Two viruses associated with a new disease of spinach in Greece

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Summary

Uncommon symptoms were observed in spinach fields in the Marathon valley, Greece. Symptoms included yellow mottling, line patterns, leaf deformation and necrosis. Seedlings from the same seed lot, grown in the greenhouse, developed similar symptoms, indicating that the causal agent(s) of the disorder is seed transmitted. Mechanical transmission to several herbaceous indicators including spinach seedlings of the same variety but a different seed lot gave two types of symptoms: Yellow mottling and deformation or necrotic spots. Double stranded RNA extractions, cloning and sequencing followed by a combination of molecular (RT-PCR, IC-PCR) and serological (ELISA) techniques verified the presence of two viruses in the diseased seedlings: *Sowbane mosaic virus* (SoMV) was present in spinach and indicators with mottling and leaf deformation, whereas *Olive mild mosaic virus* (OMMV) was found in plants with necrotic spots. The SoMV-spinach isolate shows 95% nt identities to the type isolate of the virus from *Chenopodium quinoa*. The OMMV-spinach isolate has 92% nt identity to the OMMV type isolate from olive. This is the first report of SoMV in spinach in Greece and the first record of OMMV infecting a crop other than olive and tulip, verifying the existence of OMMV as an independent species and not as an accidental recombination between *Tobacco necrosis virus* A and *Tobacco necrosis virus* D in olive.

Introduction

Spinach (*Spinacea oleracea,* L.) is susceptible to more than 100 viruses (Plant Viruses Online). Plant reaction to individual viruses depends on virus species and strains; some viruses are asymptomatic, including several members of the genus *Ilarvirus* whereas others, like *Cucumber mosaic virus* and *Tomato spotted wilt virus,* can cause severe symptoms and even plant death. The latter was the case of the disease presented in this communication.

The Marathon valley is the main spinach production area for Athens, Greece. Several viruses have been found in the area, including Cucumber mosaic, Tobacco mosaic and Tomato spotted wilt. Those viruses are normally moving in the field late in the season unlike the disease presented here.

'Spinaker' fields developed severe symptoms soon after seedlings emerged. Symptoms were widespread (over 50% of the fields infected) and included yellow mottling, line patterns, leaf deformation and necrosis (Fig. 1).

Fig. 1. 'Spinaker' spinach collected from the Marathon valley, Greece showing various degrees of virus-like symptoms.

Materials and Methods

The two major symptom variants, yellow mottling/line patterns and leaf deformation/necrosis were first inoculated to *Chenopodium quinoa* and produced chlorosis that progressively turned to systemic necrosis or local necrotic spots respectively. When material from *C. quinoa* was back inoculation to spinach the first variant caused diffused chlorotic spots and the second caused severe distortion and necrosis. The two variants were used for double stranded RNA (dsRNA) extractions according to the Yoshikawa and Converse (1990) protocol. For cloning, the method described in Tzanetakis et. al (2005a) was used. After comparison of the sequenced material to the Genbank virus database using Blastn/Blastx, oligonucleotide primers were developed and the genome sequence gaps were acquired essentially as described previously (Tzanetakis et al., 2005b). After identification of the virus species, purifications were performed using the Girgis et al. (2009) method. Viruses were also characterized for their biological properties after mechanical inoculation onto 24 indicator plant species (Table 1). Total RNA was extracted as previously reported (Tzanetakis et al., 2007) for detection using reverse transcription-polymerase chain reaction (RT-PCR). Spinach as well as indicator plants were tested for the viruses and all amplicons were sequenced to confirm the validity of the findings. Seed transmission in spinach was evaluated for both variants, and virus presence was verified using RT-PCR.

Alternative hosts

Chenopodium quinoa	*Nicotiana tabacum* cv. Turkish	*Capsicum annum*
C. amaranticolor	*N. tabacum* cv. Xanthi	*Cucumis sativus*
C. murale	*N. tabacum* cv. Samsun	*Vigna sinensis*
C. foetidum	*N. tabacum* cv. Σ 53	*Cucurbita pepo*
Sonchus oleraceus	*N. glutinosa*	*Phaseolus aureus*
Solanum melongena	*N. clevelandii*	*P. vulgaris* cv. Karmen
S. esculentum	*N. benthamiana*	*P. vulgaris* cv. Ilerda
Petunia x *hybrida*	*N. rustica*	*P. vulgaris* cv. Focus

Results and discussion

DsRNA extractions gave different electrophoresis patterns for the two variants with a ~4.5 kb predominant band for the yellowing variant and a ~4 Kb band for the distortion/necrosis variant. Virus purifications revealed the presence of spherical particles of 25-28nm in diameter in both cases (Fig. 2).

