STUDIES ON THE REACTION IN TISSUE CULTURE OF TOMATO GENOTYPES UNDER BIOTIC STRESS

EWA HANUS-FAJERSKA

Botany Department, Horticulture Faculty, Agricultural University
Al. 29 Listopada 54, 31-425 Kraków, Poland

(Received: April 20, 2000. Accepted: August 7, 2000)

ABSTRACT

Plant regeneration in vitro from virus-infected somatic tomato (*Lycopersicon* sp.) tissue was performed. Regeneration experiments were started after the determination of virus presence, using enzyme-linked immunosorbent assay, in leaves used as a source of explants. Leaf explants infected with selected strains of tomato mosaic Tobamovirus or cucumber mosaic Cucumovirus respectively, were cultured on a standardised MS agar medium to induce adventitious shoots, which were afterwards excised, rooted in vitro and cultured to plants. Explants were also screened for their ability to produce callus. Diverse effects of viral infection, ranging from stimulation to inhibition of callus formation and of morphogenesis rate, were observed. The health condition of the tissue proved to affect regeneration potential of *Lycopersicon esculentum*, whereas wild accessions did not react in that case so distinctly. In cultivated tomato was encountered the decline in competence to reproduce shoots adventitiously in infected tissue. There was also relationship between donor plant health condition and adventitious root formation in regenerated shoots. Experiments with short-term cultures of *L. esculentum* revealed also that a certain number of shoots regenerated from diseased tissue can be virus-free.

KEY WORDS: micropropagation, morphogenesis, somatic tissue, viral infection, biotic stress, tomato.

INTRODUCTION

Crops are often subjected to infection by pathogenic agents. In cultivated tomato (*Lycopersicon esculentum* Mill.) virus infections have always been an important cause of reductions in yield and of poor fruit quality. In tomato crop usually are detected mechanically transmitted tobamoviruses, among them most often – tomato mosaic, and from cucumovirus group – cucumber mosaic, whose ubiquitous occurrence is enhanced by natural transmission by aphids (Marchoux and Gebre Selassie 1992; Pelet and Hasler 1992; Watterson 1994). Breeding for disease resistance in tomato by traditional methods is rather difficult because of poor crossability with primitive species and rather small genetic reservoirs for natural resistance, especially as far as CMV-resistance is concerned (Gebre Selassie et al. 1993). Fortunately, virus resistance can also be introduced into crop plants via alternative methods, and one of proposed approaches is somaclonal variation strategy. The perpetuation of a genotype in tissue culture depends upon the regularity in growth and development of cultivated explants, so when plant tissue is removed from the stabilizing environment of the intact organism and plunged in artificial medium, any imperfection or occasional irregularity in these genetically ordered processes, or even simply metabolic adjustments to cope with the stress condition, may provide the basis for variation and evolution in such cell populations (Larkin and Sowcroft 1981; Montagno et al. 1989; Ducreux et al. 1991). Taking into account that regeneration via organogenesis of tomato somatic tissues is well documented (Sink and Reynolds 1988; Koblitiz 1991), present investiga-

tion focused on studying in what extend the presence of selected isolates of tomato mosaic virus (ToMV) or cucumber mosaic virus (CMV) respectively, will affect their regenerative potential, mainly organogenic capacities. The further objective would be, to find out if the regeneration process in chosen models, starting from leaf segments can be a source of virus-resistant clones.

MATERIAL AND METHODS

As plant material cultivars of *Lycopersicon esculentum*: Moneymaker, Potentat and Rutgers, known to be susceptible, *L. esculentum* Perou 2, carrying the gene Tm-2, and selected accessions of *L. peruvianum*, *L. glandulosum* and *L. chilense* were used. Tomato plants were grown in greenhouse. Primary leaves of young seedlings were mechanically inoculated with the isolate FvYM of cucumber mosaic Cucumovirus from *Hosta ventricosa* (Kobyłko, 1996), or tomato mosaic Tobamovirus isolate from *Physalis franchetti* (race 0-1, as determined using Pelhams method) (Bednarek et al. 1983; Kobyłko 1998) respectively. Unclarified infective sap was used as inoculum. Infectivity of ToMV and CMV in tissue samples was demonstrated on local hosts. The virus was detected in leaves before explant excision and then in regenerated shoots, using enzyme-linked immunosorbent assay (DAS-ELISA).

