Research for Practice LAIMBURG JOURNAL

Report

First Report of Tobacco ringspot virus infecting apple plants in South Tyrol

Erstnachweis von Tobacco ringspot virus am Apfel in Südtirol

Prima segnalazione del Tobacco ringspot virus in piante di melo in Alto Adige

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CITE ARTICLE AS
Gallmetzer Andreas, Springeth Christian, Reyes Dominguez Yazmid (2024). First Report of Tobacco ringspot virus infecting apple plants in Northern Italy. Laimburg Journal 06/2024.004 DOI:10.23796/LJ/2024.004.

KEYWORDS
nepovirus, apple viruses

A *Malus domestica* (Borkh.) plant conserved in the nuclear stock of mother plants at the Laimburg Research Centre (Autonomous Province of South Tyrol, Northern Italy) was tested positive to Tobacco ringspot virus (TRSV) in May 2023 during a routine phytosanitary inspection for the presence of quarantine viruses. TRSV quarantine status in the EPPO region (European and Mediterranean Plant Protection Organization, an international organization responsible for cooperation and harmonization in plant protection within the European and Mediterranean region, which currently has 52 member countries) is A2, meaning that the virus is locally present.

Following importation of the scion from North America in November 2021 and prior to grafting onto woody *Malus domestica* M9 rootstocks, the material was tested negative for the apple latent viruses Apple stem grooving virus (ASGV), Apple stem pitting virus (ASPV) and Apple chlorotic leaf spot virus (ACLSV), for the viroids Apple scar skin viroid (ASSVd) and Apple dimple fruit viroid (ADFVd) as well as for the nepoviruses Cherry rasp leaf virus (CRLV), Tomato ring spot virus (ToRSV) and TRSV. TRSV has its origin in central and eastern North America and in other parts of the world the presence of the virus is probably associated with material exported from North America [1].

As required by the regulation on protective measures against plant pests, the grafted mother plant was kept in the post-entry quarantine facility until May 2023 [2]. The plant was asymptomatic throughout the entire period.

Total RNA was isolated from the leaves of the plant in question using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Virus detection was performed by a two-step RT-PCR and the obtained PCR fragments were analysed by sequencing the amplicons with TRSVf2/TRSVr2 primers [3]. A BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) analysis was performed on the sequences and revealed 97.91% identity (E value of 9e-86) with the TRSV isolate Budblight (accession number MT210151). These results confirmed the presence of a TRSV infection in the asymptomatic *Malus domestica* plant.

Due to the wide host range of TRSV and its broad transmission profile via nematodes, thrips, seeds, sap inoculation and grafting, it represents a major risk for many agricultural crops [4]. TRSV has a wide host range and has been reported in more than 100 different herbaceous and woody plant species. The cultures that are mainly affected by infection with TRSV are *Vaccinium corymbosum*, Vitis vinifera and Glycine max and to a lesser extent Nicotiana tabacum and many plants of the Cucurbitaceae family [5]. The symptoms on the infected plants are very different depending on the infected species, for example TRSV can cause stem dieback and stunting in *Vaccinium corymbosum*, in tobacco causes ring and line patterns on the foliage and stunting, in cherry, the leaves show irregular chlorotic blotching over the whole leaf blade and the leaf margins are deformed and lobed [1].

One of the most efficient ways of spreading TRSV over longer distances is the movement of infected vegetatively propagated plants, grafts and seeds. Infected grafted plants can establish new infections when introduced to soils where the vector is present. In areas such as the EPPO region where vector transmission has so far not been known to occur, establishment of TRSV can easily be prevented by removal and destruction of infected plants [1].

The fact that many infected hosts remain symptomless means that there is a considerable risk of spreading the virus. Hence, it is of critical importance to carry out all the necessary phytosanitary controls and to establish virus-free nuclear stocks from which plants or grafts can be propagated and subsequently distributed through a certification scheme [6].

As demonstrated in this work, one of the main aims of our laboratory is to safeguard South Tyrolean agriculture through the early diagnosis of phytopathogens not only in fruit, but in every culture that is relevant to the region.

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