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TRADITIONAL CULTIVATION PRACTICES MAY EFFICIENTLY PREVENT THE VIRUS SPREAD IN SUSCEPTIBLE CROPS: A CASE STUDY OF TURNIP MOSAIC VIRUS IN UKRAINE

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ABSTRACT

Turnip mosaic virus (TuMV) is a member of the largest *Potyviridae* family of plant viruses. For domesticated *Brassica* plants, TuMV is considered one of the most damaging and economically important viruses. TuMV is mainly transmitted by many aphid species non-persistently as well as mechanically from plant to plant. TuMV probably occurs worldwide and has been found in both temperate and subtropical regions of Africa, Asia, Europe, Oceania and North and South America. In Europe, TuMV was reported from the UK, Spain, Italy, Greece, Germany, The Netherlands, Czech Republic, Hungary, Bulgaria, Poland, and Russia. Despite Ukraine geographical location and wide cultivation of different *Brassica* crops for centuries, it has been only recently that the authors have registered TuMV in this country. In this study, isolates of TuMV were collected in Ukraine from naturally infected host plants, all from *Brassicaceae* family. For the first time, TuMV was shown to be widespread in agricultural and urban regions in Ukraine where it naturally infects crops, weeds and introduced species with infection rate reaching 50%. Also, we show that urban locations and concomitant weed plants are potent factors of virus epidemiology favoring extremely high virus incidence level of 89% in susceptible hosts. Importantly, we underpin the significance of trivial cultivation practices (crop rotation and eradication of diseased plants) as preventive measures for the control of damaging pathogen of brassicas, allowing for 3 times less TuMV incidence.

Keywords: *TuMV, Brassicaceae, cultivation, field, Ukraine*

INTRODUCTION

Turnip mosaic virus (TuMV) is a member of *Potyvirus* genus belonging to the largest *Potyviridae* family of plant viruses. TuMV has flexible filamentous particles ~700-750 nm long containing a single-stranded positive sense genomic RNA of about 10,000 nt (King et al., 2012).

As many potyviruses, TuMV has an extremely wide host range but infects mostly plant species from the *Brassicaceae* family and induces persistent symptoms (mosaics, mottling, chlorotic lesions, etc.). For domesticated *Brassica* plants, TuMV is considered one of the most damaging and economically important viruses (Walsh and Jenner, 2002). TuMV is mainly transmitted by many aphid species non-persistently as well as mechanically from plant to plant. TuMV probably occurs worldwide and has been found in both temperate and subtropical regions of Africa, Asia, Europe, Oceania and North and South America (Provvidenti, 1996; Ohshima et al., 2002; Schwinghamer et al., 2014). In Europe, TuMV was reported from the UK (Pallett et al., 2008), Spain (Segundo et al., 2003), Italy (Guglielmone et al., 2000, Ohshima et al., 2002), Greece (Jenner, Walsh, 1996; Tomimura et al., 2004), Germany (Tomimura et al., 2003), The Netherlands, Czech Republic (Petrzik, Lehmann, 1996), Hungary (Horvath et al., 1975), Bulgaria (Kovachevsky, 1975), Poland (Kozubek et al., 2007), and Russia (Ohshima et al., 2002; Zubareva et al., 2012). Ukraine is one of the largest European countries enjoying strategic logistic position at the doorstep of the Northern Silk Road, between the eastern EU states and Black Sea/Middle East region, where TuMV was also recently detected in Turkey (Korkmaz et al., 2008) and Iran (Farzadfar et al., 2009). Despite Ukraine's geographical location and wide cultivation of different *Brassica* crops for centuries, it's only recently that the authors have registered TuMV in our country (Shevchenko et al., 2016). In the study reported here, we include the results of TuMV screening in various ecosystems and describe the importance of preventive measures for the control of wide-spread and damaging pathogen of brassicas.

MATERIAL AND METHODS

Sampling was carried out at the end of the growing season of 2014, and was restricted to crop-producing areas in Kyiv region (Ukraine) and different locations in the city of Kyiv where Brassicaceae plants were growing/cultivated. In Kyiv, sampling locations included two botanical gardens (Botanical garden of Taras Shevchenko National University of Kyiv and Botanical garden of the National Academy of Sciences of Ukraine), the city center, Museum of Folk Architecture and Life of Ukraine (open-air location w/o agricultural activity), and private gardens where different brassica plants were regularly cultivated. Several large fields in Kyiv region used for commercial cabbage cultivation were chosen for sampling in Kyiv region. Brassica plants were visually examined; samples were collected from plants with TuMV-like symptoms typically including mosaics, mottling, vein banding and/or leaf deformation. Collected samples were tested for TuMV by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), as described previously by Clark and Adams (1977), using specific polyclonal antibodies purchased from Loewe (Germany). Briefly, 0,5 g leaf tissue was ground to a powder with a mortar and pestle in 10 mL phosphate-buffered saline, pH 7,4, containing 0,05% Tween 20, 2,0% polyvinylpyrrolidone (MW 40 000) and 0,2% bovine serum albumin. In the meantime, microtitre plates (Maxisorb, NUNC, Denmark) were coated with TuMV-specific broad-spectrum polyclonal antibodies (1:200) in carbonate buffer according to the manufacturer's instructions. Leaf extracts were then added to the plates in duplicate wells and incubated overnight at 4°C. The presence of TuMV in the samples was detected in 200 μL homogenate by TuMV-specific antibodies conjugated to alkaline phosphatase using *p*-nitrophenyl phosphate substrate (Sigma, USA). Absorbance values at 405 nm were measured using a Multiscan-334 microtitre plate reader (Labsystem, Finland). Absorbance values, measured 60 min after adding the substrate, greater than three times those of the negative controls were considered positive.

