IDENTIFICATION OF *LYCOPERSICON* SPP. HYBRIDS ON THE BASIS OF MORPHOLOGICAL AND MOLECULAR (RAPD) PROPERTIES AS WELL AS EVALUATION OF RESISTANCE TO TOMATO SPOTTED WILT VIRUS (TSWV)

ZBIGNIEW RUSINOWSKI[1], MIECZYSŁAW ŚMIECH[1]
MARIA KAMIŃSKA[2], KATARZYNA NIEMIROWICZ-SZCZYTT[1]

[1] Department of Plant Genetics, Breeding and Biotechnology
Warsaw Agricultural University
Nowoursynowska 166, 02-787 Warszawa, Poland

[2] Research Institute of Pomology and Floriculture
Pomologiczna 18, 96-100 Skierniewice, Poland

(Received: August 6, 2001. Accepted: April 9, 2002)

ABSTRACT

The aim of the study was to evaluate the plants obtained as a result of cultivated tomato crosses with wild species meant to transfer resistance to TSWV.

Six viable plants were obtained from *L. esculentum* × *L. chilense* and *L. esculentum* × *L. peruvianum* crosses after the application of in vitro embryo culture. In terms of such morphological traits as growth habit of plants, size and shape of leaves, the length and colour of internodes in branching stems, the plants displayed intermediate traits, resembling, nonetheless, the wild form. RAPD analysis with 8 primers revealed that all the hybrids had bands typical of the paternal forms. This confirms the paternal component in hybrid development. As far as the resistance to Polish TSWV isolates is concerned, two hybrids exhibited a high level of resistance, similar to negative control, three hybrids – enhanced resistance and one hybrid was susceptible to TSWV infection.

Key words: *Lycopersicon* hybrids, source of resistance, molecular markers, DAS-ELISA test.

INTRODUCTION

For many years tomato spotted wilt virus (TSWV) affects the cultivation of tomato and other species world-wide. One of the methods to obtain resistance to the virus is the crossing of cultivated tomato with wild species of *Lycopersicon*. The crossing of *Lycopersicon* is difficult due to incompatibility barriers. One of the necessary conditions of a successful cross is the application of wild species as paternal form, with the exception of *L. pimpinellifolium* which produced F_1 hybrids being a maternal parent (Mutschler and Cobb 1985). The resistance to TSWV was reported in *L. chilense* and *L. peruvianum* (Czuber and Miczyński 1981; Stevens et al. 1992; Segeren et al. 1993; Stevens et al. 1994; Cho et al. 1996; Miczyński et al. 1997). Embryo abortion is a serious obstacle to obtain hybrids of *L. esculentum* × *L. peruvianum* or *L. chilense*. However, with the use of embryo rescue or bridge crosses some experiments proved to be successful (Smith 1944; Poysa 1990; Kozik and Dyki 2001). The results of compatibility studies on three wild species of *Lycopersicon* have been published recently (Kozik and Dyki 2001). The experiments confirm that it is possible to obtain fruits, seeds and hybrid plants from the combination of *L. esculentum* × *L. chilense*. However, out of the three wild species (*L. pennelli*, *L. hirsutum* and *L. chilense*) *L. chilense* was the most difficult parental component for crosses. In the combination *L. esculentum* × *L. chilense* only 12% of the cross pollinated flowers set fruits, all of which had 2-4 normally developed seeds per fruit. In total, 20 normal hybrid seeds were obtained and half of them germinated. Hybrid plants produced seeds and plants after self-pollination (Kozik and Dyki 2001).

The aim of our study was to determine whether the plants obtained from *Lycopersicon esculentum* × *L. peruvianum* and *L. esculentum* × *L. chilense* crosses were indeed hybrids and to assess their resistance to tomato spotted wilt virus. Nowadays the characteristics of hybrids includes not only their morphological description, but also molecular evaluation. In case of tomato especially RAPD technique (Random Amplified Polymorphic DNA) has been applied for variation assessment and genetic identification (Williams and St-Clair 1993; Rus-Kortekaas et al. 1994).

TABLE 1. Accessions of *Lycopersicon* spp. used for interspecific crosses. Source: Tomato Genetics Resources Centre, University of California, Davis.

