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THE REACTION OF CABBAGE (*Brassica oleracea* L.) BREEDING LINES AGAINST *TURNIP MOSAIC VIRUS*

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Abstract. *Turnip mosaic virus* (TuMV) is the most important and widespread virus infecting brassicas worldwide. In 2013, 2014, and 2015, twenty-three cabbage (*Brassica oleracea* L.) breeding lines from Black Sea Agricultural Research Institute (BSARI, Samsun, Turkey) were screened for their reaction to TuMV-BA isolate by mechanical inoculation under controlled conditions. On the basis of 0–5 disease rating scale and ELISA, of the lines tested, nine (39.1%) were categorized as highly resistant (Grade 1), one (4.3%) as resistant (Grade 2), seven (30.4%) as moderately resistant (Grade 3), however, two cabbage lines and four lines were found moderately susceptible and susceptible, respectively to TuMV-BA isolate under controlled conditions. The results suggested that *Brassica oleracea* L. from the BSARI contain valuable breeding lines resistant to TuMV, and the screened breeding lines in the current study could provide promising resistance sources for cabbage breeding.

Key words: Brassicaceae, viral diseases, resistance, TuMV

INTRODUCTION

Brassica is one of the most valuable vegetable families and universally important in the world that includes broccoli, cabbage, cauliflower, Brussels sprouts, collard and kale [Farzinebrahimi et al. 2012]. Cabbage (*Brassica oleracea* L.) is the most economically important brassica crop grown in Turkey [Balkaya et al. 2005]. Viral diseases are commonly found and cause serious damage to this vegetable that lead to unmarketable produce. Species of the genus Brassica may be infected by various viruses. More than six different viruses have been reported to infect brassica crops worldwide [Maskell et al.

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1999, Raybould et al. 1999]. *Turnip mosaic virus* (TuMV), *Cauliflower mosaic virus* (CaMV), *Beet western yellows virus* (BWYV), *Cucumber mosaic virus (CMV), Turnip yellow mosaic virus* (TYMV), and *Radish mosaic virus* (RaMV) were the most frequently encountered viruses in brassica crops [Spak and Kubelkova 2003, Moreno et al. 2004]. Although at least six viruses are known to infect cruciferous plants, TuMV was found to be widespread in most of the cabbage-growing fields in Turkey [Korkmaz et al. 2008, Alan 2012].

Turnip mosaic virus (TuMV) is a member of the *Potyvirus* genus in the *Potyviridae* family with a wide host range and highly variable in its biological characteristics [Murphy et al. 1995]. Virions are 15–20 nm flexuous rods and are composed of 95% coat protein (CP) and 5% RNA [Walsh and Jenner 2002]. It occurs worldwide and is considered to be one of the most economically important viruses of field vegetables. In contrast to other potyviruses, which have relatively narrow host ranges, TuMV infects over 318 plant species in 156 genera of 43 plant families, including many important crops and weed plants [Edwardson and Christie 1991].

Aphid transmitted in the non-persistent manner, by at least 89 species, including *Myzus persicae* and *Brevicoryne brassicae* [Walsh and Jenner 2002], however, an effective insecticide has not yet been found to prevent aphids transmitting TuMV to plants. Therefore, it has been suggested that the best way to stop TuMV from infecting plants would be through natural resistance of plants to the virus [Walsh et al. 2002]. Natural plant resistance is likely to be the most effective and environmentally friendly method of controlling TuMV. Several accessions of *Brassica rapa* exhibiting broad-spectrum resistance to TuMV have been identified [Liu et al. 1996]. Recently identified resistances in *B. rapa* appear to be effective against a broad range of TuMV isolates [Rusholme et al. 2007].

Therefore, to evaluate and catalogue sources of TuMV resistant Brassica lines, twenty-three breeding lines of cabbage were screened by mechanical inoculation. The level of resistance to TuMV accumulation in cabbage leaf tissues was evaluated using a combination of visual symptom observations and Enzyme-linked immunosorbent assay (ELISA).

MATERIALS AND METHODS

This study was carried out cooperatively by the University of Ondokuz Mayis and the Black-Sea Agricultural Research institute (BSARI) in 2013–2015. All experiments were run using the greenhouses of the BSARI, Samsun, Turkey.

Plant material. Twenty-three different cabbage breeding lines were obtained from Black Sea Agricultural Research Institute (BSARI) Samsun, Turkey and were used for screening purpose (tab. 1). Seeds of these cultigens were planted in plastic pots inside a growth chamber (16 h light, 24°C; 8 h dark, 18°C) for inoculation tests.

