PHYTOPLASMA ASSOCIATED WITH SHOOT PROLIFERATION IN BEGONIA

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ABSTRACT: Begonia is a very appreciated genus of ornamental plants, of economic relevancy, having species of flowers and foliage. In commercial croppings, plants exhibiting characteristic symptoms of phytoplasma infection have been observed, such as shoot proliferation, reduced plant, size small leaves and flowers, and phyllody. Leaves were sampled and total DNA was extracted to be used in nested Polymerase Chain Reaction (PCR), in order to detect and identify an expected phytoplasma. The results confirmed consistently the presence of a phytoplasma associated with symptomatic plants through the amplification of a typical genomic fragment of 1.2 kb by using the universal primers R16mF2/mR1 and R16F2n/R2. The use of specific primers R16(III)F2/R1 allowed to identify the phytoplasma detected as a representative of the group 16SrIII. This information is very expressive, because different diseases caused by fungus, bacteria, virus and nematodes have been reported for begonia, however, reports have not been found for begonia diseases associated with phytoplasmas.
Key words: Mollicutes, yellows, phytopathogenic prokaryotes

ASSOCIAÇÃO DE FITOPLASMA AO SUPERBROTAMENTO DE BEGÔNIA

RESUMO: Begônia é um gênero muito apreciado de plantas ornamentais, de relevância econômica, compreendendo espécies de flores e de folhagem. Em cultivo comercial foram observadas plantas apresentando sintomas característicos de infecção por fitoplasma, entre eles superbrotamento de ramos, redução no porte da planta, folhas e flores pequenas e filodia. A partir de amostras foliares foi feita extração de DNA para ser usado em duplo Polymerase Chain Reaction (PCR), visando 'a detecção de fitoplasma em tecido doente e a sua identificação ao nível de grupo de classificação. Os testes revelaram consistemente a associação de fitoplasma com os sintomas da doença, através da amplificação de um fragmento genômico típico de 1,2kb para os iniciadores universais R16mF2/mR1 e R16F2n/R2, usados na reação de PCR. O emprego de iniciadores específicos R16(III)F2/R1 permitiu identificar o fitoplasma detectado como um representante do grupo 16SrIII. Este é um resultado expressivo, pois diversas doenças causadas por fungos, bactérias, vírus e nematóides têm sido relatadas para begônia, no entanto, não têm sido encontrados relatos para doenças de begônia associadas a fitoplasmas.
Palavras-chave: molicutes, amarelos, procarioto fitopatogênico

INTRODUCTION

Diseases associated with phytoplasmas have been reported in a diversity of plants cultivated in Brazil, including some ornamental species as daisy (*Crysanthemum parthenium*), aster (*Collistephus chinensis*), strawflower (*Helichrysum bracteatum* (Kitajima, 1994) and periwinkle (*Catharantus roseus*) (Barros, 2002). The symptoms caused by phytoplasmas can be very similar to those incited by other pathogens, chemicals, nutritional factors and genetic abnormality, so that detection of phytoplasmas is important for disease diagnosis (Lee et al., 2000). Because phytoplasmas are not isolated in culture media, the electronic transmission microscopy and the technique of Polymerase Chain Reaction (PCR) have been useful tools used as routine to demonstrate the presence of phytoplasmas in symptomatic plants.

The plants known as begonias (*Begonia* spp.) represent more than a thousand species native from Africa and tropical regions of America (Larson, 1980). These species can be used as foliage or flowers of varied coloration, usually commercialized in pots or to constitute gardens, very appreciated among the orna-

mentals (Reynolds et al., 1998). In begonia crops, diseases caused for fungus, bacteria, viruses and nematodes demand an efficient control to avoid damages and depreciation of the economic value of specimens (Daughtrey et al., 1995).

In a commercial enterprise of begonia cultivation, located in Holambra, SP, Brazil, some plants presented reduction in size in comparison with normal plants, moderate proliferation of shoots, internode shortening and reduced size of leaves and flowers (Figure 1). The phyllody, a symptom typically attributed to phytoplasma, was also observed in diseased plants, characterized by leaves replacing petals. Thus, the objective of present study was to demonstrate the presence of phytoplasma in tissues of symptomatic plants and identify it.

MATERIAL AND METHODS

Leaf samples were collected from infected and asymptomatic plants grown in pots, of floricultural crops. DNA for use as template in PCR reactions was extracted from fresh tissue according to a methodology described elsewhere (Lee et al., 1993).

Phytoplasma detection was conducted by the nested PCR method to amplify the 16S rDNA region using the universal primer pairs R16mF2/mR1 and R16F2n/R2 (Gundersen & Lee, 1996). DNA amplified in PCR primed by the first primer pair was diluted 1:50 with water and used as template in PCR primed by the second primer pair. PCR was carried out in a final volume of 25 µL as previously described (Gundersen & Lee, 1996) for 35 cycles, following the steps: 1 min for denaturation at 94ºC (2 min for the first cycle), 2 min for annealing at 50ºC, and 3 min for primer extension at 72ºC (7 min in the final cycle). The DNA amplified fragments were analyzed by electrophoresis through 1% agarose gel, stained with ethidium bromide, and DNA bands were visualized using a ultraviolet transilluminator. DNA sample from diseased chayote plant was used as positive control and nucleic acid from asymptomatic begonia plant and water as templated were used as negative controls. DNA fragment size standard was 1kb Ladder (Life Technologies).

Figure 1 - Symptomatic (left) and asymptomatic begonia plant (right).