No.	Species	Accessions	Origin
1.	*L. peruvianum* var. Chumifusum	LA-2151	Morochupa, Cejamarco, Peru
2.	*L. peruvianum*	LA-444	Chinccha, Ica, Peru
3.	*L. peruvianum*	LA-111	Supea, Lima, Peru
4.	*L. chilense*	LA-1963	Rio Caplina Tacana, Peru
5.	*L. esculentum* „Rey de Los Tempranos"	LA-2356	South America
6.	*L. esculentum* 'Stevens × Rodade'	'ST'	Vegetable & Ornamental Plant Resarch Institute, Pretoria, Africa
7.	*L. esculentum* 'Potentat'	'PT'	Old Danish cultivar

Preliminary evaluation of plant resistance to viruses was previously done by the bio-assay and observation of symptoms on inoculated plants (Czuber and Miczyński 1981). However, more reliable results can be obtained with serological tests, such as modified enzyme linked immunosorbent assay (ELISA) (Kamińska and Korbin 1992; Korbin and Kamińska 1994; Kamińska 1995).

MATERIALS AND METHODS

Plant material

The experiments discussed in this study employed *L. esculentum* Mill., *L. peruvianum* Mill. and *L. chilense* Dun. (Table 1). Wild species accessions selected for the experiments have been reported to be resistant to TSWV. The plants obtained from the accessions were subjected to DAS-ELISA test and their resistance was verified. The variety *L. esculentum* 'Potentat' ('PT'), sensitive to the familiar TSWV isolates, served as control. All the accessions but 'ST' and 'PT' were supplied by CM Rick, Tomato Genetics Resources Centre, University of California, Davis, USA. Highly homozygous 'ST' population was obtained by courtesy of professor J. Starck from Warsaw Agricultural University.

The crosses were carried out on the plants grown in the greenhouse and *L. esculentum* served as the maternal component. One day prior to the pollination, the stamens were removed from the flower buds of the female parent. The pollination was performed the following day in the morning and was repeated after 24 hours. The seeds were extracted from the fruits 30 days after the pollination. Single large seeds, comparable in size to the self-pollinated *L. esculentum* control, were selected for further in vitro culture. After the sterilisation in calcium hypochlorite, the coat was opened carefully with a scalpel so as not to damage the embryo and the seeds were transferred to the MS medium (Murashige and Skooge 1962) containing half a dose of macro-elements without NH_4N0_3 and a full dose of micro-elements and vitamins (12% sucrose). Germinated plantlets were placed for further development in 330 ml glass jars containing 30 ml of MS basal medium supplemented with 10 g l^{-1} sucrose, 0.75% agar (Bacto Agar), pH 5.8, in a 16-h per day phtotochamber with temperatures set at 25°C (light intensity 54 E $m^{-2}s^{-1}$ provided by fluorescent tubes). Plants were multiplied in vitro and transferred to the greenhouse for observations and inoculation. All the experimental plants were cultivated in insect-proof greenhouse at 18-25°C.

TSWV resistance test

The tomato plants were mechanically inoculated twice: the first time when the seedlings had 3-4 true leaves; the second inoculation took place after 7 days. The leaves of infected tobacco plants *N. tabacum* 'Samsun', *N. rustica* or from tomato were used as a source of inoculum.

In order to enhance the response to virus inoculation, the plants were kept in darkness for 48 hours prior to the inoculation. The TSWV isolates (A1+B1 and G2) used in this study were collected during inspection of Polish greenhouses. The virus isolates were maintained in *N. rustica* plants by mechanical inoculation. Systemically infected leaves, showing mild symptoms were harvested 14 days after inoculation. The inoculum was prepared by grinding the leaves (1 : 5) in 0.02 M phosphate buffer (pH=7.5) containing 1% sodium sulphate. Three weeks after the first inoculation plant observations were made.

The following scale was adopted: 1 – non-visible symptoms, 2 – chlorotic leasions, 3 – leaf epinasty, 4 – necrotic leasions, 5 – leaf decay. The plant observations were verified using direct DAS-ELISA test according Korbin and Kamińska (1994). IgG and TSWV conjugate were produced in Research Institute of Pomology and Floriculture – Skierniewice (Korbin and Kamińska 1994; Korbin 1995).