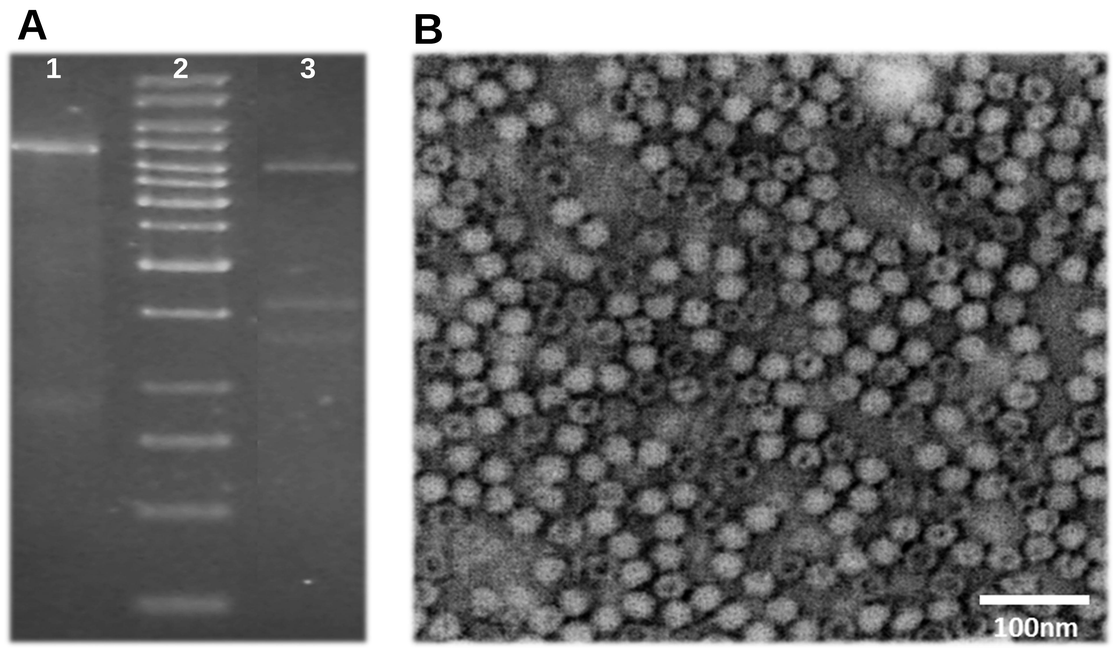

Fig. 2. A. DsRNA patterns of the yellowing (1) and distortion/necrosis (3) variants. Lane 2 - 1kb NEB marker. B. Purified virions of the yellowing variant, proved to be an isolate of *Sowbane mosaic virus*.

Indicator inoculations gave an array of symptoms on *Chenopodium* species and petunia for the yellowing variant ranging from yellowing to plant death whereas the distortion/necrosis variants gave local necrotic lesions to all indicators other than *N. glutinosa* where concentric necrotic lesions where observed.

The shotgun cloning revealed that the yellowing variant was infected with *Sowbane mosaic* virus (SoMV). The spinach isolate had about 95% nt identity to the *C. quinoa* and raspberry isolates. Symptoms were typical of the virus on indicators and spinach seed transmission was 43%, similar to the percentage observed in the original and only other report of the virus in spinach (Bos and Huijberts, 1996). Those results are indicative that the greek SoMV spinach isolate has similar properties to the previously studied isolates.

The distortion/necrotic variant proved to be an isolate of *Olive mild mosaic virus* (OMMV). The spinach isolate had 93 and 92% nt identities to the tulip and olive isolates of the virus respectively, the only other natural hosts of the virus identified to date. The seed transmission of the virus in spinach was 9% and this is the first proof of OMMV seed transmission.

The disease presented in this communication caused major economic losses to the growers, estimated to over 50%. Two seed-borne viruses were associated with the disease making them a potential problem for the seed industry. Although this is only the second report of SoMV infecting spinach, its worldwide distribution makes it less of a problem for international movement of material. OMMV is a new virus, first described in 2005. Its natural host range is limited to olive, tulip and now spinach but given the limited knowledge available on the virus biological properties there are soon more to come. A member of the genus *Necrovirus,* OMMV can be transmitted by fungi in the genus *Olpidium* and can, based on observations of its close relatives, persist in the soil for several decades. For this reason, rigorous monitoring for the presence of virus in seed fields is essential so as to minimize the possibility that OMMV moves to new areas were a vector is present and can affect field health for several decades.

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