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The first report of a phytoplasma associated with pot marigold phyllody in Iran

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Abstract

Pot marigold phyllody was observed in a Botanical Garden inYazd province of Iran. The disease agent was transmitted from pot marigold to pot marigold and periwinkle via dodder inoculation. Polymerase chain reaction using phytoplasma-specific primer pair P1/P7 or nested PCR using primer pair P1/P7 followed by R16F2n/R16R2, amplified products of expected size (1.8 and 1.2 kbp, respectively) from symptomatic pot marigold in the field and from symptomatic dodder inoculated pot marigold and periwinkle plants. Restriction fragment length polymorphism analysis of R16F2n/R16R2 primed nested PCR products indicated association of a peanut witches' broom related phytoplasma (16SrII) in naturally and experimentally infected pot marigold plants. This is the first report of pot marigold phyllody in Iran.

Key words: Pot marigold phyllody, 16SrII group, Yazd, Iran.

Introduction

Pot marigold (*Calendula officinalis* L.) is a plant in the genus *Calendula* in the family Asteraceae native to southern Europe and cultivated in temperate regions around the world. It is a herbaceous ornamental plant with many medicinal, culinary and cosmetic uses.

Phytoplasmas belonging to aster yellows (16SrI) group were identified in diseased pot marigold from Italy (Marcone *et al.*, 1997) and Canada (Wang and Hiruki, 2001). During a survey in 2006 in Yazd Botanical Garden, 30 km North west of Yazd (Yazd province, Iran), pot marigold phyllody (PMP) was observed (Esmailzadeh-Hosseini *et al.*, 2008). In the present study the identification of phytoplasmas associated with PMP was carried out.

Materials and methods

A pot marigold plant with typical phyllody symptoms was selected in Yazd Botanical Garden, transferred to greenhouse and used as the source of the disease agent for dodder (*Cuscuta campestris* Yunck.) transmission and molecular studies. Infection of dodder-inoculated plants was verified by PCR. Total DNA was extracted from midrib tissue of 10 naturally phyllody affected pot marigold, dodder inoculated and healthy plants using Zhang *et al.* (1998) procedure. DNA samples were tested for presence of phytoplasma by direct PCR using P1/P7 (Schneider *et al.*, 1995) and nested PCR using P1/P7 and R16F2n/R16R2 (Gundersen and Lee, 1996) primer pairs. For identification of phytoplasma associated with pot marigold phyllody, R16F2n/R16R2 primed nested PCR products from naturally infected marigold and experimentally infected marigold and periwinkle plants were digested with *Alu*I, *Hin*fI, *Mse*I, and *Rsa*I restriction enzymes and digestion profiles were compared with those of known phytoplasmas. Furthermore, ELISA test using polyclonal antibody against Yazd alfalfa witches' broom phytoplasmas (Esmailzadeh-Hosseini *et al.*, 2003) was also used for identification of PMP agent.

Results and discussion

Characteristic symptoms of the PMP disease were leaf size reduction, yellowing, phyllody, virescence, proliferation and sterility in the flower, proliferation of axillary buds along the stem, witches' broom and stunting (figure 1).

Up to 12% of the pot marigold plants were found infected in the field. Under greenhouse conditions, the agent of PMP was transmitted from naturally infected pot marigold to pot marigold and periwinkle via dodder inoculation.

Figure 1. Pot marigold phyllody in Yazd Botanical Garden of Yazd province.
(In colour at www.bulletinofinsectology.org)

Figure 2. Virescence, phyllody and witches' broom in a pot marigold plant dodder inoculated with PMP agent (left) compared with a healthy plant (right). (In colour at www.bulletinofinsectology.org)

Four of 5 pot marigold and all 5 periwinkle plants parasitized by dodder from infected pot marigold developed disease symptoms. The duration of the latent period in dodder-inoculated plants ranged from 6 to 11 weeks. In dodder inoculated marigold (figure 2), disease symptoms were similar to those of naturally infected pot marigold plants. The major symptoms shown by experimentally infected periwinkle plants were small leaves, virescence, phyllody, yellowing and stunting. DNA fragments of approximately 1,800 and 1,200 bp were amplified by direct and universal primer pairs P1/P7 and R16F2n/R16R2, respectively by direct and nested PCR from total nucleic acid samples extracted from 10 naturally phyllody-affected pot marigold and all symptomatic experimentally inoculated plants. No amplification was observed in DNA samples from symptomless plants and water control. R16F2n/R16R2 primed nested PCR products (1.2 kbp) were analyzed by digestion with *Alu*I, *Hinf*I, *Mse*I and *Rsa*I enzymes. Collectively, RFLP patterns analyzed with these enzymes were similar to those of peanut witches' broom, 16SrII group phytoplasmas (Lee *et al.,* 1998). ELISA test using polyclonal antibody prepared against Yazd alfalfa witches' broom (YAWB) phytoplasma showed that PMP phytoplasma is serologically related to YAWB agent, a 16SrII group related phytoplasma.

On the basis of disease symptoms, dodder transmission and positive reaction in PCR and ELISA tests, PMP in Yazd has phytoplasmal etiology. This is the first report of pot marigold phyllody disease in Iran. On the basis of RFLP analysis, this phytoplasma is related to '*Candidatus* Phytoplasma aurantifolia', 16SrII group phytoplasma. In other countries, an aster yellows–related phytoplasma (16SrI) was associated with the same disease (Marcone *et al.*, 1997; Wang and Hiruki, 2001) but no reports were found of pot marigold as a host for the 16SrII phytoplasma group. Alfalfa witches' broom is a peanut witches' broom related phytoplasma (16SrII) that is prevalent in Yazd province (Salehi *et al.*, 1995). It is yet to be determined whether the agents of YAWB and PMP are the same phytoplasma.

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