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Evaluation of the infectivity of selected turnip mosaic virus isolates towards white cabbage cultivars

Karol Gładysz, Ewa Hanus-Fajerska***

*Department of Genetics, Plant Breeding and Seed Science
**Botany Department
Faculty of Horticulture, University of Agriculture in Krakow
29 Listopada 54, 31-425 Kraków, Poland
e-mail: karglad@ogr.ar.krakow.pl, ehanus@ogr.ar.krakow.pl

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ABSTRACT

Experiments were carried out to evaluate the reaction of cabbage cultivars to mechanical inoculation with selected isolates of the turnip mosaic virus (TuMV). Simultaneously we aimed for the assessment of TuMV pathogenicity towards cultivars chosen to be transformed in order to obtain the resistance trait. The TuMV-CAR37A and TuMV-CAR39 isolates from horseradish proved to be infective towards 'Amager' and 'Langedijker' *B. oleracea* subsp. *capitata* f. *alba.* The course of symptom expression was assessed and the results of virus detection in symptomless leaves, using DAS-ELISA, were documented. 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 the turnip mosaic virus.

INTRODUCTION

Brassica crops are grown worldwide, and cabbage (*Brassica oleracea* L.), a member of the *Brassicaceae* family (*Brassicaceae* Burnett, syn. *Cruciferae* Juss.), is a significant cash crop. This species, characterized by unusual form diversity, includes many horticultural yields belonging to several varieties with numerous cultivars. One of them is Dutch white cabbage (*B. oleracea* L. subsp. *capitata* (L.) Duchesne, f. *alba)*. Among countries belonging to the EU, Poland is the greatest producer of Dutch white cabbage, which has become a highly valued vegetable (Kaniszewski 2006).

Viral diseases are a significant threat to cabbage plantations due to the impossibility of constraining them through the use of chemical protection. At the same time, the turnip mosaic virus (TuMV) is ranked among the major pathogens infecting brassica crops, inducing a disease known as the mosaic of *Cruciferae*. TuMV has a very widespread host range, including many popular weeds and horticultural crops. The most important are oilseed rape, cauliflower, broccoli, cabbage, numerous ornamentals and medicinal herbs. The variability of isolates, relating to virulence and host range, implies the necessity of research concerning the susceptibility of individual cultivars to respective isolates of the turnip mosaic virus (Shukla et al. 1994, Robak and Wiech 1998, Ohshima et al. 2002, Walsh and Jenner 2002).

In Poland, the turnip mosaic virus is frequently diagnosed in different species and cultivars classified as part of the *Brassicaceae* family, especially in the *Brassica* species (Błaszczak 1968, Kobyłko and Maj 1989, Fiedorow 1993, Twardowicz-Jakusz 1999) and horseradish (Twardowicz-Jakusz et al. 1977, Kochman and Rondomański 1997, Kozubek et al. 2007). Due to the common occurrence of TuMV, infections in production plantations and in plants growing in natural environment are quite frequent. Successes in attempts to control TuMV spread by means of insecticides have proved to be very limited, because aphids transmit the virus in the nonpersistent manner. Additionally, it can be also spread by contact with regular agricultural cultivation treatments. Therefore, in order to reduce crop loss caused by TuMV infection, the only efficient solution is control through resistant cultivars. Biotechnological approaches rank among the most effective techniques in attempts to generate disease resistance in cultivated plants. Techniques of genetic engineering can be especially helpful in efforts to obtain lines with improved resistance to viral diseases. However, detailed knowledge about pathogens and transformed plant species is necessary in this case (Petri and Burgos 2005, Bonos et al. 2006).

In the study reported here we focused on the reaction of two Dutch white cabbage cultivars upon inoculation with TuMV isolates. Afterwards, they will be applied in transformation experiments. Cabbage cultivars were chosen because of

their good tolerance to contact with *Agrobacterium* and high regeneration ability *in vitro*, which is essential to obtain transgenic lines. The possession of properly selected isolates, which infect such cabbage genotypes, will allow resistance tests to be conducted in the population of transgenic plants.

MATERIAL AND METHODS

Plant material

Two cultivars of white cabbage (*B. oleracea* L. subsp. *capitata* (L.) Duchesne, forma *alba*) were assessed for their reaction to infection by the turnip mosaic virus (TuMV). The cultivars chosen were 'Amager' and 'Langedijker'. Seeds of each cultivar were sown into pots containing steam-sterilized horticultural soil. Plants were grown for eight weeks in an environmental chamber at 24/22°C day/night temperature, and a 16-h photoperiod with 100 μmol m^{-2} s^{-1} fluorescent illumination. As indicators used to control the inoculum infectivity *Nicotiana tabacum* L. 'Samsun' and *Chenopodium amaranticolor* Coste et Reyn. were selected. Afterwards, plants employed in the biological tests and cabbage plants were transferred to an isolated insect-proof glasshouse. The glasshouse temperature was 19-24°C, and regular routine insecticide spraying was conducted.

