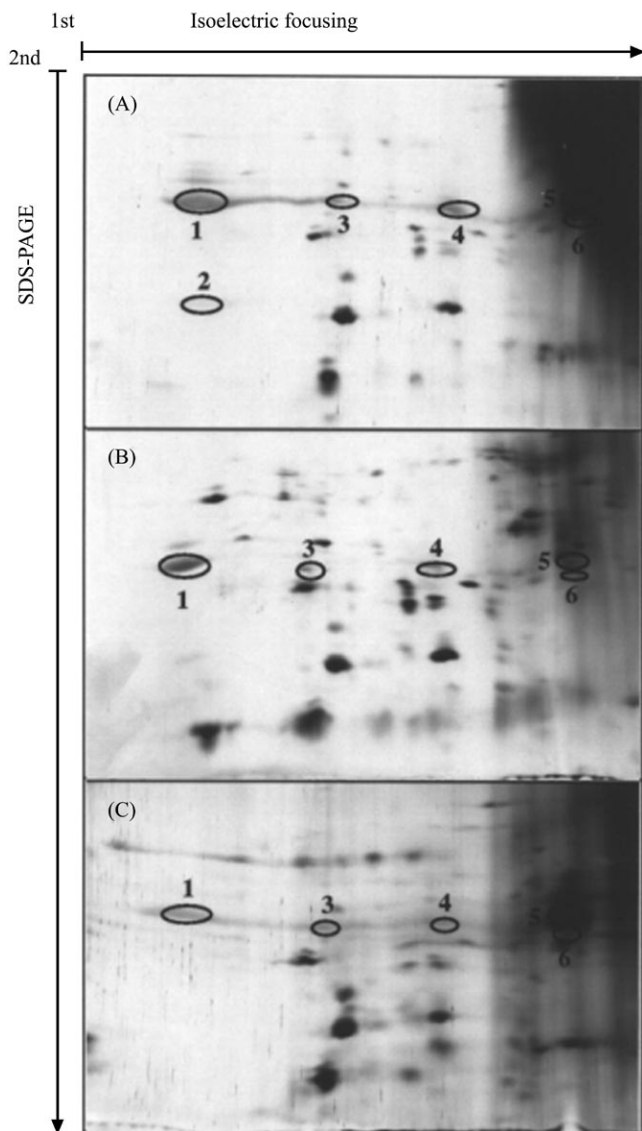
Garland chrysanthemum plants grown in green house at 25 °C for 30 days (A) and 45 days (B) after inoculation, which were not inoculated (H), pseudo-inoculated (Ps), and inoculated with OY-phytoplasma (W).
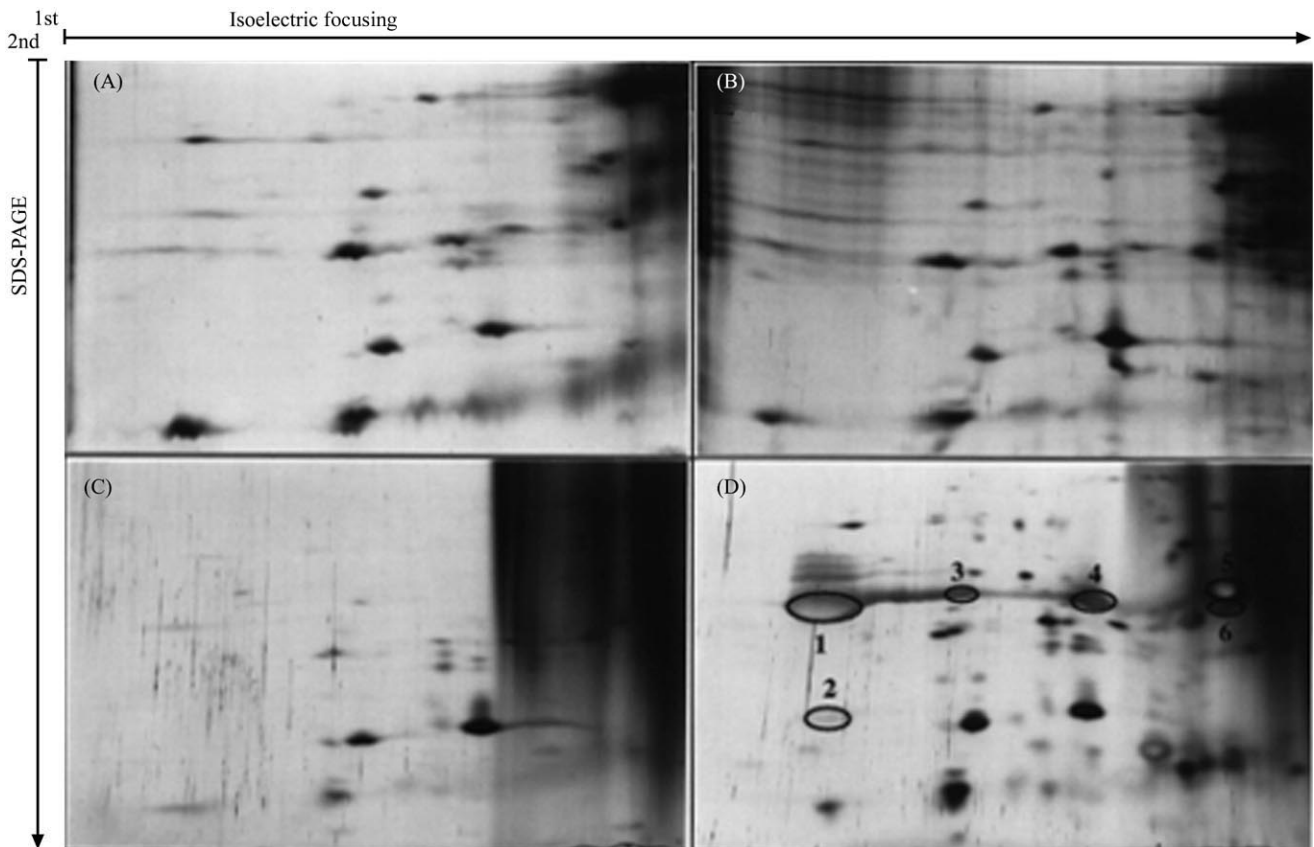


