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ELIMINATION OF *TOMATO MOSAIC VIRUS* (ToMV) FROM PEPINO (*SOLANUM MURICATUM*) PLANTS

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Abstract

The effect of thermotherapy combined with meristem tip culture on elimination of *Tomato mosaic virus* (ToMV) from pepino plants was examined. Heat-treatment duration (day 38°C and 36°C night) was established for 40, 60, 80 and 100 days. High temperature was applied to young plants and to older plants (in the bush stage) of two pepino clones (clone No. 3, No. 6) exploited for the breeding program of new cultivars of pepino. From among 721 excised explants 560 plantlets were regenerated and 26 of them were virus free. Plantlets were indexed by DAS-ELISA. The effect of the prolonged thermotherapy duration (over 60 days) on virus elimination was not observed.

Key words: pepino, ToMV, thermotherapy, meristem tip culture

Introduction

Pepino (*Solanum muricatum*) is a small, perennial woody shrub which produces large fruits with a sweet smell, subtle flavour, and attractive yellow/golden skin colour, often with purple stripes. The original cultivation of *S. muricatum* extended along the Andes, from southern Colombia to Bolivia and the Peruvian coast. Commercial crops produced with advanced technology are known in Chile, Mexico and United States but also in New Zealand (Pepino 1989). The species *S. muricatum* displays wide intraspecific variability. Morphological variation is evident in the division of the leaf lamina, the pubescence of the stems and leaves and the shape, colour and consistency of the fruit. Physiological variation has been detected in the formation of the fruits and seeds, since there are certain biotypes that produce fruits after pollination and contain fertile seeds and others which, owing to the sterile pollen, form parthenocarpic fruit without seeds. Because usually pepino does not produce plants that are true-to-type from seed, so it must be propagated

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vegetatively (Prohens et al. 1996). The trials on *S. muricatum* cultivation in Poland and the breeding programmes on selecting new clones and maybe new cultivars have been undertaken at the Department of Vegetable and Medicinal Plants, Warsaw University of Life Sciences (Kowalczyk and Kobryń 1999, 2000, 2003). Unfortunately, all plants of the most interesting and promising clones of pepinos used in the Polish breeding programme expressed disease symptoms (mosaics and deformations of leaves) resembling those caused by viruses. There are several viruses and one viroid described on pepinos: *Pepino mosaic virus, Tomato mosaic virus, Alfalfa mosaic virus, Cucumber mosaic virus, Potato leafroll virus, Potato virus S, Tobacco mosaic virus, Tomato spotted wilt virus* and *Potato spindle tuber viroid* but only the first two are considered most important by plant breeders and pathologists (Prohens et al. 1998, Pérez-Benlloch et al. 2001, Descriptors... 2004). During the previously conducted investigations on affected pepino plants it was found that they harboured *Tomato mosaic virus* – ToMV (*Tobamovirus*) and the viral infection was confirmed by bioassays and the immunoelectronmicroscopic techniques (Szyndel, unpublished results, 2004/05). For the breeding purposes we decided to use thermotherapy and meristem tip cultures to clean the infected clones of pepino. Prohens et al. (1996) effectively used thermal seed disinfection and sodium hypochlorite treatment to inactivate seed-borne ToMV in affected pepino seeds but our generatively propagated *S. muricatum* clones show very high genetic variability. For some breeding programmes as well as for commercial production of pepinos many different techniques of micropropagation of those plants were used (Pauli et al. 1988, Pierik 1997, Pérez-Benlloch et al. 2001, Leiva-Brondo et al. 2006) including meristem tip cultures (Cai et al. 1999).

The main aim of this work was elimination of ToMV from selected pepino clones to obtain virus-free mother stock plants which could be used in breeding programmes of pepino plants performed in the Department.

Materials and methods

Two different clones from the Department of Vegetable and Medicinal Plants' collection of *S. muricatum* (clone No. 3 originated from New Zealand and clone No. 6 originated from Israel that had been grown for a few seasons on the greenhouse benches) were selected for experiments according to their suitability to further breeding programme.

The cuttings were taken from stock plants and after rooting in perlite planted to the pots with peat substrate. Thermotherapy was applied to plants in two growths stages:

1) in the young stage (10–15 cm height), 25 plants from each of clone,
2) in the bush stage (45–50 cm height), 10 plants from each of clone.

All plants selected for the experiment displayed typical mosaic symptoms on leaves and presence of ToMV infection was confirmed by DAS-ELISA using Loewe Biochemica GmbH kits and procedures of test recommended by kits' producer.