Virus source and maintenance. A Turkish isolate of cabbage infecting *Turnip mosaic virus* (TuMV-BA) was used as virus source for mechanical inoculation in this study. TuMV-BA was isolated from white cabbage (*Brassica oleracea* var. *capitata*

subvar. *alba* L.) in the Bafra District of Samsun Province, Turkey in 2013. The virus was propagated and maintained in *B. oleracea* plants.

Table 1. Breeding lines used in the research and reaction of lines against TuMV in a climate--controlled room

Breeding lines	Infection index (1–5)	The mean ELISA absorbance values	Symptoms observed	Reaction type
W-7	1.2	0.104	NS	HR
W-8	1.5	0.081	M	HR
W-24	1.7	0.079	M	HR
W-29	4.0	0.154	M	S
W-34	3.2	0.104	SM, Mo, Y	MR
W-35	2.0	0.082	LC, N, D	HR
W-38	1.5	0.089	NS	HR
W-40	3.7	0.218	M, LD	MR
W-42	2.2	0.185	Mo	MR
W-43	2.0	0.176	M, LD	MR
W-44	2.2	0.111	M	MR
W-45	3.8	0.284	SM	MS
P-27	1.0	0.083	NS	HR
P-63	1.3	0.126	M, NLL	R
P-83	3.3	0.381	M, LC	S
P-87	2.2	0.174	M, LC, NLL	MR
P-93	1.8	0.192	M	MR
HBF-4/2	1.0	0.083	NS	HR
23/1	3.8	0.159	M, N	S
44-F3	4.0	0.496	SM, NLL	S
102-1	1.0	0.075	NS	HR
508-T	1.0	0.085	NS	HR
541	2.8	0.238	M, NLL	MS
Control	1.0	0.062	NS	–

M = mosaic, Mo = mottling, LD = leaf deformation, LC = leaf curling, Y = yellowing, SM = severe mosaic, NS = no symptom; NLL = necrotic local lesion, N = necrosis; HR = highly resistance, R = resistance, MR = moderately resistance, MS = moderately susceptible, S = susceptible

Plant inoculation process. Fresh symptomatic leaves of cabbage were harvested for use as inoculum sources at 2–3 weeks after TuMV inoculation and macerated in 0.01 M phosphate buffer, pH 7.0, in a pre-chilled mortar and pestle [Korkmaz et al. 2008]. The seedlings of each 15- to 21-day-old cabbage breeding line were lightly dusted with carborundum (600 mesh) and rub-inoculated with virus-infected sap (1:5) and grown for 45 days. The date of first symptom appearance and subsequent symptom development in non-inoculated upper leaves were recorded daily for 45 days.

Host response. Phenotypic data of host reaction was recorded in terms of symptom manifestation following mechanical inoculation on plants of each lines, placed under climate-controlled conditions four weeks post inoculation. The host reaction was recorded according to disease rating scale of Shah et al. [2011], and Gładysz and Hanus-Fajerska [2009] with some modifications. A modified version of (0–5) scale was adopted for the study and breeding lines were categorized in a five-degree scale as highly resistance (HR), resistance (R), moderately resistance (MR), moderately susceptible (MS) and susceptible (S) on the basis of host reaction and ELISA results. All plants in each breeding line were scored, and the ratings totalled and divided by the number of plants to give a disease index for the breeding line.

Testing lines for virus infection. Double Antibody Sandwich- ELISA tests, described by Clark and Adams [1977], were used for investigation of virus in leaves of cabbage breeding lines after four weeks of inoculation. Polystyrene 96-well plates and a polyclonal antiserum kit for TuMV (Bioreba AG, Switzerland) was used in the study. ELISA plates were coated (100 µl per well) with anti-TuMV antibodies diluted 1:1000 in coating buffer and incubated at 30°C for 4 h, followed by three washings at 3 min intervals with PBS plus 0.05% (v/v) Tween-20 (PBS-T). Samples for ELISA were prepared by grinding of leaf tissue in phosphate buffered saline, pH 7.4 with 2% PVP, 0.05% Tween-20 and 0.2% of bovine albumin, in the ratio 1/5 (w/v). Exactly 100 µl of the extracted sap of each sample was then added to the coated polystyrene plate and incubated overnight at 4°C. After washing ELISA plates with PBS-T, Alkaline phosphatase conjugated TuMV-specific antibodies in antibody buffer was added at a 1:1000 dilution and incubated at 30°C for 4 h. ELISA plates were washed with PBS-T and *p*-nitrophenyl phosphate (Sigma, 1 mg/ml) in 0.1 M diethanolamine buffer, pH 9.8 was added as a substrate. Plates were incubated at room temperature after pipetting the substrate buffer, and the absorbance values were read at 30, 60, and 120 min following the addition of substrate at 405 nm using a microplate reader (Tecan Spectra, Grodig/ Salzburg, Austria) and also confirmed visually after incubation for 2 h at room temperature. All samples tested in two replicate wells and the absorbance value greater than three times that of a negative control [Winiarczyk et al. 2014] and with a visually detectable yellow colour was rated as positive [Iqbal et al. 2012]. Commercial positive and negative controls (Bioreba) were included in TuMV ELISA kit.