The absorbance value was recorded using Multiscan II reader (Labsystems) with 405 nm filter. The score was judged positive if the absorbance value (A_{405}) was higher than the mean negative control plus 3 times standard deviation. The test was repeated twice.

RAPD analysis

The leaves for DNA isolation were taken from young plants grown in optimal greenhouse conditions and frozen in liquid nitrogen. DNA was isolated from 4 gm tissue according to the procedure by Van der Beek et al. (1992) with some modifications.

PCR reaction was conducted in Perkin Elmer thermocycler. The 25 µl volumes of the mixture contained 35 ng template DNA, 25 ng starter, 1,5 units of Taq polymerase, 100 µM of each dNTP, 50 mM KCl, 10 mM Tris-HCl, 1,5 mM $MgC1_2$. It was overlaid with 2 drops of mineral oil. The amplification proceeded in the following conditions: 1 cycle – 3 min. at 94°C (DNA denaturation), 45 cycles consisting of 1 min – 94°C (DNA denaturation), 1 min – 35°C (attaching the starter), 2 min – at 72°C (synthesis of complementary DNA), 1 cycle – 10 min at 72°C (completion of DNA synthesis). The amplification products were separated on 1.5% agarose gel, stained with ethidium bromide and photographed in UV light.

The analysis of DNA polymorphism of all the materials with RAPD method was made using the starters from Ramson Hill Bioscience Inc. and polymerase from MBI Fermentas.

RESULTS

Crossing of cultivated and wild TSWV resistant Lycopersicon species

In the crosses with *L. esculentum* (three cultivated forms: RT, ST, PT) a plant from one *L. chilense* accession (LA-1963) and from three *L. peruvianum* accessions (LA-111, LA-444, LA-2151) was used (Table 1). As a result of 852 crosses 259 fruits were obtained which means 30% of successful pollination. Only 58 fruits contained large seeds (Fig. 1). Out of these seeds 11 plants were grown, however, only 6 were capable of further growth until maturity. The highest number of viable hybrids was obtained from *L. esculentum* cv. Potentat × *L. chilense* (LA-1963).

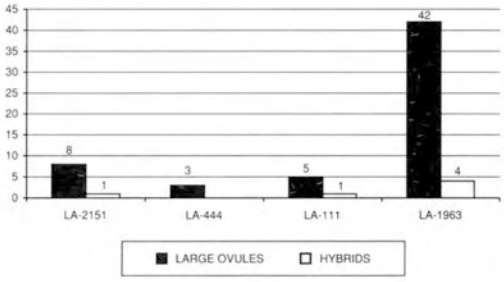

Fig. 1. The number of enlarged ovules and hybrids obtained from crosses *L. esculentum* × *L. chilense* and *L. peruvianum*.

Morphological description of the hybrids

The growth habit of the hybrids resembled that of the wild parental form. It was manifest particularly in the traits of the leaves, inflorescence, shoots and hairs (Table 2). The last were numerous in the hybrids which is typical of the wild parental forms. The majority of the hybrids had approximate hair length on the lower surface of the leaf. Most hybrids (except of no 3) formed additional longer hairs on the nerves, which were, nevertheless, shorter than those of cultivated tomato.

All the plants but hybrid no 6 had a number of morphological traits in common with wild tomato species and some traits typical of *L. esculentum*. The leaf blade shape of the hybrids was similar to that of the wild species or had intermediate features. The leaves of hybrids no 1, 2 and 4 revealed the closest similarity to *L. chilense* (Fig. 3). The fruits of hybrids were also more related to *L. chilense* (Fig. 4). The first two of the above mentioned hybrids additionally displayed growth habit closely resembling male parent *L. chilense*. The wild tomato plant from *L. chilense* LA-1963 as well as two hybrids derived from it, exhibited a considerable tolerance to the growth conditions in the greenhouse.