For heat treatment the plants were kept in growth chamber at 38°C – day temperature with 12 h irradiance light 200–250 μE/m^2/s and at 36°C during night. After exposure to the alternating temperatures for a period of 40, 60, 80 and 100 days, shoots fragments with terminal buds were taken from the plants and surface disinfected with 70% ethanol, 10% solution of Domestos (sodium hypochlorite) and then rinsed with sterile distilled water. The meristem tip explants with primordial leaves (0.1–1.0 mm long) were isolated under stereoscopic microscope. The explants were cultured on MS medium (Murashige and Skoog 1962) with 0.1 mg/l BA (6-benzylaminopurine) at temperature of 24°C and 16-hour-long day with irradiance light 160 μE/m^2/s and 8 h night. After four weeks the healthy-looking and viable explants were transferred to MS medium without plant growth regulators. The plantlets obtained *in vitro* were tested for the presence of ToMV using DAS-ELISA as above. Indexing was performed three times, starting from the moment when we had enough plant tissue to perform a test, and it was repeated every five weeks of plantlets growth. The percentage of developed plants and, from those of virus-free plants, was calculated. Test χ^2 with Statgraphics Plus 4.1. software was used for testing the hypothesis of independence of classifications.

Results

The younger plants survived only 40 days of heat treatment. The older plants were more resistant to long lasting thermotherapy. However, at the beginning of thermotherapy the leaves and some terminal buds died, so that the number of meristem tip explants available was restricted. After 40 and 60 days of heat treatment the older plants continued growth but some changes in their appearance were observed. The plants formed a great number of side branches with small-sized leaves.

After 40 days of thermotherapy the survival of plantlets obtained from meristem tips depended on the pepino clone and the age of plant ($\chi^2(3) = 12.69$; $\alpha = 0.01$). More plantlets were obtained from clone No. 6 in both combinations from younger (81.7%) and older plants (83.8%), respectively (Table 1). But after this period of thermotherapy many meristem tips formed non-regenerating callus. The highest rate of contaminated meristem cultures (16.4%) was observed in explants obtained from younger plants of clone No. 3 (Table 1).

A great number of meristem cultures obtained from the older plants after 40 and 60 days of heat treatment produced only callus, particularly in clone No. 3 (Table 1). After 80 and 100 days of thermotherapy over 90% of isolated meristem tips regenerated plantlets. The total number of necrotic and contaminated cultures obtained from the older plants was very low, 2.9% and 1.0% respectively. The ratio of the obtained plantlets number to the number of isolated meristem tips after 40 and 60 days of thermotherapy was lower than after the longer thermotherapy period (Table 1).

Table 1

The survival of plantlets obtained by meristem tip culture from two pepino clones after different duration of thermotherapy

Age of plants	No. of clone	Duration of heat treatment (days)	The number of cut meristems	The number and percentage of			
				necrotic meristems	contaminated cultures	callus non-regenerating	obtained plantlets
Young plants*	3	40	73	5 (6.8%)	12 (16.4%)	7 (9.6%)	49 (67.2%)
	6	40	131	6 (4.6%)	4 (3.2%)	14 (10.7%)	107 (81.7%)
Total			204	11 (5.6%)	16 (7.8%)	21 (10.3%)	156 (76.5%)
Old plants**	3	40	54	2 (3.7%)	1 (1.9%)	17 (31.5%)	34 (62.9%)
		60	59	2 (3.4%)	1 (1.7%)	27 (45.8%)	29 (49.1%)
		80	63	2 (3.2%)	0	9 (14.3%)	52 (82.5%)
		100	60	1 (1.7%)	0	3 (5.0%)	56 (93.3%)
	Total		236	7 (3.0%)	2 (0.8%)	56 (23.7%)	171 (72.4%)
	6	40	68	6 (8.8%)	0	5 (7.4%)	57 (83.8%)
		60	70	1 (1.4%)	0	10 (14.3%)	59 (84.3%)
		80	85	1 (1.2%)	1 (1.2%)	1 (1.2%)	82 (96.4%)
		100	58	0	2 (3.4%)	3 (5.2%)	53 (91.4%)
	Total		281	8 (2.8%)	3 (1.1%)	19 (6.8%)	251 (89.3%)
Total			517	15 (2.9%)	5 (1.0%)	75 (14.5%)	422 (81.6%)
Total			721	26 (3.6%)	21 (2.9%)	96 (13.3%)	578 (80.2%)

*Plants in the young stage (10–15 cm height).
**Plants in the bush stage (45–50 cm height).

Generally, the regeneration of plantlets from excised meristems was very high (80.2%) and the survival of the obtained plantlets depended significantly on pepino clone and heat treatment duration as was verified and confirmed by the chi-square test ($\chi^2(9) = 47.43$; $\alpha = 0.01$). The highest number of plantlets obtained from meristem tips was observed for clone No. 6 after thermotherapy duration of 40 days and longer than 60 days.

