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First report of maize redness disease in Hungary

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Abstract

During 2010, several maize production areas in Hungary were surveyed for the occurrence of maize redness (MR) disease symptoms associated with stolbur phytoplasma, as well as for the presence of the known vector of the disease, a planthopper *Reptalus panzeri* (Low). Incidence of maize plants with symptoms of reddening was low in all surveyed areas. Altogether, 25 symptomatic maize plants were collected at 9 localities and tested for phytoplasma presence. In addition, from one locality specimens of cixiids *R. panzeri* and *Hyalesthes obsoletus* Signoret were collected and PCR analyzed. Presence of stolbur phytoplasma in MR symptomatic maize plants and stolbur-infected *R. panzeri* was identified at the single locality Monorierdő in central Hungary. This finding represents the first report of MR presence in Hungary.

Key words: corn, *Hyalesthes obsoletus*, maize reddening, *Reptalus panzeri*, stolbur.

Introduction

Maize redness (MR) is a severe disease of maize associated with stolbur phytoplasma (16SrXII-A) which is transmitted to maize by the cixiid planthopper *Reptalus panzeri* (Low) (Jović *et al.*, 2007). Disease is characterized by midrib, leaf and stalk reddening, followed by reddening and desiccation of the whole-plant and abnormal ear development. Although MR has been reported from Serbia, Romania and Bulgaria for more than 50 years, the associated agent, a vector and the epidemiological cycle of the disease have only recently been determined (Jović *et al.*, 2009). MR can cause significant yield losses in the years of epiphytotic appearance, as was recorded during the 2002/03 outbreak in and around the South Banat district of Serbia.

Recent reports of maize with reddening symptoms in Italy (Calari *et al.*, 2010), as well as possible host-shift of *R. panzeri* to maize as a preferable host plant (Jović *et al.*, 2010) are suggesting an ongoing, and in the future expected spread of the disease and of its insect vector. Previously reported presence of *R. panzeri* in Hungarian vineyards and their natural infection with stolbur phytoplasma (Palermo *et al.*, 2004), in addition to economic importance of maize production, increased the need for a survey of MR presence in the territory of Hungary. Here we present the first results of this survey.

Materials and methods

During August and September of 2010 selected maize fields in certain production areas of Hungary were surveyed for the occurrence of reddening symptoms on maize. Plants expressing symptoms such as midrib, leaf and stalk reddening were collected from nine sampling sites in south, southwest, northwest and central Hungary (figure 1). On all surveyed localities only individual plants were symptomatic, and no presence of epiphytotic appearance of the symptoms was recorded. During July *R. panzeri* and *Hyalesthes obsoletus* Signoret cixiids were collected from a single maize field in the vicinity of village Monorierdő in central Hungary, which was later sampled for MR symptomatic maize plants. Insects were collected by sweep nets in the vicinity of maize fields.

DNA was extracted from fresh leaf midribs and adventitious roots of MR symptomatic maize plants and individual insects according to previously reported CTAB protocols (Daire *et al.*, 1997; Clair *et al.*, 2003). Phytoplasma identification was conducted using a nested PCR on the 16S rRNA gene with primer pairs P1/P7 and R16F2n/R16R2 (Lee *et al.*, 1998). The obtained nested-PCR products were digested with *Tru1*I restriction enzyme and RFLP profiles were compared with reference strains (Rep9 and WRp5 MR from J. Jović; EAY, 16Sr-B, MOL, 16SrXII-A from A. Bertaccini and AY27, 16SrI-A, CPh, 16SrI-C, PaWB, 16SrI-D from I-M. Lee). In parallel, all samples were tested with TaqMan real-time PCR amplifying phytoplasma nonribosomal *map* gene of the 16SrXII-A subgroup, applying plant endogenous control (EC) (Pelletier *et al.*, 2009) with slight modification of probe labelling and PCR conditions.

Results and discussion

Only in three out of 25 symptomatic maize plants, collected from nine sampling sites in Hungary, was the stolbur phytoplasma (16SrXII-A) identified by PCR/RFLP based analysis of the 16S rRNA gene. All three stolbur infected corn samples originated from the same locality (Monorierdő) in central Hungary (figure 1) where the potential planthopper vectors were identified. In the case of analyzed insects, two out of six *R. panzeri*

and three out of eight *H. obsoletus* specimens tested positive for the presence of stolbur phytoplasma. The results were confirmed by real-time PCR. The Ct values were measured in all the stolbur positive samples ranging from 31.1-33.8. Amplification curves of EC were observed in each sample with Ct values between 18.2-22.5.

None of the other maize samples, collected at other localities (figure 1) were positive for any phytoplasma. Although these plants were symptomatic, absence of phytoplasma was not surprising, taking into account that reddening of maize can also occur as a consequence of different biotic and/or abiotic factors, as well as the absence of the MR vector *R. panzeri*. These results lead to the conclusion that presence of MR disease can be identified only when a three-parameter identification of symptoms, phytoplasma and vector is performed.

Figure 1. Map of maize sampling sites in Hungary.

Since the first records of MR appearance in the South Banat district of Serbia in 1957 (Marić and Kosovac, 1959) it has been noted that the disease has periodical variations in symptom intensity and incidence. Due to specificities of the disease epidemiological cycle and the insect vector life cycle (Jović *et al.*, 2009), environmental factors play a significant role both in the intensity and incidence of the disease. More severe disease and higher incidence of symptoms are associated with warm springs and summers which facilitates earlier emergence of the vector adults and consequently earlier infection of maize with the stolbur phytoplasma. Thus, if the vector and stolbur phytoplasma as agent associated with the disease are present in a certain geographic area, risk of epiphytotic appearance of MR is also present, whenever favorable environmental conditions are.

Based on these results we conclude that stolbur phytoplasma associated with maize redness disease, and the identified vector of the disease *R. panzeri* are present in corn in Hungary. Presence of the stolbur-infected vector of MR *R. panzeri* is in agreement with previously determined role of this cixiid as a major vector of MR in the South Banat district of Serbia (Jović *et al.*, 2009). However, the role of *H. obsoletus* in MR epidemiology in Hungary is yet to be studied, since this cixiid was not reported to play a significant role in MR epidemiology in Serbia.

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