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Molecular evidence of phytoplasmas in winter oilseed rape, tomato and corn seedlings

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Abstract

To evaluate the seed transmission of phytoplasmas in three herbaceous hosts material from different geographical origin but all collected from infected or symptomatic mother plants was used. Almost 1,000 seeds were germinated under controlled conditions and 652 seedlings were obtained. Samples were grouped in 214 samples and 74 of them resulted positive to phytoplasma presence after three to 90 days from germination by nested PCR assays on 16S ribosomal gene. All the tested species i.e. winter oilseed rape, tomato and corn resulted carrying phytoplasmas belonging to the ribosomal groups retrieved in the infected mother plants.

Key words: Phytoplasma, seed transmission, aster yellows, stolbur, elm yellows.

Introduction

Phytoplasma seed transmission is still a controversial issue considering the very poor connection of embryo with the mother plant. However it is well known that flower structures are the most colonized by phytoplasmas in many plant species. Most likely the seed production in infected plants is quite reduced, however recent studies have highlight the fact that late infections, especially in herbaceous crops, allow the normal seed production increasing chances of transmission of the pathogens though the seeds (Olivier e Galka, 2008). After the fist report of 16SrII phytoplasma in alfalfa seedlings (Khan *et al*., 2002), recently stolbur phytoplasmas were again identified in pea seedlings (Zwolinska *et al*., 2010). To verify the seed transmissibility trials in sterile conditions on a relevant number of seedlings collected from field infected mother plants of tomato, oilseed rape and corn were carried out.

Materials and methods

Winter oil sees rape seeds were collected in Veneto (Northern Italy) from aster yellows and stolbur infected mother plants (Mori *et al*., 2010; Contaldo *et al*., unpublished). Two batches of seeds were analyzed Excalibur 2008 (same batch used to seed the field where infection was detected) and Excalibur 2009 (produced from the infected plants). A total of 50 and 325 seed respectively for each batch were employed. A total of 128 tomato seeds from Bulgaria (varieties Rila, Marti, Kristi, Trapesitza, Milyana, and UC 82-A) collected from symptomatic tomato plants (figure 1) were tested. For corn from Serbia 186 seeds tested were from 4 cobs collected from plants showing reddening (Duduk and Bertaccini, 2006) and resulted positive to stolbur phytoplasmas (Mitrovic *et al*., unpublished). Further 287 seeds from 11 Italian cobs were collected in Mantova province (Northern Italy) and derived from plants showing reddening (Calari *et al*., 2010) resulted either positive or negative to phytoplasmas presence. Surface sterilized seed were grown in insect proof environments, *in vitro* or in sterile wet substrate (figure 2) for 10 days to 3 months before testing for phytoplasmas presence. Batches of 3 to 20 individual seedlings (about 100 mg at least each) clean from all maternal tissues were subjected to DNA extraction, for some of the corn seedlings a separation into roots and leaflets was also carried out for testing. The DNaesy Plant Minikit (Qiagen) and a CTAB method (Angelini *et al*., 2001) were employed for nucleic acid extraction and nested PCR with 16S ribosomal primers was carried out under reported conditions (Calari *et al*., 2010). Phytoplasma identification was performed by RFLP analyses and/or sequencing of the obtained amplicons.

Figure 1. In each picture: healthy (left) and stolbur infected (right) tomato from which seeds were used.

Results

Germinating percentages for winter oilseed rape and tomato seeds ranged from 30 to 100%. For corn there was a difference in germination rate between the Serbian seeds (average 70%) and the Italian onces (average 54%).

In seedlings from Excalibur 2009 a total of 30 seed batches was tested and both 16SrI-B and stolbur (16SrXII) phytoplasmas were identified: 7 batches resulted infected with 16SrXII phytoplasmas and 3 with 16SrI-B phytoplasmas. Seedling from Excalibur 2008 five out of the seven batches tested resulted positive for 16SV and 16SrII phytoplasmas.

In tomato 23 batches were tested and only Rila and Milyana resulted negative to phytoplasmas presence, two batches were positive to 16SrXII (stolbur) and 5 to 16SrI-B (aster yellows) phytoplasmas.

The tested batches of Italian corn were 55 and 17 batches resulted positive to phytoplasmas presence, seedling from 5 of the tested cobs resulted all negative. Phytoplasmas identified in these samples were 16SrI-B, 16SrXII and 16SrV in some cases in mixed infections, phytoplasmas were detected in both leaves and roots. The 37 batches tested from seedling germinated from Serbian cobs show phytoplasma presence in all samples but 7, all cobs were positive.

Figure 2. Corn seeds under germination process before testing.

Discussion

The long distance spread of phytoplasmas is quite easily achieved for woody plants of agricultural interest by infected propagation material such as cutting, corms and micropropagated plant or insect vectors accidentally carried with plant material. On the contrary epidemic outbreak associated with phytoplasmas not present in a certain eco-system such as small island or similar in annual herbaceous crops allow to hypotize a role of the phytoplasma seed transmission. Considering the ability of seed producing of plants that are infected by phytoplasmas in late stages or their life (De La Rue *et al*., 2002; Cordova *et al*., 2003), and the viability of malformed seeds (Nečas *et al*., 2008; Nipah *et al*., 2007; Olivier e Galka, 2008) it is possible to assume that a low percentage of seed transmission should be considered. In this work the presence of phytoplasmas in seedling was not correlated to the germination percentages, however evident differences in the number of batches positive to phytoplasmas infection was found for example between corn in Italy and in Serbia. To better define the statistical relevance of the reported results work is in progress in oilseed rape to verify the ability of the phytoplasmas seed transmitted to induce symptoms in mature plants.

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