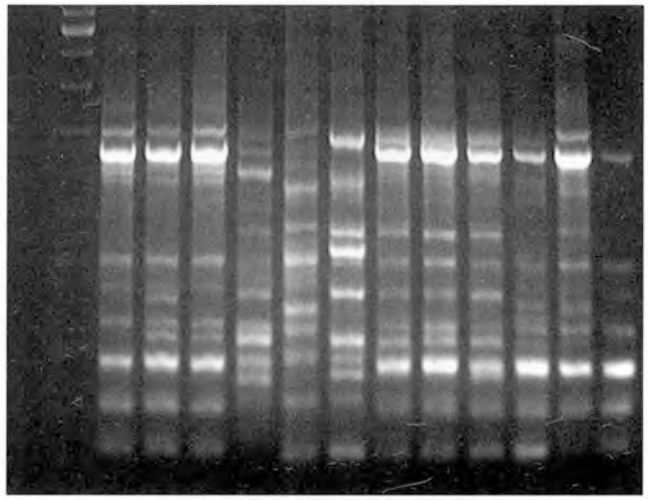

Fig. 2. Amplification profiles obtained with the use of M9 primer: maternal forms (from the left) – 'ST', 'RT', 'PT'; paternal forms – LA-2151, LA-111, LA-1963, hybrids – 2, 1, 4, 3, 5, 6.

The hybrids obtained in these experiments produced viable pollen grains and seeds. The seeds germinated, although the percentage of the viable seeds differed depending on the hybrid.

Molecular traits (RAPD) of the hybrids

RAPD analyses were carried out to confirm the hybrid origin of the plants from interspecific crosses. Eight primers, noted for a large repetitiveness of the results and observed to differentiate *L. esculentum* in the previous experiments, were used (Table 3). All the hybrids were found to have bands typical of the female and male forms *L. chilense* or *L. peruvianum* (Fig. 2).

In 48 combinations (6 hybrids × 8 primers) only one band characteristic of the male form was observed in 17 cases. In 19 combinations 2 specific bands were noted,

TABLE 2. Morphological properties of *L. esculentum* × *L. chilense* and *L. peruvianum* hybrids (mean of 10 plants measurements).

Hybrid cod number	Shape	Branch stem		Leaves		Fruits		Seeds germination***
		Length (cm)	Color*	Leaf shape	Length (cm)	Diameter (cm)	Color**	
1	*L. chilense*	150	vg	*L. chilense*	25	2.0	o	n
2	*L. chilense*	170	vg	*L. chilense*	35	2.0	w	n
3	intermediate	70	bg	intermediate	15	2.8	o	n
4	*L. chilense*	30	vg	*L. chilense*	27	3.1	o	p
5	*L. peruvianum*	180	gy	intermediate	25	1.3	o	p
6	*L. esculentum* 'ST'	50	bg	*L. esculentum* 'ST'	30	6.4	r	n

* vg violet-green, lg – light green, gy green-yellow
** o – orange, w – white, r – red
*** n – normal, p – poor

Fig. 3. Leaves of paternal forms and the hybrid (from the left: *L. esculentum* cv. Potentat, hybrid no 2, *L. chilense* (LA-1963). The hybrid leaf exhibits intermediate traits of parental forms.