Leaf pieces were taken out from about 6 week old plants raised in the greenhouse, sterilized and cultured on modified Murashige-Skoog agar medium in order to regenerate shoots (Murashige and Skoog 1962; Lech et al. 1996). Explants were

isolated from the leaves precisely after the detection of virus in their tissues and from leaves of healthy tomato plants simultaneously. The cultures were incubated at 24°C under white fluorescent light ($80 \mu\text{mol m}^{-2}\text{s}^{-1}$) with 16 h photoperiod and were transferred every three weeks onto fresh medium. For each experiment, data on percent explants developing callus and morphological descriptions of induced callus including: size, colour and texture, were recorded. Appropriate data concerning morphogenesis were recorded and micropropagation coefficients were calculated for every tested species during the whole course of the experiment. Rooting was performed in vitro, still on MS supplemented with $0.25 \mu\text{M}$ IAA (van den Bulk et al. 1990). The regenerated plantlets were transferred to the soil and cultured to plants.

Experiments were carried out in 20 replications for virus-infected and 20 replications for healthy material. Each replication was one Erlenmeyer flask with seven explants. Selected results were evaluated statistically, using ANOVA.

RESULTS

Each lump of tissue had produced callus from their periphery, not before eight days after placing the explants upon nutrient medium. Overall, callus induced was compact and brown in cultivated tomato, whereas greenish in wild accessions. Callus initiation on explants of *L. esculentum* 'Money-maker' and 'Rutgers' infected with CMV isolate occurred at about the same time as on the control healthy explants, but at other instances of cultivated tomato a retardation of callogenesis for about a week was observed. There was no visible differences between infected and healthy-control tissue in wild accessions reaction to the propagation medium used, their callus was rather similar in the proliferation rate, appearance and organization, except that usually infected tissue had revealed a certain dissimilarity in pigmentation. As far as cultivars are concerned, healthy and infected tissue responded differently to the new environment. The common feature of most healthy callus tissue was relatively stable proliferation, whereas callus from virus infected *Lycopersicon esculentum* tissue exhibited both increase and decrease in the rate of growth, depending on the host/virus model used in the experiment. Cultivar 'Potentat' infected with ToMV was characterized, as an instance, by stimulation of callus formation in preliminary stage of cultivation irrespective light conditions employed (Fig. 1). On Figure 1 are demonstrated sharply outlined differences in fresh weight of both 'Potentat' (shown as *L. escul.*) and *Lycopersicon glandulosum* (shown as *L. gland.*) during successive weeks of cultivation of infected and non-infected (healthy) tissues respectively. In contrast, cucumber mosaic virus infection of *L. esculentum* usually significantly inhibited the growth of proliferating callus tissue. The results of ex-

Fig. 1. Comparison of the fresh weight increase in ToMV-infected and healthy callusing tomato explants.

TABLE 1. The response of proliferating *Lycopersicon esculentum* explants to the biotic stres.

<i>L. esculentum</i> material	Diameter of callus-forming explants [cm]		
	Money-maker	Potentat	Rutgers
CMV-infected	1.09* a	1.62 d	1.51 c
healthy	1.22 b	1.82 e	1.59 cd

*Mean from 20 replications (140 explants); P=0.05

plant diameter measurements showing mathematically proved differences between virus-infected and healthy tissues are summarized in Table 1. There were either observed differences in the morphological aspects of such callus tissues, referring to their structure and pigmentation or even vitrification, eventually necrotization ('Money-maker'/CMV; 'Perou'/ToMV), whereas healthy tissue had rather uniform appearance.