**Fig. 2    The 2D-E pattern of the soluble proteins from young leaves of the garland chrysanthemum infected by the OY-phytoplasma**

2D-E pattern of the proteins from young leaves of pseudo-inoculated (A,B) and OY-infected (C,D) garland chrysanthemum plants 30 days (A,C) or 45 days (B,D) after inoculation.

soluble proteins, CTLP-1 (CTLP means traumatic-like protein in phytoplasma-infected *Chrysanthemum coronaries*) with pI 7.35 and MW 29.6 kD, CTLP-2 with pI 7.35 and MW 21.0 kD, CTLP-3 with pI 6.26 and MW 28.8 kD, CTLP-4 with pI 5.40 and MW 29.0 kD, CTLP-5 with pI 4.75 and MW 29.9 kD, and CTLP-6 with pI 4.75 and MW 27.6 kD were clearly observed in large quantities. As shown in Fig. 3, specific accumulations of

these six CTLPs were also observed in axillary shoots, apical shoots, and stems of OY-infected garland chrysanthemum, but the accumulation of CTLP-2 was absent in apical shoots and stems.

## N-terminal amino acid sequence of CTLPs

No N-terminals of any CTLPs were blocked. N-terminal ammo acid sequences of CTLP-1, 2, 3, and 4 (CTLP-1–4) were completely identical with each other in residue 1–43. The search results for amino acid sequence similarity in protein databases revealed that the CTLPs' N-terminal amino acid sequences shared high similarity with those of PR-5 proteins reported previously (Fig. 4). N-terminal amino acid sequences of CTLP-1–4 with 43 residues also shared sequence similarities with thaumatin-like proteins from *Arabidopsis thaliana* [34], and *Prunus*



**Fig. 3      The 2D-E pattern of the soluble proteins from different organs and tissues of the Garland chrysanthemum infected by the OY-phytoplasma**

2D-E pattern of the proteins extracted from axillary shoots (A), apical shoots (B), and stems (C) of the OY-infected garland chrysanthemum plants 45 days after inoculation.