Data analysis. All statistical analyses were done using the statistical software package SPSS v. 21.0 (SPSS, release V.21.0 for Windows; SPSS, Chicago, Illinois, USA).

RESULTS

Results on reaction of the twenty-three *B. oleracea* breeding lines against Turkish isolate of *Turnip mosaic virus* (TuMV) under controlled conditions are given in Table 1. Seventeen of the twenty-three cabbage breeding lines tested showed symptoms of TuMV including mosaic (fig. 1), mottling, yellowing, leaf curling, leaf deformation, necrotic local lesion (fig. 2), necrosis, and death of the plant (tab. 1). The lines W-7, W-38, P-27, HBF-4/2, 102-1 and 508-T, have not shown any symptoms and were found virus free after testing with DAS-ELISA against TuMV.

Fig. 1. *Brassica oleracea* var. *capitata* subvar. *alba* L. showing mosaic symptoms

Fig. 2. *Brassica oleracea* var. *capitata* subvar. *alba* L. showing necrotic spots symptoms

On the basis of disease rating scale (0–5) and ELISA tests, breeding lines were grouped as highly resistance (HR), resistance (R), moderately resistance (MR), moderately susceptible (MS) and susceptible (S). Of the lines tested, 9 (39.1%) were categorized as highly resistant, 1 (4.3%) as resistant, 7 (30.4%) as moderately resistant, 2 (8.6%) as moderately susceptible and 4 (17.3%) as susceptible (tab. 1).

The reaction of these cabbage breeding lines against TuMV isolate has been summarized in Table 1. Based on both disease rating scale and ELISA results indicated that breeding lines W-7, W-8, W-24, W-35, W-38, P-27, HBF-4/2, 102-1, and 508-T were

highly resistance (HR); P63 was resistance (R); W-34, W-40, W-42, W-43, W-44, P-87, and P-93 were moderately resistance (MR); W-45, and 541 were moderately susceptible (MS) and W-29, P-83, 23/1, and 44-F3 were susceptible (S).

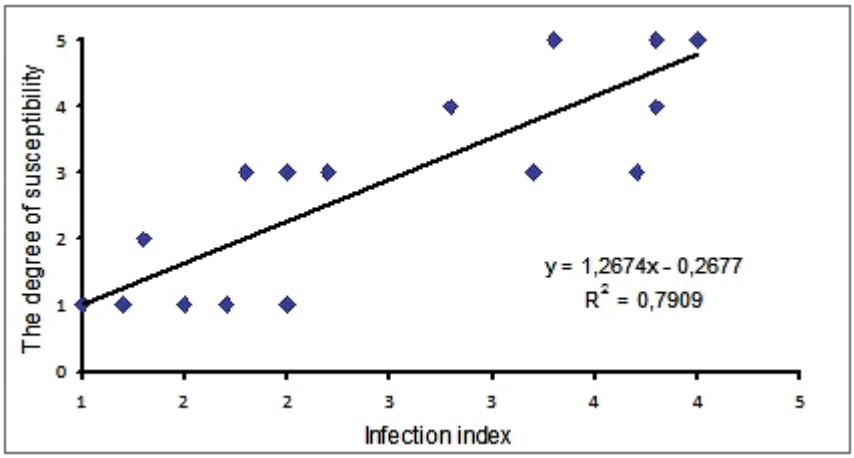

Fig. 3. Relationship between infection index and the degree of susceptibility to TuMV

There were significant differences ($p < 0.05$) among the breeding lines with respect to the degree of susceptibility to TuMV using the Tukey's Studentized Range (HSD) Test at $P < 0.05$. Multiple linear regression analyses showed that there was a positive correlation between infection index and the degree of reaction against TuMV (fig. 3).