At least two weeks after the isolation of the explants, rapidly growing primordia emerged from the superficial cells of callus mass and the formation of differentiated structures has begun. Precisely, differentiation of cultivated somatic tissue started with the appearance of meristematic nodules, distributed irregularly over the surface of callus, which usually become the centres of shoot growth, but in infected tissue might have also been induced to form roots. Still, shoot buds predominated, however expanded shoots exhibited not only the range of types typical of these organs but there have been noted cases of extremely anomalous structures differentiated from infected tissue. As suggestive example can serve Figure 2, where on the right are shown leaf-like structures observed on cultures infected with cucumber mosaic virus derived from cultivar 'Money-maker'. Regenerative potential was distinctly higher in wild accessions (Fig. 3) and there were no evident differences in morphogenic capacities between virus-infected and control healthy tissues. To all examined tomato varieties the rate of buds development on infected tissue was different from those, observed in healthy control. In this case, formation of shoots started mainly after third week of cultivation in vitro, and after fourth one a certain number of shoots on diseased material was much higher than those on healthy series. First after five weeks rate of growth of healthy shoots gradually surpassed that of the diseased material. Nevertheless at model: Money-maker/CMV even after six weeks of cultivation the number of diseased explants with longest shoots exceeded always that of healthy explants (Fig. 4).

Fig. 2. Distinct shoots morphology on CMV-infected (right side) and healthy (left side) tissue of *Lycopersicon esculentum*.

Fig. 3. Comparable stage of regeneration of *Lycopersicon chilense* (20), *L. glandulosum* (17), *L. peruvianum* (4) accessions and of *Lycopersicon esculentum* Perou (22), Potentat (P).

Fig. 4. Number of explants isolated from cv. Moneymaker with regenerated shoots of given height, during successive weeks of cultivation.

The another aspect, considered here, was the possible correlation between the health condition of the tissue and the regeneration potential of somatic tissue. There were observed evident differences in regeneration capabilities between healthy and infected tissues of *L. esculentum* (Fig. 5). In this case, again the reaction of wild accessions was more uniform, even so not quite similar. Especially CMV infection induced a significant decrease in number of regenerated shoots of: 'Moneymaker', 'Potentat' and 'Rutgers' (Table 2).

TABLE 2. The effect of cucumber mosaic virus infection on regeneration potential in *Lycopersicon esculentum*.

cultivar	material	number of regenerated shoots	micropropagation coefficient
Moneymaker	CMV-infected	350	2.53* c
	healthy	504	3.55 d
Potentat	CMV-infected	238	1.66 b
	healthy	364	2.63 c
Rutgers	CMV-infected	98	0.74 a
	healthy	182	1.34 b

* Mean from 20 replications (140 explants); P=0.05

Fig. 5. Adequate phase of virus-infected (right side) and healthy (left side) *Lycopersicon esculentum* tissue cultivation on regeneration medium.

Fig. 6. Spontaneous rhizogenesis on virus-infected callus (right side). On the left healthy control.

Also noteworthy is necrotic reaction observed in differentiated buds and expanded shoots of *L. esculentum* Perou infected with tomato mosaic virus. There was also observed a phenomenon of spontaneous rhizogenesis during cultivation on the propagation medium at 16% of CMV-infected and 9% of healthy explants of cultivar 'Moneymaker' and at 5% of diseased explants of 'Rutgers' (Fig. 6). On the contrary – after transfer of excised from this tissue shoots onto the rooting medium, the initiation of roots on diseased material have usually occurred some weeks later. Early rhizogenesis was characterised by the development of small nodules on the surface of shoot basis put into the rooting medium. In 37% of infected shoots callus proliferation was first observed and only after regeneration of adventitious roots has begun. A part of such shoots has developed roots only from callus. In such cases it took almost six weeks more to form plantlets. The number of diseased shoots developing roots during the course of experiment was significantly smaller, so the rooting percentage of such shoots dramatically declined, in some cases such differences were statistically proved (Table 3).