| Protein | Amino acid sequence | Identity |
|---------|---------------------|----------|
| **(A)** | | |
| CTLP-1–4 | AVFTVRNNXPYSIAPGVLTGG–GAAA–STTGFQLAPGXSXNVNVP | |
| AT-PR5K | TNFTIENKCDYTVWPGFLTMT–TAVSLPTNGFSLKKGESRVINVP | 41.9% |
| PA-TLP | ATISFKNNCPYMVWPGTLTSDQKPQ–LSTTGFELASQASFQLDTP | 41.9% |
| AT-PR5 | TDFTLRNNCPTTVWAGTLAGQ–GPK–LGDGGFELTPGASRQLTAP | 39.5% |
| AT-TLR | TVFTLQNSCAYTVWPGTLSGN–SIT–LGDGGFPLTPGASVQLTAP | 37.2% |
| | | |
| **(B)** | | |
| CTLP-5 | ANFDIINQXPYTVXAAASPGGGRRLETGQSXXLQVAPGTTXAAI | |
| AT-OLP | ATFEILNQCSYTVWAAASPGGGRRLDAGQSWRLDVAAGTKMARI | 65.9% |
| NT-TLPe22 | ATFDIVNKCTYTVWAAASPGGGRRLDSGQSWSINVNPGTVQARI | 63.6% |
| NT-TLPe2 | ATFDIVNQCTYTVWAAASPGGGRQLNSGQSWSINVNPGTVQARI | 63.6% |
| CA-TLR | ANFEIVNNCPYTVWAAASPGGGRRLDRGQTWNLWVNAGTSMARI | 63.6% |
| | | |
| **(C)** | | |
| CTLP-6 | AIFTIRNNXQQTVXAGAVPVGGGQXLDXGQTXTLD | |
| AS-TLR | ATFTITNNCGYTVWPAAIPVGGGQQLDQGQTWTLN | 62.9% |
| HV-PRS | ATFTVINKCQYTVWAAAVPAGGGQKLDAGQTWSIX | 57.1% |
| TA-TLP | ATFNIKNNCPYTVWPAATPIGGGRQLNTGETWTLD | 51.4% |
| NT-OSM | ATIEVRNNXPYTVWAASTPIGGGRRLDRGQTXVIN | 51.4% |

**Fig. 4      The homology analysis of the thaumatin-like proteins in the Garland chrysanthemum**

Alignment of the N-terminal amino acid sequence of CTLP-1–4 (A), CTLP-5 (B), and CTLP-6 (C) and partial sequences of known PR-5 group proteins. AT-PR5K, PR5K of *A. thaliana* (ATU48698_l, positions 26 aa to 68 aa); PA-TLP, thaumatin-like protein of *P. avium* (P50694, positions 24 aa to 67 aa); AT-PR5, PR-5 of *A. thaliana* (P28493, positions 24 aa to 66 aa); AT-TLP, thaumatin-like protein of *A. thaliana* (ATU83490_l, positions 24 aa to 66 aa). AT-OLP, osmotin-like protein of *A. thaliana* (P50700, positions 23 aa to 66 aa); NT-TLPe22, thaumatin-like protein e22 of *N. tabacum* (P13046, positions 26 aa to 69 aa); NT-TLPe2, thaumatin-like protein e2 of *N. tabacum* (P07052, positions 26 aa to 69 aa); CA-TLP, thaumatin-like protein of *C. arietinum* (CAR010501_1, positions 22 aa to 65 aa). AS-TLP, thaumatin-like protein of *A. sativa* (P50698, positions 22 aa to 56 aa); HV-PRS, PR-5 of *H. vulgate* (P33045, positions 1 aa to 35 aa); TA-TLP, thaumatin-like protein of *T. aestivum* (TATHAUMLP_1, positions 26 aa to 60 aa); NT-OSM, osmotin of *N. tabacum* (CAA64620, positions 22 aa to 56 aa).

*avium* [35]. The CTLP-5 with 44 residues shared similarity with tabacco thaumatin-like proteins from *Nicotiana tabacum* [36] and *Cicer aestinum*, and osmotin-like protein from *A. thaliana*. The CTLP-6 with 35 residues shared 80% similarities with thaumatin-like protein from *Avena sativa*, *Hordeum vulgare* [22] and *Triticum aestivum* [37], and the osmotin from *N. tabacum*.