The identification of phytoplasma was carried out by the nested PCR using group-specific primer pairs. Products of PCR primed by R16mF2/mR1, were diluted 1:50 in water and used as template in PCR reaction primed by primer pairs R16(I)F1/R1, R16(III)F2/R1 and R16(V)F1/R1, specific for groups 16SrI, 16SrIII and 16SrV, respectively (Lee et al., 1994). Controls were represented by maize bushy stunt phytoplasma (group 16SrI), chayote witches'-broom phytoplasma (group 16SrIII) and crotalaria proliferation phytoplasma (group 16SrV). The conditions of PCR and the electrophoresis analysis were carried out as previously described. The DNA fragment size standard was 1kb ladder.

RESULTS AND DISCUSSION

Based on the amplification of 16S rDNA, using the primer pairs R16mF2/mR1 and R16F2n/R2, phytoplasma was consistently detected in symptomatic begonia samples. The presence of phytoplasma was demonstrated by amplification of a fragment characteristic 1.2kb DNA, visualized in agarose gel (Figure 2). The DNA bands were typical for phytoplasmas, when the universal primer pairs are used to amplify 16Sr DNA (Gundersen & Lee, 1996). No amplification was observed in PCR containing DNA obtained from asymptomatic plant and water as template. Repeated PCR assays confirmed the same results. These findings demonstrated the expected association of a phytoplasma with diseased begonia exhibiting reduction in size, shoot proliferation, internode shortening, phyllody, and small leaves and flowers.

Molecular identification indicated the occurrence of a group 16SrIII phytoplasma. PCR products amplified by the specific primer pair R16(III)F2/R1 yielded DNA fragments of 0.8kb in agarose gel, after electrophoresis (Figure 3). These kinds of DNA bands were identified as typical of phytoplasma belonging to group 16SrIII, according to Lee et al., 1994. Bands corresponding to 0.8 kb were also observed when DNA from chayote plant was amplified by the same specific primer pairs. No amplification was observed

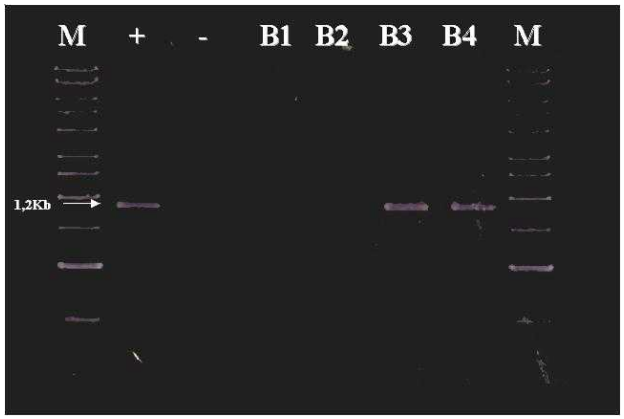

Figure 2 - Amplified fragments (1.2kb) corresponding to 16S rDNA of phytoplasma present in begonia plants, using universal primers R16mF2/mR1 and R16F2n/R2 in nested Polymerase Chain Reaction. M = DNA fragment size standard; + = positive control (chayote); - = negative control (water); B1, B2 = asymptomatic plants; B3, B4 = symptomatic plants.

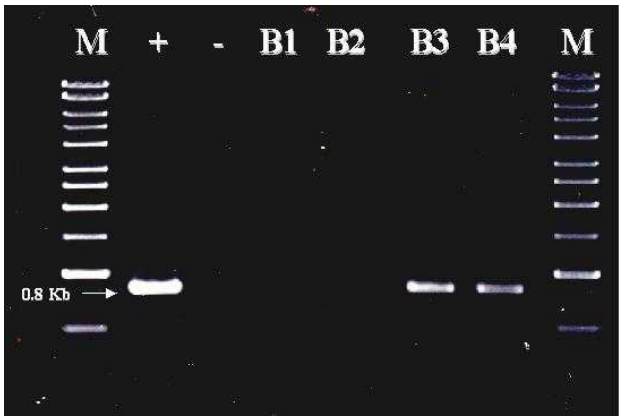

Figure 3 - Amplified fragments (0.8kb) corresponding to 16S rDNA of phytoplasma detected in begonia plants, using specific primer pair R16(III)F2/R1 in nested Polymerase Chain Reaction. M = fragment size standard; + = Positive control (chayote); - = negative control (water); B1, B2 = asymptomatic plants; B3, B4 = symptomatic plants.

when DNA extracted from symptomatic begonia samples was used as template in reactions primed by specific primers R16(I)F1/R1 and R16(V)F1/R1. However, from DNA of corn and crotalaria plants, used as positive controls for both prime pairs, fragments of 1.1kb were amplified, characteristic for phytoplasmas belonging to groups 16SrI and 16SrV, respectively.

Diseases caused by fungus, bacteria, viruses and nematodes have usually been reported for *Begonia* (Daughtrey et al., 1995). However, even in recent searches, no information has been found concerning to begonia diseases associated with phytoplasmas. Thus, the findings presented in this study provide a new information on the association of a phytoplasma of the group 16SrIII with proliferation of begonia. The occurrence of phytoplasmas belonging to group 16SrIII is very often found in the Brazilian territory and the phytoplasma present in begonia can be clustered with group 16SrIII phytoplasmas reported in distinct species of plants, such as tomato (Amaral Mello, 2004), eggplant (Barros, 2002; Amaral Mello, 2004), manihot (Barros, 2002), chayote and *Momordica charantia* (Montano et al., 2000). Diseases associated with phytoplasmas in cultivated, wild and weed plants are not widely known in Brazil and some diseases have been usually attributed to various biotic or abiotic agents (Amaral Mello, 2004). Thus, the demonstration that a phytoplasma may be capable of inducing disease in begonia, as revealed in the present work, indicate that phytoplasmas must also be considered as a possible pathogen in begonia crops, as well as fungi, bacteria, viruses and nematodes.

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