This observations were followed by ELISA tests of representative probe of newly formed shoots. It should be stressed, that in *Lycopersicon esculentum* short-term cultures not all the shoots regenerated from diseased tissue were found to be infected. It is consistent with 'Moneymaker'/CMV model in the culture condition employed. Rooted shoots were

TABLE 3. Percentage of rooted *Lycopersicon esculentum* shoots in cucumber mosaic virus-infected and healthy cultures.

material	cultivar		
	Moneymaker	Potentat	Rutgers
CMV-infected	49.4 a	69.5 b	73.7 b
healthy	100.0 c	96.0 c	97.2 c

Means followed by the same letter do not differ significance ($P=0.05$)

transferred to vermiculite-containing pot, and when regenerated plantlets were about 10 cm, they were transferred to the greenhouse. Plants derived from virus-infected and healthy cultures demonstrated distinct acclimatization responses, those obtained from virus-free material have exhibited greater survival percentage.

DISCUSSION

The excision and cultivation of tissue formed from quiescent cells, promote changes in their structure and metabolism, afterwards is followed by a phase of active synthesis, which give rise to promotion of cell divisions. Divisions are initiated by plant growth substances and specific proteins action, and a mass of undifferentiated cells is formed. Those proteins, that participate in forming, stabilizing and guiding the cell plate to correct division site have recently been identified. Callus induction usually takes place along the wounded fragments of explant, and similary dedifferentiation of leaf tomato explants was first observed along their cut edges. In the course of callus development, its growth rate may either be rapid or slow and restricted to the wounded area, depending on genotype, induction medium and the physiological status of donor plant (Yeoman and Forche 1981; Sylvester 2000). Inhibition of callus proliferation in tomato leaf cucumber mosaic virus-infected tissue and on the other hand ToMV influence on initially increased *L. esculentum* 'Potentat' callus growth do not seem unusual, considering that number of microorganisms that interact with plants use the same hormonal signals as plants, modifying endogenous hormone balance. Proliferation, mediated by growth factors, is subjected of that growth factor stimulation presence, so the response of cultured tissues was surely influenced by changes in endogenous levels of growth regulating substances (Hagege 1993; Gaudin et al. 1994).

Cultured tissues despite being isolated from physiological control of the donor plant, are able to differentiate and form organs de novo. The main objective of the reported experiments was precisely to study thoroughly the organogenetic responses of tomato tissues subjected to viral infection. Indirect obtention of plantlets on solid media is well documented in tomato. Leaf tissue proved to be pretty well responsive and was therefore frequently used as a source of explants (Zenkteler 1972; Padmanabhan et al. 1974; Behki and Lesley 1976; Tal et al. 1977; Kurtz and Lineberger 1983; Dwivedi et al. 1990; Selvi and Khader 1993; Villers et al. 1993; Lech et al. 1996). The effect of viral infection on host plants has also been studied thoughtfully; viruses are usually reported to be an important limiting factor of plant growth (Sequeira 1973; Grant and Mansfield 1999). During the past several years have been made studies of many biotrophic parasites in tissue cultures, but such cultures seldom respond to infection by sharply outlined changes in growth rate, like was observed in the case of Moneymaker, Potentat, Rutgers/CMV models.