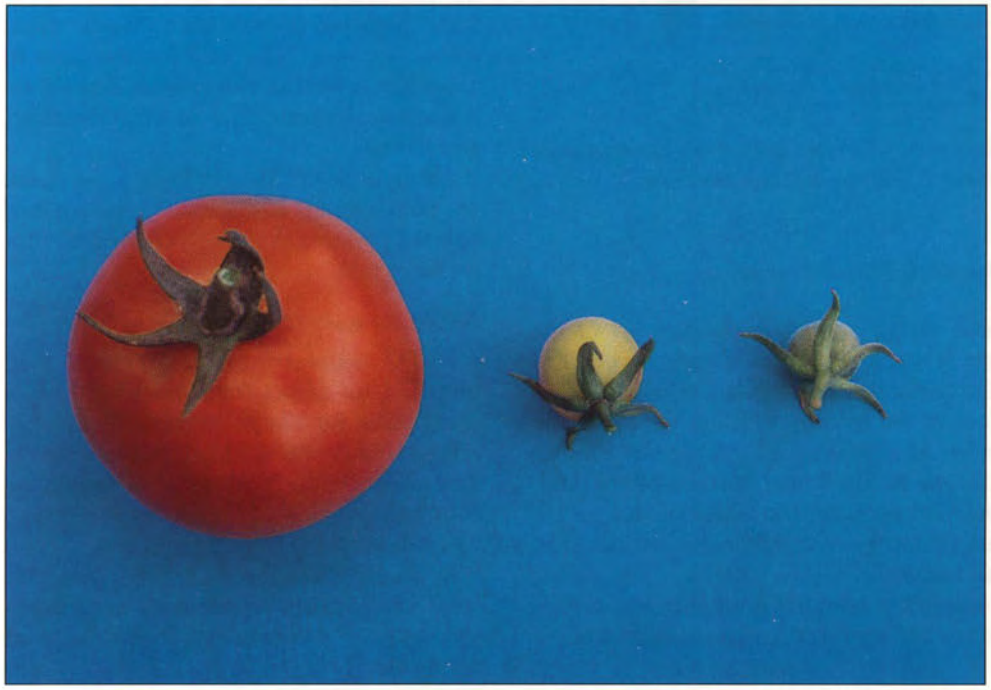

Fig. 4. Fruits of parental forms and the hybrid (from the left: *L. esculentum* cv. Potentat, hybrid no 3, *L. chilense* (LA-1963). Hybrid fruit slightly bigger than that of wild form (shown on the right side of the photograph).

while 3 or 4 DNA fragments, typical of paternal component, were found in 5 and 3 combinations respectively. The greatest number of DNA amplification products enabling to determine the hybrid origin of the plants was generated by G5 primer in three hybrids (no 1, 2 and 4).

Hybrid resistance

The study showed that one (no 5) out of six tomato hybrids mechanically inoculated with TSWV isolates became infected and developed systemic brown lesions 10 days after inoculation. The other inoculated tomato hybrids did not develop disease symptoms. In tomato plants 'Potentat' (positive control) inoculated with TSWV isolates local symptoms appeared 3-5 days after inoculation; systemic 5-7 days later.

Experiments with TSWV infected tomato hybrids indicated that virus serological activity measured in DAS-ELISA was differentiated depending on the tested plants and virus

TABLE 3. Number of bands in hybrids typical of the paternal *L. chilense* and *L. peruvianum* obtained in RAPD analyses with eight primers.

Hybrid cod number	Male forms	Primers							
		M19	M9	G5	K16	B19	MM19	J17	A12
1	*L. chilense* LA-1963	2	2	4	1	1	2	1	3
2	*L. chilense* LA-1963	2	2	4	2	1	2	3	2
3	*L. chilense* LA-1963	1	1	2	3	0	1	1	2
4	*L. chilense* LA-1963	1	2	4	2	2	2	2	3
5	*L. peruvianum* LA-2151	0	2	0	1	0	1	1	2
6	*L. peruvianum* LA-111	3	1	1	1	1	1	2	2

TABLE 4. Values of A_{405} DAS-ELISA estimated for selected isolates and hybrids in four series of tests.

Hybrid cod number	Parents		Plant seedling (A_{405} DAS-ELISA)			
			Isolate A1+B1		Isolate G2	
	female	male	I	II	III	IV
1	*L. esculentum* 'PT'	*L. chilense* LA-1963	0.189	0.184	0.251	0.243
2	*L. esculentum* 'PT'	*L. chilense* LA-1963	0.460*	0.483*	0.413*	0.381*
3	*L. esculentum* 'PT'	*L. chilense* LA-1963	0.328*	0.279*	0.322*	0.240
4	*L. esculentum* 'PT'	*L. chilense* LA-1963	0.364*	0.250	0.176	0.190
5	*L. esculentum* 'RT'	*L. peruvianum* LA-2151	1.998*	0.732*	1.918*	2.029*
6	*L. esculentum* 'ST'	*L. peruvianum* LA-111	0.187	0.176	0.198	0.184
positive control			2.024*		1.565*	
negative control			0.176			

* positive result

isolate (Table 4). The A_{405} of TSWV reached the highest value in plants of 'Potentat' (positive control) and hybrid no 5 showing symptoms of virus infection. In spite of lack of visible symptoms, the increased A_{405} was also stated in hybrids no 2,3 and 4 especially inoculated with isolates A1+B1. The A_{405} of other tested hybrids was on the level of negative control.

DISCUSSION

The interspecific crosses resulted in 6 viable hybrids which were planted in the greenhouse. Their morphological traits were evaluated and found to be intermediate in comparison with parental forms, on the whole, however, wild form traits prevailed.