## Accumulation of CTLPs in garland chrysanthemum plants infected with other phytoplasmas

As shown in Fig. 5, comparisons of 2D-E patterns revealed that CTLPs observed in OY-infected garland chrysanthemum plants also accumulated in leaves of garland chrysanthemum plants infected with SY, SW1, SW2, LY1,
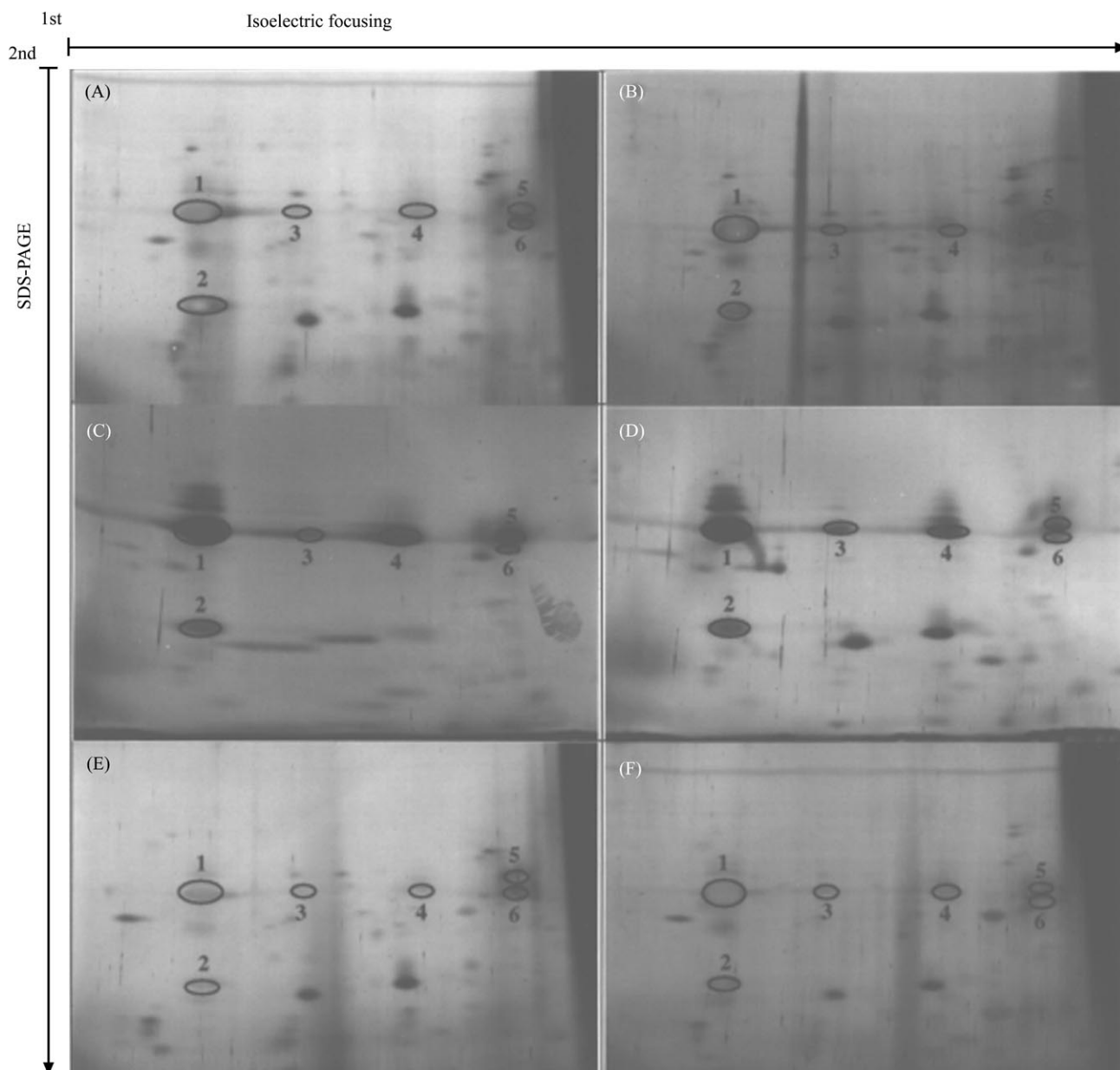


**Fig. 5        The 2D-E pattern of the soluble proteins from young leaves of the Garland chrysanthemum infected by different types of the phytoplasma**

2D-E pattern of the proteins from young leaves of garland chrysanthemum plants infected with SWl (A), SW2 (B), LY1 (C), LY2 (D), SY (E), and GW (F) phytoplasma 45 days after inoculation.