Virus material

The TuMV isolates used in this study, kindly provided by Dr. Lehmann (Institute of Plant Genetics, Polish Academy of Sciences, Poznań), CAR37A and CAR39, were obtained from horseradish *Armoracia rusticana* P. Gaertn., B. Mey & Scherb, syn. *Cochlearia armoracia* L. (Kozubek et al. 2007). The viral material was propagated by mechanical inoculation in susceptible line S6 of rapeseed (*Brassica napus* L.). The inoculum of each isolate respectively was prepared by grinding S6 rapeseed leaf tissue expressing clear symptoms of systemic infection (Fig. 1 c). Leaf tissue was grinded in a phosphate buffer: 100 ml H_2O dest. + 1.31 g $K_2HPO_4 \times H_2O$, pH = 7.5, using cooled mortar (1 g leaf tissue : 3 ml buffer solution). Inoculum prepared in the same way from healthy plants was used as the control. The cabbage plants were twice inoculated mechanically, first during a cotyledon stage, and afterwards in a three-leaf stage. Unclarified infective sap was used as the inoculum. Simultaneously, six *N. tabacum* 'Samsun' and *Ch. amaranticolor* plants were inoculated. The diagnostic verification was performed on local hosts and using enzyme-linked immunosorbent assay.

The scheme of the experiment

For each cabbage cultivar/TuMV isolate combination 20 plants were inoculated, and six were used as the control. The entire experiment was conducted twice. For

each experiment regular observations were done for 140 days, in order to detect potential symptoms and growth disorders of the plants. Symptoms were scored in a three-degree scale, describing occurrence intensity of necrotic spots and mosaics: (1) a few small necrotic spots and/or mosaics on single leaves of inoculated plants, (2) 3-10 sites of necrotic spots and/or mosaics on 2-3 leaves, (3) more than 10 sites of necrotic spots and/or mosaics on more than four leaves.

Six weeks after the first inoculation, the double antibody sandwich (DAS-ELISA) procedure was applied to detect TuMV presence in young cabbage leaves not showing any detectable symptoms, which were taken from all inoculated plants. Tests were performed with a Loewe Biochemica GmbH TuMV complete commercial kit (cat. no 07049C/96). Incubations with polyclonal antibodies and conjugate were carried out at 37°C for four hours each. The absorbance was measured at 405 nm using the Labsystem Multiscan MS microplate reader model 325.

RESULTS AND DISCUSSION

The results of the infectivity tests in leaf tissue samples verified on *Nicotiana tabacum* 'Samsun' and *Chenopodium amaranticolor* were clear necrotic spots occurring on inoculated leaves from five to 14 days after inoculation (Figs 1 a and b), whereas cabbage plants were symptomless for about five weeks, and clear symptoms were observed 40 days after inoculation as necrotic spots visible on leaves, sometimes accompanied by mosaics (Fig. 2). In comparison to the control there were no disorders of plant growth or even leaf shape regardless of cultivar/TuMV isolate combination.

The number of infected plants and average intensity of symptoms expressed in the three-degree scale is presented in Table 1. From the moment of symptom appearance they demonstrated a rather stable level for the next eight weeks, but 5.2 percent of the plants lost their distinct mosaic symptoms during the following period. In next 20 percent of the plants expressing symptoms in newly grown leaves, free from necrotic spots, the only symptoms observed was mild mosaic attenuated with time. Only a part of the material tested using DAS-ELISA was shown to be infected (Table 2). In 'Amager' TuMV presence was detected in 42.5 percent of plants inoculated with CAR37A isolate and in 50 percent of plants inoculated with CAR39 isolate. For the 'Langedijker' cultivar the percentage of infected plants was 35 and 50, respectively, for CAR37A and CAR39 isolates.

Fig. 1. Symptoms on the leaves of: a) *Nicotiana tabacum* 'Samsun', b) *Chenopodium amaranticolor*, c) *Brassica napus* S6

Fig. 2. Symptoms on *Brassica oleracea* 'Langedijker'

Brassica plants were proven to be susceptible to TuMV, which is counted among the most important pathogens infecting vegetable crops, as infection with the turnip mosaic virus is usually connected with serious yield reductions (Błaszczak 1968, Tomlinson 1987, Kobyłko and Maj 1989, Shattuck 1992, Fiedorow 1993, Twardowicz-Jakusz 1999). As far as TuMV resistance research is concerned, the important point is the possibility to exclude false positive results caused by an improperly done selection of isolates.