Organ formation in callus is preceded and accompanied by numerous processes, both at the biochemical and the morphological level. Virus-infected and healthy tissues were not alike both in the trend to meristemization and the dynamics of organogenesis. Examination of possible reactions created by stress inducing viral infection of tomato has revealed that it may affect the developmental process and regeneration potential of cultures, in other words, there is a relationship between donor plant health condition, callus initiation on excised explants, its proliferation rate, morphogenic potential and even adventitious roots formation in regenerated shoots. As a rule, it has been possible to notice distinct differences between healthy and infected tissue, especially excised from *Lycopersicon esculentum* genotypes. Infected cultures have to certain degree lost the ability to differentiate and to produce organized structures or have demonstrated a certain shift in the morphogenic pattern. The expected negative effect of viral infection were even in some cases proved by statistical analysis. For the organized development of a plant tissue the differentiation of any one cell must be integrated with the others so that information in the form of chemical material must be transmitted. Process of virus replication interferes with the information which is exchanged between nucleus and cytoplasm, and similary as each kind of stress, stimulate the alteration of the expression of genetic programme in plants. Obviously the condition of culture and other non genetical factors are also very important, because they might facilitate or prevent the manifestation of genom encoded information (Attfield and Evans 1991; Gaspar 1995). That it was mainly the manipulation of the growth regulators level, resulting from infection of donor plants, which had an effect on cell competence for regeneration seems to be obvious, as undisturbed development was the rule in healthy cultures, while in infected there were observed dramatic deviations.

Different morphogenic potential was noted between cultivated tomato and wild *Lycopersicon* accessions, much the same previous work by Lech et al. (1996), which is also well documented phenomenon (Tal et al. 1977; Wijbrandi et al. 1988; Koornneef and Hanhart 1990; Takashina et al. 1998). The virus existence apparently decreased regeneration capacity of *Lycopersicon esculentum* explants, which, in turn, is in agreement with results obtained by others with tobacco, asparagus, plum and raspberry (Reinert 1966; De Vries-Pateron et al. 1992; Nowak 1999; Tsao et al. 1999). Wild tomato species are known to serve as a source of resistance traits, which function in cultures in vitro and had given uniformity in organogenetic response both in infected and healthy tissues (Rick 1993). Thus, many of changes observed has reflected the metabolic condition of the tissue.

Another interesting aspect is selection of mutant lines using numerous tissue culture protocols, considering that some differences acquired in culture may be perpetuated. These differences may result either from a change in genetic expression, or even in genetic information. Physiologically or morphologically different tissue lines can be isolated from cultured cells (Davidonis et al. 1982; Graham et al. 1993) which is consistent with conducted experiments. Beachy and Murakishi (1971) have reported a local lesion response of tobacco clones infected with tobacco mosaic virus. Also in the present author experiments necrotic response was observed in *L. esculentum* 'Moneymaker' tissue infected with CMV and in *L. esculentum* 'Perou' tissue infected with ToMV. Response to tomato mosaic virus of a tomato cultivar Perou, heterozygous for resistance gene Tm-2 was local hypersensitive reaction. According Hirth and Durr (1971) healthy cells

present in infected cultures may be either susceptible or may show some resistance to viral infection, so the occurrence of necrotic areas in callus tissue of susceptible *L. esculentum* 'Moneymaker' can suggest the ability of hypersensitive response to be acquired during the time of cultivation. It must be stressed that only 96% of shoots regenerated from diseased cultures of this cultivar were CMV-infected, whereas virus could not be detected in the remaining 14/(350) shoots as determined by enzyme-linked immunosorbent assay. The pathogen-free clones from infected cultures have also been obtained by others (Mori and Hosokawa 1977; Pojnar et al. 1977) This again raises the question of how far could be the biological activity of a virus affected by culture conditions.

Tissue culture may be used to select and recover spontaneous mutants or variants from suitably treated or directly from untreated material, exhibiting particular characteristics, hence differing from the parental lines. The evidence available so far, indicates that this technique is promising for uncovering variation in differentiated structures, allowing for the regeneration of plants with different level of resistance.