LY2, and GW. The N-terminal amino acid sequence identity of these proteins with corresponding CTLPs was confirmed by a protein sequencer (data not shown).

## Discussion

2D-E analysis proved that CTLPs accumulated in OY-infected garland chrysanthemum plants accompanied the development of systemic symptoms. The accumulation was also recognized in garland chrysanthemum plants infected with other five isolates of the 16SrI and one of the 16SrlII group phytoplasma. Accumulation of CTLPs did not induced by insect feeding or leaf senescence, because the accumulation was not observed in any pseudo-inoculated sample. Therefore, it was concluded that phytoplasmal infection induced the accumulation of CTLPs in garland chrysanthemum.

The N-terminal amino acid sequences of CTLP-1–4 were identical, but their pIs and molecular weights were different. One possible explanation is that genes encoding CTLP-1–4 constitute a group like a multi-gene family; the other possibility is that CTLP-1–4 originate from gene isoforms produced by post-translational modifications. Further analysis of the full sequence of CTLP-1–4 genes is necessary to clarify the relationships among CTLP-1–4.

CTLP-1–4 showing the highest sequence similarity to the N-terminal amino acid sequence of proteins which were known thaumatin-like proteins, and CTLP-5/6 also showing higher similarities to thaumatin-like or osmotin-like proteins were known as the PR-5 protein members. The determined molecular weights of CTLPs were within the range of known PR-5 proteins. Three isoforms, distinguished by their pIs (basic, neutral and acidic), of PR-5 proteins have been reported, and genes encoding them have been isolated in tobacco and *A. thaliana* [16,19,38]. In our case, CTLP-1–4 and CTLP-5/6 correspond to the neutral and acidic isoform of PR-5 proteins, respectively. These results show that CTLPs are member of the PR-5 protein group. Some PR-5 proteins have been known to possess antifungal activity [15,16,22,39,40], but CTLPs might not influence phytoplasmal infection or multiplication because their accumulation increase with the development of symptoms and no difference is recognized between the 2D-E pattern of LY1 and LY2.

It is interesting that phytoplasmal infection induces only PR-5 like proteins in garland chrysanthemum while TMV infection induces nine types of PR protein in tobacco [19]. No hypersensitive reaction was exhibited in the appearances of phytoplasma-infected garland chrysanthemum plants, though cell necrosis was usually observed in the phloem [29]. The accumulation of CTLPs was assumed to be induced by the disturbance of the normal plant hormone balance or of regular translocation of substances though phloem, which were supposed to be caused by phytoplasmal infection, whose effects were similar to those of ethylene treatment or salt stress inducing PR-5 proteins in tobacco.

## Acknowledgements

## References

1   Gundersen DE, Lee IM, Rehner SA, Davis RE, Kingsbury DT. Phylogeny of mycoplasmalike organisms (phytoplasmas): A basis for their classification. J Bacteriol, 1994, 176: 5244–5254

2   Kirkpatrick BC, Smart CD, Gardner SL. Gao JL, Ahrens U, Mäurer R, Schneider B *et al*. Phylogenetic relationships of plant pathogenic MLOs established by16/23S rDNA spacer sequences. Int Org Mycoplasmol Lett, 1994, 3: 228–229

3   Seemüller E, Schneider B, Mäurer R, Ahrens U, Daire X, Kison H, Lorenz KH *et al*. Phylogenetic classification of phytopathogenic mollicutes by sequence analysis of 16S ribosomal DNA. Int J Syst Bacteriol, 1994, 44: 440–446

4   McCoy RE, Caudwell A, Chan CJ, Chen TA, Chiykowski LN, Cousin MT, Dale L *et al*. Plant diseases associated with mycoplasma-like organisms. In: Whitcomb RF, Tully JG eds. The Mycoplasmas. New York: Academic Press, 1989, V: 545–640