Table 1. Evaluation of the pathogenicity of TuMV-CAR37A and TuMV-CAR39 isolates in white cabbage plants, scored six weeks after the first inoculation

Cultivar	Isolate			
	CAR37A		CAR39	
	Number of inoculated plants / Number of infected plants	% infected / Infection index	Number of inoculated plants / Number of infected plants	% infected / Infection index
'Amager'	40/15	37.5/1.4	40/18	45.0/1.7
'Langedijker'	40/13	32.5/1.6	40/19	47.5/1.8

Table 2. Infectivity of TuMV-CAR37A and TuMV-CAR39 isolates towards two cultivars of white cabbage evaluated with DAS-ELISA conducted six weeks after the first inoculation

Isolate		Cultivar	
	Evaluated parameters	'Amager'	'Langedijker'
CAR37A	Number of inoculated plants / Number of infected plants	40/17	40/14
	% infected	42.5	35.0
	Min. abs. / Max. abs.	0.015/2.8	0.04/0.13
CAR39	Number of inoculated plants / Number of infected plants	40/20	40/20
	% infected	50	50
	Min. abs. / Max. abs.	0.018/2.2	0.15/2.8

This threat is a serious problem brought about by the large diversity among TuMV pathotypes (Stavolone et al. 1998, Oshima et al. 2002). In this study, the infectivity of two turnip mosaic virus isolates with a good molecular background was evaluated. Their nucleotide sequences are available in genebank accessions DQ648591 and EF374098 for isolate 37A and 39, respectively (Kozubek et al. 2007). It is also necessary to take into consideration the solid leaf structure of cabbage, what is an obstacle for efficient inoculation with a viral pathogen (Jan et al. 2000). Therefore, to make inoculation with carborundum powder easier, the first inoculation was conducted onto cotyledons of 14 day-old seedlings. The results of the experiment proved that 'Langedijker' and 'Amager' were cultivars susceptible to both tested TuMV isolates. Six weeks after inoculation, symptoms of infection were observed in approximately 50 percent of the tested population. 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 (Pink and Walkey 1986). In order to evaluate the susceptibility level, in addition to such factors as inoculum concentration and genotype describing host properties, the growth phase of plant material during inoculation and environmental factors – mostly temperature and lightning – should also be considered. The next problem is the slow development of infection and the virus uneven distribution in the plants, so cabbage can be a symptomless host of TuMV for months (Walkey and Pink 1988). Symptoms of the disease develop rapidly during cold storage, making the cabbage heads unmarketable (Walkey and Webb 1978, Shattuck 1992). This could significantly affect the symptoms observed, and the obtained number of infected plants. Another reason for uneven infection can be population character of both tested cultivars; their genetic diversity might have an influence on the diversity of plant/pathogen interaction. Observations carried out on inoculated plants indicate a slow development of the disease and limited spatial distribution of the virus in plant tissues, even if TuMV infection resulted in characteristic symptoms, like necrotic spots and mosaics, corresponding to those observed by other authors (Pink and Walkey 1990, Hunter et al. 2002). Another point is that the intensity of the symptoms and the course of a disease could be different when observed in the field, because infection with TuMV usually makes cabbage more prone to coinfection by other pathogens, resulting in greater damage to the crop (Hardwick et al. 1994, Hunter et al. 2002, Spence et al. 2007). It can be concluded that both tested TuMV isolates were infective to 'Amager' and 'Langedijker' cabbage cultivars. Even though in the early phase of plant growth we did not observe a high pathogenicity level of the isolates used, they can be employed in the selection for resistance of material with inserted Nib gene fragments from the turnip mosaic virus. It is recommended that the observation period should be extended to the harvest time.

CONCLUSIONS

1. White head cabbage infection with TuMV-CAR37A and TuMV-CAR39 resulted in symptom expression not before 40 days after inoculation.
2. The tested cultivars showed similar levels of susceptibility to TuMV-CAR37A and TuMV-CAR39 isolates.
3. The absorbance level detected with DAS-ELISA was not correlated with an intensity of symptoms in the tested populations.

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OCENA INFEKCYJNOŚCI WYBRANYCH IZOLATÓW WIRUSA MOZAIKI RZEPY W STOSUNKU DO ODMIAN UPRAWNYCH KAPUSTY GŁOWIASTEJ BIAŁEJ

Przeprowadzono doświadczalną inokulację mechaniczną izolatami wirusa mozaiki rzepy z zamiarem oceny podatności odmian uprawnych kapusty głowiastej białej *B. oleracea* subsp. *capitata* f. *alba* 'Amager' oraz 'Langedijker'. Jednocześnie prowadzono ocenę patogeniczności izolatów TuMV-CAR37A i TuMV-CAR39 w stosunku do odmian mających posłużyć do uzyskania linii transgenicznych wykazujących podwyższony stopień odporności na TuMV. Izolaty TuMV-CAR37A i TuMV-CAR39 otrzymane z roślin chrzanu w początkowym okresie wzrostu wegetatywnego infekowały badane odmiany, wywołując objawy chorobowe na porażonych roślinach bądź bezobjawowo. Przy użyciu testu DAS-ELISA wykazano porażenie jedynie części materiału, przy czym wartości absorbancji nie były skorelowane z intensywnością występowania objawów chorobowych ocenianej populacji.

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