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Responses of six Turkish apricot cultivars to '*Candidatus* Phytoplasma prunorum' under greenhouse conditions

Çiğdem ULUBAŞ SERÇE, Mona GAZEL, Songül YALÇIN, Kadriye ÇAĞLAYAN
Mustafa Kemal University, Agriculture Faculty, Plant Protection Department, Hatay, Turkey

Abstract

Turkey leads in apricot, *Prunus armeniaca* L., production in the world. In Turkey, apricot is mainly produced for table and dry consumption. Both local and foreign cultivars are grown for table production while local cultivars dominate drying. Although there are several reports for '*Candidatus* Phytoplasma prunorum' infections focusing on coastal regions, there are no reports, to our knowledge, indicating the response of Turkish cultivars to this pathogen. In this study, we observed the responses of six Turkish cultivars (Hacihaliloğlu, Kabaaşı, Tokaloğlu, Şekerpare, Alkayısı and Karacabey) to '*Ca.* P. prunorum' under greenhouse conditions. For each cultivar, 11 one-year-old trees were potted in 15 lt containers; eight of these trees were inoculated by three chip-buddings for each tree in spring of 2005 and three trees were used as control. Next spring, the presence of '*Ca.* P. prunorum' was tested by PCR using phytoplasma specific universal primers P1/P7 and R16F2n/R2, and the plants were monitored for symptom development. The '*Ca.* P. prunorum' infections were detected in one to four trees of each cultivar. The infected trees exhibited visual symptoms in early summer of 2006 and 2007. The first year's symptoms include longitudinally upward rolling of the leaves along the mid-vein. In the second year, some of the infected trees sprouted and flowered earlier when compared to the controls. Most of the infected trees died at the end of the second summer. These preliminary results demonstrate that although there are some differences among the cultivars for visual symptoms caused by '*Ca.* P. prunorum' inoculation and PCR testing, all of the cultivars tested appear to be susceptible to '*Ca.* P. prunorum'.

Key words: ESFY, Turkish apricot cultivars, PCR, phytoplasmas.

Introduction

Turkey is the centre of wide range of fruit trees arising from its ecological conditions. Most of the fruit species like apricot, apple, plum and cherries are indigenous to the area. Turkey is the most important producer and importer country of apricot, *Prunus armeniaca*, which is an important temperate fruit. In general, apricot is produced for either table or dry consumptions. Turkey's table apricot production is usually practiced with foreign-origin cultivars on Mediterranean and Aegean regions while Malatya is the most important region for dry apricot where its production is almost a monoculture. Hacıhaliloğlu and Kabaaşı cultivars dominate Malatya's dry apricot production.

Recently, growers reported decline and off-season flowering in winter for apricots. These symptoms are related to symptoms caused by '*Candidatus* Phytoplasma prunorum' (European stone fruit yellows; ESFY) which affects mainly apricot and plum trees causing quick decline of susceptible plants in one to two years. This disease was observed in three main fruit growing regions in Turkey (Mediterranean, Marmara and Aegean) symptomatically and it has been proved by molecular tools in preliminary works (Çaglayan *et al*., 2004; Ulubaş Serçe *et al*., 2006; Jarausch *et al*., 2000). ESFY has been spreading very quickly, causing quick decline and it is a quarantine disease.

Management of fungal and bacterial diseases as well as insects can be achieved by using different chemicals. However, chemical control is impossible or not economical for virus, viroid and phytoplasma diseases. These diseases can only be controlled by using healthy plants, and vector control to avoid dissemination. Using resistant cultivars is also an effective strategy against these diseases. Although Turkey is centre of origin for apricot and has many important cultivars, the responses of these local cultivars against ESFY have not been studied. In this study, we tested the responses of six leading Turkish cultivars against ESFY.

Materials and methods

Six apricot cultivars grafted on seedlings (Cvs. Hacihaliloğlu, Kabaaşı, Tokaloğlu, Şekerpare, Alkayısı and Karacabey) were selected. For each cultivar, 11 one-year-old trees were potted in 15 lt containers and maintained in greenhouse.

ESFY inoculum originated from an infected apricot plant (Ulubaş Serçe *et al*., 2006). Eight of these trees were inoculated by three chip-buddings for each tree at the beginning of September of 2005 and three trees were used as control.

Following graft inoculation, the trees were observed for 2 years for early bud burst, development of leaves before flowering, small chlorotic rolled leaves, foliar yellowing and/or reddening.

In early spring the year following grafting the trees were examined to determine whether the inoculum had bee successful or not. The presence of ESFY was tested by PCR using phytoplasma specific universal primers P1/P7 (Deng and Hiruki, 1991; Smart *et al*., 1996) and R16F2n/R2 (Lee *et al*., 1995) in April 2006. DNA extraction was carried out from leaf midribs (Doyle and Doyle, 1990).

Nested PCR products (1200 bp) of phytoplasma 16S rDNA gene obtained from samples as well as reference

isolates were subjected to RFLP analysis. Five µl aliquots of each PCR product were separately digested overnight at 37 °C with restriction endonucleases *Rsa*I and *Ssp*I, at 65 °C with restriction endonuclease *Mse*I (MBI Fermentas, Lituania; GmbH, Germany). The digested products were analyzed by electrophoresis using 2% agarose gel and the products were visualized with a UV transilluminator after staining with ethidium bromide and then photographed.

Results

ESFY was detected by PCR in six cultivars of apricot trees: in one tree of cv Tokaloğlu, in two trees of cv Alkayısı, in four trees of cv Şekerpare, in two trees of cv Karacabey and in one tree of cv Hacıhaliloğlu. No infection was found in cv Kabaaşı. Despite detection of the pathogen in April, no symptom appeared on the trees. The first symptoms were monitored in all ESFY infected cultivars in May 2006 and these symptoms were maintained during the summer. These symptoms were up-rolling of leaves and reduced size of leaves.

In the following winter (February 2007), early bud break and development of leaves before flowering occurred. Phloem necrosis was also observed on the infected cultivars followed by yellowing of trees and decline in summer. Two ESFY infected trees of cv Alkayısı, two of four infected trees of cv Şekerpare, one of two infected trees of cv Karacabey and one tree of cv Hacıhaliloğlu died at the middle of summer.

Discussion

Phytoplasmas are limited to functional phloem tissues and their transmission by grafting is difficult due to the low concentration (Jarausch *et al*., 1999). Therefore, the reason of unsuccessful inoculation of all trees might be the inefficient phytoplasma concentration in the inoculated plant and/or no inoculation. The phytoplasmas were eliminated in the above-ground parts of the trees during winter but not in the root system from which they re-colonized the stem in spring (Jarausch *et al*., 1999). The pathogen can be readily detected by PCR in various *Prunus* species during winter (Jarausch *et al*., 1999) but for symptom appearance a certain concentration of the colonizing pathogen is needed. Therefore, although ESFY was detected in inoculated plants, symptom emerging was observed later.

Off-season growth of all infected apricot cultivars is related to the activity of the remaining sieve tubes of the late season phloem under mild climatic conditions (Jarausch *et al*., 1999).

In this preliminary study, all tested Turkish apricot cultivars were evaluated to be susceptible to '*Ca.* P. prunorum'. The observation of all inoculated plants is still in progress, since phytoplasmas might be equally distributed in the tree only after longer periods after the inoculation (Jarausch *et al*., 1999).

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Corresponding author: Çiğdem ULUBAŞ SERÇE (e-mail: culubas@mku.edu.tr), Mustafa Kemal University, Faculty of Agriculture, Plant Protection Department 31034, Hatay, Turkey.