5   Doi Y, Teranka M, Yora K, Asuyama H. Mycoplasma or PLT group-like microorganism found in the phloem elements of plants infected with mulberry dwarf, potato witches' broom, aster-yellows or panlownia witches' broom. Ann Phytopathol Soc Jpn, 1967, 33: 259–266

6   Shiomi T, Tanaka M, Sawayanagi T. A symptomatic mutant of onion yellows phytoplasma derived from a greenhouse-maintained isolate. Ann Phytopathol Soc Jpn, 1998, 64: 501–505

7   Namba S, Oyaizu H, Kato S, Iwanami S, Tsuchizaki T. Phylogenetic diversity of phytopathogenic mycoplasmalike organisms. Int J Syst Bacteriol, 1993, 43: 461–467

8   Okuda S, Prince JP, Davis RE, Dally EL, Lee IM, Mogen B. Two groups of phytoplasmas from Japan distinguished on the basis of amplification and restriction analysis of 16S rDNA. Plant Disease, 1997, 81: 301–305

9   van Loon LC. Pathogenesis-related proteins. Plant Mol Biol, 1985, 4: 111–116

10  White RF. Acetylsalicylic acid (aspirin) induces resistance to tobacco mosaic virus in tobacco. Virology, 1979, 99: 410–412

11  Yamakawa H, Kamada H, Satoh M, Ohashi Y. Spermine is a salicylate-independent endogenous inducer for both tobacco acidic pathogenesis-related proteins and resistance against tobacco mosaic virus infection. Plant Physiol, 1998, 118: 1213–1222

12  Antoniw JF, Kueh JSH, Walkey DGA, White RF. The presence of patho-genesis-related proteins in callus of *Xanthi*-no tobacco. Phytopathol Z, 1981, 101: 179–184

13  Gianinazzi S, Martin C, Vallee JC. Hypersensitivity to viruses, temperature soluble protein in *Nicotiana Xanthi* n.c. Appearance of new macromolecules at the repression of viral synthesis. C R Acad Sci [Hebd Seances Acid Sci D], 1970, 270: 2383–2386

14  van Loon LC, van Kammen A. Polyacrylamide disc electrophorsis of the soluble leaf proteins from *Nicotiana tabacum* var. 'Samsun' and 'Samsun NN' II. Changes in protein constitution after infection with tobacco mosaic virus. Virology, 1970, 40: 190–211

15  Cheong NE, Choi YO, Kim WY, Kim SC, Cho MJ, Lee SY. Purification of an antifungal PR-5 protein from flower buds of *Brassica campestris* and cloning of its gene. Physiol Plant, 1997, 101: 583–590

16  Hu X, Reddy ASN, Cloning and expression of a PR5-like protein from *Arabidopsis*: Inhibition of fungal growth by bacterially expressed protein. Plant Mol Biol, 1997, 34: 949–959

17  Minic Z, Brown S, de Kouchkovsky Y, Schultze M, Staehelin C. Puri-fication and characterization of a novel chitinase-lysozyme, of another chitinase, both hydrolysing *Rhizobium meliloti* Nod factors, and of a patho-genesis-related protein from *Medicago sativa* roots. Biochem J, 1998, 332: 329–335

18  Morris SW, Vernooij B, Titatarn S, Starrett M, Thomas S, Wiltse CC, Frederiksen RA *et al*. Induced resistance responses in maize. Mol Plant Microbe Interact, 1998, 11: 643–658

19  van Loon LC, Pierpoint WS, Boller Th, Conejero V. Recommendations for naming plant pathogenesis-related proteins. Plant Mol Biol Rept, 1994, 12: 245–264

20  Antoniw JF, Ritter CE, Pierpoint WS, van Loon LC. Comparison of three pathogenesis-related proteins from plants of two cultivars of tobacco infected with TMV. J Gen Virol, 1980, 47: 79–87

21  Camacho Henriquez A, Sanger HL. Analysis of acid-extractable tomato leaf proteins after infection with a viroid, two viruses and a fungus and partial purification of the 'pathogenesis-related' protein p14. Arch Virol, 1982, 74: 181–196