LITERATURE CITED

- ATTFIELD E.M., EVANS P.K. 1991. Developmental pattern of root and shoot organogenesis in cultured leaf explants of *Nicotiana tabacum* cv. Xanthi nc. Jour. Exp. Bot. 42: 51-57
- BEACHY R.N., MURAKISHI H.H. 1971. Local lesion formation in tobacco tissue culture. Phytopathol. 61: 877-878
- BEHKI R.M., LESLEY S.M. 1976. In vitro plant regeneration from leaf explants of *Lycopersicon esculentum* (tomato). Can. Jour. Bot. 2409-2413
- VAN DEN BULK R.W., LOFFLER H.J.M., LINDHOUT W.H., KOORNNEEF M. 1990. Somaclonal variation in tomato: effect of explant source and a comparison with chemical mutagenesis. T.A.G. 80: 817-825
- BEDNAREK J., CZUBER B., MAJ Z., KOBYLKO T. 1983. Identification of Tobacco Mosaic Virus races isolated from two species of the winter cherry (*Physalis*) and the susceptibility to infection by the former of some cultivars of the field tomato (*Lycopersicon esculentum* Mill.). Acta Agraria et Silv. Series Agraria Vol. XXII: 3-18
- DAVIDONIS G.H., HAMILTON R.H., MUMMA R.O. 1982. Evidence for compartmentalization of conjugates of 2, 4-dichlorophenoxyacetic acid in soybean callus tissue. Plant Physiol. 70: 939-942
- DUCREUX G., ROSSIGNOL L., SIHACHAKR D. 1991. Exploitation of genetic and physiological variability in *Solanaceae*. Acta Hort. 289: 65-75
- DWIVEDI K., SRIVASTAVA P., VERMA H.N., CHATURVEDI H.C. 1990. Direct regeneration of shoots from leaf segments of tomato (*Lycopersicon esculentum*) cultured in vitro and production of plants. Indian Jour. of Exp. Biol. 28/1: 32-35
- GASPAR T. 1995. The concept of cancer in vitro plant cultures and the implication of habituation to hormones and hyperhydricity. Plant Tiss. Cult & Biotechnol. 1/3: 126-136
- GAUDIN V., VRAIN T., JOUANIN L. 1994. Bacterial genes modifying hormonal balances in plants. Plant Physiol. Biochem. 32 (1): 11-29
- GEBRE SELASSIE K., LATERROT H., MARCHOUX G. 1993. Breeding tomatoes for resistance to cucumber mosaic virus, potato virus Y and tomato spotted wilt virus in France. Abstr. VIth Int. Congr. Plant Pathol.: 178
- GRAHAM M.J., HEAVNER D.L., NICKELL C.D., WIDHOLM J.M. 1993. Response of soybean genotypes to boron, zinc and manganese deficiency in tissue culture. Plant and Soil 150: 307-310
- GRANT M., MANSFIELD J. 1999. Early events in host-pathogen interactions. Curr. Opinion in Plant Biol. 2/4: 312-319
- HAGEGE D. 1993. Proto-oncogenes in plants: Widespread conserved genes for which roles? Plant Physiol. Biochem. 31/4: 621-629
- HIRTH L., DURR A. 1971. Donnees recentes sur la multiplication du virus de la mosaïque du tabac dans des tissus de tabac cultivés in vitro. Coll. Inter. Centre Nat. De la Recherche Scient. No 193: 481-498
- KOBLITZ H. 1991. Cell, tissue and organ culture of *Lycopersicon*. In: Genetic improvement of tomato. G. Kallo ed., Springer-Verlag, Berlin: 231-244
- KOBYLKO T. 1996. Choroby wirusowe funkcji (*Hosta* Tratt.). Zeszyty Naukowe AR w Krakowie, Rozpr. Nr 207
- KOBYLKO T. 1998. Zimowanie wirusa mozaiki pomidora na bylinach ozdobnych ogrodu działkowego. Zeszyty Naukowe AR w Krakowie Nr 333: 693-696
- KOORNNEEF M., HANHART C.J. 1990. The genetics of regeneration capacity in tomato. Abs 7th Int. Congr. Plant Tiss & Cell Cult., Amsterdam: B6-47
- KURTZ S.M., LINEBERGER R.D. 1983. Genotypic differences in morphogenic capacity of cultured leaf explants of tomato. J. Amer. Soc. Hort. Sci. 108/5: 710-714
- LARKIN P.J., SCOWCROFT W.R. 1981. Somaclonal variation – a novel source of variability from cell cultures for plant improvement. T.A.G. 60: 197-214
- LECH M., MICZYŃSKI K., PINDEL A. 1996. Comparison of regeneration potentials in tissue cultures of primitive and cultivated tomato species (*Lycopersicon* sp.). Acta Soc. Bot. Pol. 65/1-2: 53-56
- MARCHOUX G., GEBRE SELASSIE K. 1992. Nouveaux et anciens virus affectant la tomate. PHM Revue Hort. 323: 57-65
- MONTAGNO T.J., LINEBERGER R.D. BERRY S.Z. 1989. Somaclonal and radiation induced variation in *Lycopersicon esculentum*. Env. & Exp. Bot. 29/3: 404-408
- MORI K., HOSOKAWA D. 1977. Localization of viruses in apical meristem and production of virus-free plants by means of meristem and tissue culture. Acta Hort. 78: 389-396
- MURASHIGE T., SKOOG F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15: 473-479
- NOWAK B. 1999. Regeneration capacity of *Prunus domestica* cv. Węgierka Zwykła explants infected with plum pox virus. Acta Physiol. Plant. Vol. 21 No. 3 Suppl.: 60
- PADMANABHAN V., PADDOCK E.F., SHARP W.R. 1974. Plantlet formation from *Lycopersicon esculentum* leaf callus. Can. Jour. Bot. 52/6: 1429-1432
- PELET F., HASLER T. 1992. Viroses et maladies analogues de la tomate. Rev. Suisse Vitic. Arboric. Hortic. Vol. 24 (1): 29-36
- POJNAR E., KOBYLKO T., LECH M. 1977. Regeneracja roślin tytoniu (*Nicotiana tabacum* v. Samsun) z protoplastów porażonych wirusem mozaiki tytoniu. Zesz. Probl. Post. Nauk Roln. 195: 23-26
- REINERT R.A. 1966. Virus activity and growth of infected and healthy callus tissues of *Nicotiana tabacum* grown in vitro. Phytopathol. 56: 731-733
- RICK C.M. 1993. Wild tomato germplasm: source of disease resistance and other economic traits. Abs. 6th Int. Congr. Plant Pathol.: 22
- SINK K.C., REYNOLDS J.F. 1988. Tomato (*Lycopersicon esculentum* L.) In: Biotechnol. in Agric. and Forestry., Y.P.S. Bajaj ed., Springer-Verlag: 319-344
- SELVI D.T., KHADER M.A. 1993. In vitro morphogenetic capacity of tomato (*Lycopersicon esculentum* Mill.). South Ind. Hortic. 41/5: 251-258
- SEQUEIRA L. 1973. Hormone metabolism in diseased plants. Ann. Rev. Plant Physiol. 24: 353-380
- SYLVESTER A.W., 2000. Division decisions and the spatial regulation of cytokinesis. Curr. Opinion in Plant Biol. 3/1: 58-66
- TAKASHINA T., SUZUKI T., EGASHIRA H., IMANISHI S. 1998. New molecular markers linked with the high shoot regeneration capacity of the wild tomato species *Lycopersicon chilense*. Breeding Scie. 48/2: 109-113
- TAL M., DEHAN K., HEIKIN H. 1977. Morphogenetic potential of cultured leaf sections of cultivated and wild species of tomato. Ann. Bot. 41: 937-941
- TSAO C.V., POSTMAN J.D., REED B.M. 1999. Single and multiple virus infections reduce in vitro multiplication of 'Malling Landmark' raspberry. Abs. Int. Congr. On In Vitro Biol., New Orleans, Luiziana, In: In vitro 35/3, 41 A: 1013