Literature findings as well as the present study, report the occurrence of hybrids exhibiting intermediate traits in comparison with their parents after *L. esculentum* × *L. peruvianum* cross (Segeren et al. 1993). Plant variation was observed in the size and shape of the leaves, hairs, the size and colour of the fruits.

Our observations on the hybrid fertility are similar to those made by Kozik and Dyki (2001). However, there are other reports in the literature pointing out to some difficulties with obtaining seeds (Segeren et al. 1993). Self-incompatibility of hybrids (*L. esculentum* × *L. chilense*) was evaluated (Martin 1961) on plants obtained in vitro. It was found out that there are two dominant genes regulating self-incompatibility. On the other hand, it is known that some plants of *L. chilense* are self-incompatible, while others are not (Barten 1992).

The interspecific cross character of the plants was confirmed after the application of RAPD analysis with the use of 8 primers, which were selected because they generated polymorphism in cultivated tomato (female parents). In the DNA amplification profiles of the hybrids additional bands, typical of *L. chilense* and *L. peruvianum,* were observed, which indicates the paternal contribution in hybrid production.

Although it is not easy to select the primers which can generate polymorphic bands in tomato, the analysis made within this experiment proved effective and gave clear results.

In the experiments a few hybrids resistant to Polish isolates of TSWV were obtained. The resistance ranged from the high level of negative control (two instances) to susceptibility (one hybrid). However, even the non-resistant plant grown over a longer period of time in the greenhouse did not exhibit very severe symptoms and manifested only slight brown necrotic lesions. In similar conditions the plants of a sensitive cultivar Potentat were strongly infected and shoved pronounced symptoms. The resistance confirmed by these experiments was only verified for the two Polish isolates so that further tests are needed with isolates from other countries. Taking advantage of a relatively high fertility of hybrids it will be possible to cross them with cultivated forms in order to transfer the genes of resistance.

It can be supposed that the resistance factors in our hybrids differed from those transferred by Stevens et al.

(1992) from *L. peruvianum* because the source material was of different origin.

ACKNOWLEDGMENTS

This study was financially supported by the Polish State Committee for Scientific Research (KBN) project No 5 PO6A 004 16.

LITERATURE CITED

BARTEN J.H.M. 1992. Breeding for resistance to sweetpotato whitefly and tomato mottle geminivirus. Proceedings for the Tomato Quality Workshop and Tomato Breeders Roundtable. Sarasota, FL, University of Florida, pp. 72-73.

CHO J.J., CUSTER D.M., BROMMONSCHENKEL S.H., TANKSLEY S.D. 1996. Conventional breeding: host-plant resistance and the use of molecular markers to develop resistance to tomato spot wilt virus in vegetables. Acta Hort., 431: 367-378.

CZUBER B., MICZYŃSKI K., 1981. Reaction of cultivars and some wild species of tomato to infection by tomato spotted wilt virus. Zesz. Probl. Post. Nauk Roln. 244: 109-116.

KAMIŃSKA M., KORBIN M. 1991. The occurrence of tomato spotted wilt virus in polish glasshouses. Proc. Conf. Polish Plant Virologists, Warsaw, 19-20 September 1991. Phytopatologica Polonica. XII: 9-14.

KAMIŃSKA M. 1995. Delectability of tomato spotted wilt virus in mechanically inoculated tomato and pepper by DAS-ELISA. Phytopathol. Pol. 11: 135-141.

KORBIN M. 1995. Charakterystyka i wykrywanie izoltów wirusa brązowej plamistości pomidora (TSWV) występujące w Polsce. PhD thesis. Research Institute of Pomology and Floriculture Skierniewice. (in Polish)

KORBIN M., KAMIŃSKA M. 1994. Detection of tomato spotted wilt virus using DAS-ELISA and indirect F (ab')2-ELISA. Phytopathol. Pol. 8: 79-85.

KOZIK E., DYKI B.2001. Compatibility studies in three wild species of *Lycopersicon*. Acta Physiol. Plant. 23: 65-72.

MARTIN F. W. 1961. The inheritance of self – incompatibility in hybrids of *Lycopersicon esculentum* Mill. × *L. chilense* Dunn. Genetics 46: 1443-1454.