DISCUSSION

Turnip mosaic virus (TuMV) is the most important and widespread virus infecting cabbage plants in Turkey [Korkmaz et al. 2008]. TuMV can cause serious epidemics and yield reductions in some years. Natural plant resistance is likely to be the most effective and environmentally friendly method of controlling TuMV. In this study, twenty-three breeding lines of *B. oleracea* var. c*apitata* were evaluated in the greenhouses of the BSARI, Samsun, Turkey in 2013–2014 for the source of resistance against TuMV-BA. The majority of tested breeding lines were resistant to TuMV. Nine out of the twenty-three *B. oleracea* lines tested showed highly resistance to TuMV-BA. One breeding line and seven lines were regarded as resistant and moderately resistant, respectively, based on both disease rating scale and ELISA tests. Resistance has been reported in a number of different species. Recently identified resistances in *Brassica rapa* appear to be effective against a broad range of TuMV isolates [Walsh et al. 2002]. A range of different extreme forms of resistance to TuMV have been found in *B. rapa* and *B. napus*. Most are dominant and effective against specific TuMV isolates/ genotypes [Walsh and Jenner 2002].

The breeding lines differed in their reaction to TuMV in the current study. This suggested susceptibility variations of the lines [Usta et al. 2014]. Similar work carried out by Gładysz and Hanus-Fajerska [2009] to evaluate the reaction of white cabbage cultivars to mechanical inoculation with selected isolates of the TuMV. The TuMV-CAR37A and TuMV-CAR39 isolates from horseradish proved to be infective towards 'Amager' and 'Langedijker'. Both tested cultivars showed a similar level of susceptibility. TuMV-CAR37A and TuMV-CAR39 can be useful in the selection of cabbage lines with resistance to TuMV.

In the present study, of the lines tested, two (W-45, and 541) breeding lines were detected as moderately susceptible and four (W-29, P-83, 23/1, and 44-F3) as susceptible. Similar results were reported by Pink and Walkey [1990] with 'Polinius F1' cultivar of white cabbage infected with TuMV-UK-NVRS isolate. *B. oleracea* subsp. *capitata* f. *alba* 'Amager' was also recognized as susceptible to TuMV isolates. Statistical analyses showed that there was a positive correlation between infection index and the degree of reaction against TuMV. Symptoms of virus infected plants show a linear correlation between symptom severity and the virus concentration [Shah et al. 2011].

The non-persistent mode of transmission for TuMV means that it is very difficult to control using insecticides because brief probes by aphids are enough to cause virus infection. Strategies for the management of viral diseases normally include control of vector populations using insecticides, use of virus-free propagating material, appropriate cultural practices and use of resistant cultivars [Ashfaq et al. 2007]. However, each of the above methods has its own drawbacks. The use of conventional phytosanitary practices is often inefficient against Potyviruses as they spread rapidly in the field through non-persistent transmission by aphids, therefore resistant cultivars remain the most economical and reliable method of control [Hughes et al. 2002, Shah et al. 2011].

CONCLUSION

Screening under controlled conditions with artificial inoculation with different isolates of the virus and pyramiding genes conferring resistance, will help in breeding for durable resistance against TuMV in *Brassica* spp. The tested breeding lines showed different levels of susceptibility to TuMV-BA isolate. The present findings suggest that the breeding lines showing resistance to the virus should be need to be maintained for further studies for locating resistance sources under field conditions and for genetic manipulations and breeding purpose.

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REAKCJA LINII HODOWLANYCH KAPUSTY (*Brassica oleracea* L.) NA *TURNIP MOSAIC VIRUS*

Streszczenie. *Turnip mosaic virus* (TuMV) jest najważniejszym i najbardziej rozpowszechnionym wirusem zakażającym kapustę. W latach 2013, 2014 i 2015 dwadzieścia trzy linie hodowlane kapusty (*Brassica oleracea* L.) z Czarnomorskiego Instytutu Badań Rolniczych (BSARI, Samsun, Turcja) przetestowano pod względem ich reakcji na izolat TuMV-BA za pomocą inokulacji mechanicznej w warunkach kontrolowanych. Na podstawie 0–5 skali oceny choroby oraz ELISA stwierdzono, że dziewięć (39,1%) z testowanych linii jest wysoce odporne (39,1%) (stopień 1), jedna (4,3%) jest odporna (stopień 2), a siedem (30,4%) jest umiarkowanie odpornych (stopień 3). Jednak stwierdzono, że – odpowiednio – dwie i cztery linie kapusty są umiarkowanie podatne i podatne na izolat TuMV-BA w warunkach kontrolowanych. Na podstawie wyników badania wnioskuje się, że *Brassica oleracea* L. z BSARI zawierają wartościowe linie hodowlane odporne na TuMV-BA, a linie hodowlane w niniejszym badaniu mogą być cennym źródłem odporności w uprawie kapusty.

Słowa kluczowe: Brassicaceae, choroby wirusowe, odporność, TuMV

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