There was no correlation between the pepino clone, age of treated plants or duration of heat treatment and the number of received *Tomato mosaic virus* free plants in meristem tip cultures, although the highest number of healthy plants was obtained after 40 days of thermotherapy (Table 2). The greatest number of all healthy plants was obtained from the young plants from clone No. 6 after 40 days of thermotherapy (10 virus-free plants per 103 tested) but also very good results were obtained for older plants from clone No. 6 (7/54). Higher number of healthy plants was obtained from clone No. 6 than from clone No. 3. The duration of thermotherapy over 60 days did not increase the efficiency of virus elimination from infected pepino plants.

Table 2

The efficiency of ToMV elimination from young and old plants of two pepino clones by heat-therapy and meristem tip culture

No. of clone	Duration of heat treatment (days)	The number of tested plantlets		The number and percentage of obtained ToMV-free plantlets	
		young plants*	old plants**	young plants*	old plants**
3	40	49	35	2 (4.7%)	1 (2.9%)
	60	0	26	0	2 (7.7%)
	80	0	50	0	1 (2.0%)
	100	0	52	0	0
Total		49	163	2 (4.7%)	4 (2.4%)
6	40	103	54	10 (9.7%)	7 (13.0%)
	60	0	57	0	2 (3.5%)
	80	0	81	0	0
	100	0	53	0	1 (1.9%)
Total		103	245	10 (9.7%)	10 (4.1%)
Total		152	408	12 (7.8%)	14 (3.4%)

*Plants in the young stage (10–15 cm height).
**Plants in the bush stage (45–50 cm height).

Discussion

Heat therapy and meristem tip culture have been known for long as methods of virus elimination from infected plants (Hollings 1965, ten Houten et al. 1968, Quak 1972, Walkey 1991). The viruses, including tobamoviruses, were found to be present in meristem tips of plants (Toussaint et al. 1984). Therefore, a combination of heat therapy and meristem tip culture was used in our experiments, which is a common practice in such situation. Prohens et al. 1998 suggested that regeneration of virus-free plants from meristems obtained from heat-treated pepino plants was rather difficult. Confronted with this statement, our results seem to be successful. We excised 721 meristem tips from heat-treated pepino plants and we obtained 578 plantlets that regenerated from these meristem cultures. Many of them, namely 560, grew into plants that could be tested for ToMV presence. Only 26 of them (4.6%) were shown to be virus-free. This could have been the effect of heat therapy or a result of a situation in which not all the meristem tips of infected plant had obviously to be infected (Hull 2004).

The aim of our work was not to elaborate a good method of *Tomato mosaic virus* control in pepino. We were aware of the opinion that the best strategy against the virus is to develop new virus-resistant pepino cultivars (Prohens et al. 1998). As an example 'Turia' is a salad pepino cultivar tolerant to *Tomato mosaic virus* (Rodríguez-Burruezo et al. 2004). At present there are no pepino cultivars registered in Poland. On the other hand, we have some promising pepino selections and clones

at the Department of Vegetable and Medicinal Plants of WULS but they were totally infected with ToMV. Trying to eliminate the virus by heat treatment and meristem tip culture seemed to be the only way of obtaining healthy breeding material (Walkey 1991) for further breeding work. This will open the prospects to create some breeding programmes directed to obtain pepino cultivars resistant to most important pepino viruses causing substantial losses in pepino fruit yields and quality (Prohens et al. 1999, Prohens and Nuez 1999, Pérez-Benlloch et al. 2001, Leiva-Brondo et al. 2006).

Prolonged heat treatment (longer than 40 days) caused death of explants excised from treated plants, especially in the case of young plants. Still, it is possible to use the higher temperature (up to 42°C) for a shorter time (four–five weeks). Some heat treatment programmes could make it possible to cut bigger shoot-tips for culture which could improve the survival of the explants without decreasing the efficiency of virus elimination (Quak 1972, 1977, ten Houten et al. 1968, Walkey 1991, Cai et al. 1999, Hull 2004).

Streszczenie

UWOLNIENIE ROŚLIN PEPINO (*SOLANUM MURICATUM*)
OD WIRUSA MOZAIKI POMIDORA (*TOMATO MOSAIC VIRUS*, ToMV)

Aby uzyskać rośliny pepino (*Solanum muricatum*) wolne od wirusa mozaiki pomidora (*Tomato mosaic virus*, ToMV), dwa klony pepino (nr 3 i nr 6), szczególnie ważne w prowadzonym programie hodowlanym, poddano zabiegowi termoterapii, a następnie zastosowano hodowlę stożków wzrostu. Termoterapię (dzień: 38°C, noc: 36°C) prowadzono przez 40, 60, 80 i 100 dni, traktując rośliny w fazie rozsady (1) lub starsze, silnie rozkrzewione (2). Z tak traktowanych roślin wycinano stożki wzrostu (721 sztuk) do hodowli na pożywkach MS i uzyskano 560 zregenerowanych roślin, z których 26 było wolnych od ToMV. Nie zaobserwowano korzystnego wpływu przedłużania termoterapii, powyżej 60 dni, na efektywność eliminowania wirusa.

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