MICZYŃSKI K., KOBYŁKO T., CZUBER B., CYBULARZ T., GAJEWSKI Z., HANUS-FAJERSKA E. 1997. Screening of wild tomato species to infections with isolates of tomato mosaic, cucumber mosaic, and tomato spotted wilt virusess, occuring in the region of Krakow. Folia Horticulture 9: 51-52.

MURASHIGE T., SKOOG F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol. Plant. 15: 473-497.

MUTSCHLER M.A., COBB E. 1985. Crosses of *Lycopersicon penellii* and *L. esculentum* using *L. penellii* as female parent. TGC Raport 35: 14.

POYSA V. 1990. The development of bridge lines for interspecific gene transfer between *Lycopersicon esculentum* and *L. peruvianum*. Theor. Appl. Genet. 79: 187-192.

RUS-KORTEKAAS W., SMULDERS M.J.M., ARENS P., VOSMAN B. 1994. Direct comparison of levels of genetic variation in tomato detected by a GACA-containing microsatellite probe and by random amplified polymorphic DNA. Genome 37: 375-381.

SEGEREN M.I., SIQUEIRA W.J., SONDAHL M.R., LOURENCAO A.L., MEDINA FILHO H.P., NAGAI H. 1993. Tomato breeding: 2. Characterization of F1 and F2 hybrid progenies of *Lycopersicon esculentum* × *L. peruvianum* and screening for virus and insect resistance. Revista Brasileira de Genetica. 16: 773-783.

SMITH P. G. 1944. Embryo culture of tomato species hybrid. Proc. Amer. Soc. Hort Sci. 44: 413-416.

STEVENS M.A., RICK C.M. 1986. Genetics and breeding. In The tomato crop. Atherton J.G. and Rudish J. Chapman and Hall, London, New York, pp. 35-109.

STEVENS M.R., SCOTT S.J., GERGERICH R.C. 1992. Inheritance of a gene for resistance to tomato spotted wilt virus (TSWV) from *Lycopersicon peruvianum* Mill. Euphytica 59: 9-17.

STEVENS M.R., SCOTT S.J., GERGERICH R.C. 1994. Evaluation of seven *Lycopersicon* species for resistance to tomato. Euphytica 80: 79-84.

VAN DER BEEK J.G., VERKERK R., ZABEL P., LINDHOUT P., 1992. Mapping strategy for resistance genes in tomato based on RFLPs between cultivars: Cf9 (resistance to *Cladosporium fulvum*) on chromosome 1. Theoretical and Applied Genetics 84: 106-112.

WILLIAMS C.E., ST-CLAIR D.A. 1993. Phenetic relationships and levels of variability detected by restriction fragment length polymorphism and random amplified polymorphic DNA analysis of cultivated and wild accessions of *Lycopersicon esculentum*. Genome. 36: 619-630.

IDENTYFIKACJA MIESZAŃCÓW *LYCOPERSICON* SPP. NA PODSTAWIE CECH MORFOLOGICZNYCH I MOLEKULARNYCH (RAPD) JAK RÓWNIEŻ OCENA ODPORNOŚCI NA WIRUSA BRĄZOWEJ PLAMISTOŚCI POMIDORA (TSWV)

STRESZCZENIE

Celem pracy była ocena roślin powstałych w wyniku krzyżowania pomidora uprawnego z dzikimi gatunkami dla przeniesienia odporności na TSWV.

Po zastosowaniu kultury zarodków in vitro otrzymano sześć żywotnych roślin z krzyżowania *L. esculentum* × *L. chilense* i *L. esculentum* × *L. peruvianum*. Pod względem takich cech jak pokrój roślin, wielkość i kształt liści, długość i barwa pędów bocznych rośliny wykazały cechy pośrednie, bliższe jednak formom dzikim. Analiza RAPD z użyciem 8 starterów wykazała, że wszystkie mieszańce miały prążki charakterystyczne dla form ojcowskich. Wskazuje to na udział formy ojcowskiej w rozwoju mieszańca. Wysoki poziom odporności na polskie izolaty TSWV zbliżony do kontroli negatywnej wykazały dwa mieszańce, trzy podniesioną odporność a jeden nie wykazał odporności, był na poziomie kontroli pozytywnej.

SŁOWA KLUCZOWE: mieszańce *Lycopersicon*, źródło odporności, markery molekularne, test DAS-ELISA.