- VILLIERS R.P., VUURENS R.J., VAN FERREIRA D.J., STADEN J. 1993. Regeneration of adventitious buds from leaf discs of *Lycopersicon esculentum* cv. Rodade. Optimalization of culture medium and growth conditions. Journ. South. Af. Soc. Hort. Scien. 3/1: 24-27
- DE VRIES-PATERSON R.M., EVANS T.A., STEPHENS C.T. 1992. The effect of asparagus virus infection on asparagus tissue culture. Plant Cell Tiss. & Organ Cult. 31: 31-35
- WATTERSON J.C. 1994. Diseases. In: Tomato crop – a scientific base for improvement. J.G. Atherton & J. Rudich eds., Chapman & Hall, London: 467-484
- WIJBRANDI J., VOS J.G.M., KOORNNEEF M. 1988. Transfer of regeneration capacity from *Lycopersicon peruvianum* to *L. esculentum* by protoplast fusion. Plant Cell Tiss. & Organ Cult. 12: 193-196
- YEOMAN M.M., FORCHE E. 1981. Cell proliferation and growth in callus cultures. Int. Rev. Cytol. Suppl. 11A: 2-24
- ZENKTELER M. 1972. In vitro formation of plants from leaves of several species of the *Solanaceae* family. Biochem. Physiol. Pflanz. 163: 509-512