22  Hejgaard J, Jacobsen S, Svendsen I. Two antifungal thaumatin-like proteins from barley grain. FEBS Lett, 1991, 291: 127–131

23  Liu D, Raghothama KG, Hasegawa PM, Bressan RA. Osmotin overexpression in potato delays development of disease symptoms. Proc Natl Acad Sci USA, 1994, 91: 1888–1892

24  Ruiz-Medrano R, Jimenez-Moralla B, Herrera-Estrella L, Rivera-Bustamante RF. Nucleotide sequence of an osmotin-like cDNA induced in tomato during viroid infection. Plant Mol Biol, 1992, 20: 1199–1202

25  Woloshuk CP, Meulenhoff JS, Sela-Buurlage M, van den Elzen PJ, Cornelissen BJ. Pathogen-induced proteins with inhibitory activity toward *Phytophthora infestans*. Plant Cell, 1991, 3: 619–628

26  Miyahara K, Matsuzaki M, Tanaka K, Sako N. A new disease of onion caused by mycoplasma-like organism in Japan. Ann Phytopath Soc Japan, 1982, 48: 551–554

27  Osada S, Tanaka M, Uehara T, Matsuda I. Phytoplasma diseases of vegetable occurring in Miyagi Prefecture 2. Occurrence of spinach yellows. Ann Rept Plant Prot North Japan, 1998, 49: 69–72

28  Tanaka M, Shiomi T, Tairako K. Uehara T, Matsuda I. Occurrence of glaucidium witches' broom. Ann Phytopath Soc Japan, 1998, 64: 205–207

29  Uehara T, Tanaka M, Shiomi T, Namba S, Tsuchizaki T, Matsuda I. Histopathological studies on two symptom types of phytoplasma associated with lettuce yellows. Ann Phytopath Soc Japan, 1999, 65: 465–469

30  Shiomi T. Host range of gentian witches' broom MLO and its mode of transmission by *Scleroracus fiavopictus*. Proc Kansai Pl Prot, 1988, 30: 31–36

31  Zhong B, Karibe H, Komatsu S, Ichimura H, Nagamura Y, Sasaki T, HiranoH. Screening of rice genes from a cDNA catalog based on the sequence data-file of proteins separated by two-dimensional electrophoresis. Breeding Sci, 1997, 47: 245–251

32  O'Farrell PH. High resolution two-dimensional electrophoresis of proteins. J Biol Chem, 1975, 250: 4007–4021

33  Zhong B, Weng H, Fang W. Preparation of protein samples for gel electro-phoresis by sequential extraction. J Zhejiang Univ Science, 2002, 3: 606–610

34  Uknes S, Mauch-Mani B, Moyer M, Potter S, Williams S, Dincher S, Chandler D *et al*. Acquired resistance in Arabidopsis. Plant Cell, 1992, 4(6): 645–656

35  Fils-Lycaon BR, Wiersma PA, Eastwell KC, Sautiere P. A cherry protein and its gene, abundantly expressed in ripening fruit, have been identified as thaumatin-like. Plant Physiol, 1996, 111: 269–273

36  Cornelissen BJC, Hooft van Huijsduijnen RAM, Bol JF. A tobacco mosaic virus-induced tobacco protein is homologous to the sweet-tasting protein thaumatin. Nature, 1986, 321: 531–532

37  Mingeot D, Jacquemin JM. A wheat cDNA coding for a thaumatin-like protein reveals a high level of RFLP in wheat. Theor Appl Genet, 1998, 95: 822–827

38  Sato F, Kitajima S, Koyama T, Yamada Y. Ethylene-induced gene expres-sion of osmotin-like protein, a neutral isoform of tobacco PR-5, is mediated by the AGCCGCC *cis*-sequence. Plant Cell Physiol, 1996, 37: 249–255

39  Koiwa H, Kato H, Nakatsu T, Oda J, Yamada Y, Sato F. Purification and characterization of tobacco pathogenesis-related protein PR-5d, an antifungal thaumatin-like protein. Plant Cell Physiol, 1997, 38: 783–791

40  Roberts WK, Selitrennikoff CP. Zeamatin, an antifungal protein from maize with membrane-permeabilizing activity. J Gen Microbiol, 1990, 136: 1771–1778

Edited by
**Zu-Xun GONG**