RESULTS AND DISCUSSION

A total of 54 plant samples with TuMV-like mosaic and mottling symptoms were collected in different districts of the city of Kyiv and Kyiv region. Sampling areas included both agricultural sites (two cabbage producing fields and private gardens) and urban locations where no agricultural activity was carried out (different sites in the City of Kyiv, two botanical gardens and open-air Museum of Folk Architecture and Life of Ukraine). On cabbage plants, TuMV typically induced systemic mosaics, vein banding and leaf deformation (Fig.1/2), whereas systemic mosaics and mottling were common for naturally infected radish and mustard plants.

A

Figure 1. TuMV-positive cabbage (*B. oleracea* var. capitata)


B

Figure 2. TuMV-positive cabbage (*B. oleracea* var. capitata) (A) with vein banding/clearing and mustard (*Brassica juncea*) (B) showing symptoms of systemic mottling (source: photos made by authors during the sampling)

Using ELISA, TuMV was detected in samples from cabbage, red radish, mustard, radish, white mustard, gold of pleasure, weed species (hill mustard), etc. (Table 1).

Table 1. Double-antibody enzyme-linked immunosorbent assay for the detection of *Turnip mosaic virus* by hosts (source: authors' elaboration based on the obtained results)

Plant	**No of samples**	**Positives**	**Incidence of TuMV infection (%)**
Brassica oleracea (**cabbage**) *Brassica sp.*	23	8	35
Raphanus sativus (**red radish**) *Raphanus sp.*	12	11	92
Brassica juncea (**mustard**)	4	2	50
Sinapis alba (**white mustard**)	3	3	100
Other brassicas	5	3	60
Other non-brassicas (*Asteraceae*, *Primulaceae*, *Papaveraceae*, *Malvaceae*)	7	0	0
TOTAL	**54**	**27**	**50**

TuMV has been detected in 27 samples of plants (overall 50% incidence rate in symptomatic hosts) including *B. oleracea* var. capitata, *R. sativus*, *S. alba*, *B. juncea*, *Camelina sativa*, and *Bunias orientalis* (identified as the weed host for TuMV in Ukraine). Cabbage, radish and mustard were the predominant hosts for TuMV in sampled areas which probably reflected the virus host range for the country in general.
TuMV was found in the main brassica-crop fields, private gardens and urban locations of Ukraine, with a high overall incidence of 50%. Importantly, the agricultural sites used for plant sampling were characterized with different level of incidence of TuMV infection varying from 17% and 42% for two crop fields, and to as much as 58% for private gardens (Table 2).

Table 2. Survey for *Turnip mosaic virus* by sampling sites continuously used for crop cultivation (source: authors' elaboration based on the obtained results)

Sampling site	**No of samples**	**Positives**	**Incidence of TuMV infection (%)**
Commercial cabbage producing field 1	6	1	17
Commercial cabbage producing field 2	12	5	42
Private gardens	12	7	58
Total for agricultural sites	**30**	**13**	**39**

Several sampling sites within the Kyiv city (i.e. where no agricultural activity was carried out) demonstrated even higher incidence rate of TuMV with the minimum value of 33% for symptomatic plants (Table 3). Apparently, urban sites play an important role in virus epidemiology serving as dormant 'nests' for virus populations. These results suggest that TuMV is widespread in both agricultural and urban locations but remained undetected for a long time.

Table 3. Survey for *Turnip mosaic virus* by sampling sites in urban areas (no cultivation) (source: authors' elaboration based on the obtained results)

Sampling site	**No of samples**	**Positives**	**Incidence of TuMV infection (%)**
City of Kyiv	9	3	33
Botanical garden of Taras Shevchenko National University of Kyiv	3	1	33
Botanical garden of the National Academy of Sciences of Ukraine (Kyiv)	9	8	89
Museum of Folk Architecture and Life of Ukraine (Kyiv)	3	2	67
Total for urban locations	**24**	**14**	**55**

Expectedly, different locations demonstrated high but varying level of TuMV occurrence. However, several aspects were of special interest in this regard. For the two fields used for commercial cabbage production in Kyiv region and situated in neighboring villages just 5 km apart, the TuMV incidence rate varied from 17% to 42%. This probably reflects the efficiency of the confirmed regular eradication of diseased plants in the former case (field 1) and underpins the significance of long-known simple approach – elimination of virus inocula – for the disease control.
In turn, rather high rate of TuMV infection in private gardens (58%) may be explained by both growing of infected plants and repeated cultivation of susceptible crops, as reported by the landowners. Another approach allowing to limit virus spread – crop rotation – was also missing in this case.
Obtained results clearly demonstrate that trivial measures for crop cultivation (known for decades but often thoroughly disregarded) remain highly efficient in controlling the spread of the mechanically and aphid-transmitted virus and reducing consequential damages.

CONCLUSIONS

In summary, the survey indicated high occurrence of TuMV in urban and agricultural regions in Ukraine where it naturally infects crops, weeds and introduced species with average infection rate reaching 50%. Urban locations and concomitant weed plants are potent factors of virus epidemiology favoring extremely high virus incidence level of 89% in susceptible hosts.
Wide range of infected plant species in surveyed areas obviously demonstrates lack of virus screening in Ukraine. Obtained data also suggests a just discovered long-term coexistence of the virus and the hosts in Ukraine.

Importantly, trivial cultivation practices (crop rotation and eradication of diseased plants) are shown as effective preventive measures for the control of damaging pathogen of brassicas, allowing for 3 times less TuMV incidence.

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