REAKCJA KILKU GENOTYPÓW POMIDORA NA STRES BIOTYCZNY W KULTURACH IN VITRO

STRESZCZENIE

Badano wpływ stresu biotycznego na proces kalogenezy oraz na potencjał organogenetyczny tkanek somatycznych pomidora (*Lycopersicon* sp.). Izolowano eksplantaty z liści roślin porażonych systemicznie odpowiednio wirusem mozaiki pomidora lub wirusem mozaiki ogórka, bezpośrednio po potwierdzeniu obecności wirusa w tkance przy użyciu testu enzymatyczno-immunosorpcyjnego. Regenerację prowadzono w sposób pośredni, stosując zmodyfikowany skład pożywki Murashige'a i Skooga; namnożone pędy ukorzeniano w warunkach in vitro a następnie ukorzenione regeneranty aklimatyzowano do warunków uprawy szklarniowej. Obserwowano zróżnicowany efekt infekcji wirusowej na kultywowane tkanki, obejmujący zarówno stymulację jak i inhibicję proliferacji i różnicowania komórek. Efekt ten był bardzo wyrazisty w przypadku pomidora uprawnego, natomiast nieznaczny w kulturach dzikich gatunków pomidora. Pomimo, iż we wszystkich wariantach doświadczenia doszło do regeneracji pędów, *L. esculentum* reagował na stres biotyczny wyraźnym obniżeniem potencjału morfogenetycznego, a dla trzech odmian: 'Moneymaker', 'Potentat' i 'Rutgers' infekcja wirusem mozaiki ogórka powodowała istotne zmniejszenie liczby zregenerowanych pędów w porównaniu do zdrowej kontroli. Pędy zregenerowane w zdrowych kulturach kontrolnych po przeniesieniu na pożywkę ukorzeniającą wytwarzały od kilku do kilkunastu dobrze rozwiniętych korzeni przybyszowych, natomiast pędy pochodzące z kultur porażonych ukorzeniały się w różnym, często niewielkim procencie i to niejednokrotnie dopiero po wytworzeniu kalusa. Niewielka liczba roślin zregenerowanych z zainfekowanych krótkoterminowych kultur pomidora uprawnego okazała się wolna od infekcji wirusowej.

SŁOWA KLUCZOWE: mikrorozmnażanie, morfogeneza, tkanki somatyczne, infekcja wirusowa, stres biotyczny, pomidor.