COMPARISON OF REGENERATION POTENTIALS IN TISSUE CULTURES OF PRIMITIVE AND CULTIVATED TOMATO SPECIES (*LYCOPERSICON* SP.)

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ABSTRACT

Regeneration capacities of two tomato cultivars: Potentat and Rutgers, and of three accessions of wild tomato species: *Lycopersicon peruvianum* PI 128650, *L. peruvianum* var. *dentatum* PI 128655 and *L. glandulosum* were studied using an universal medium suitable for regeneration of those plants from leaf pieces in tissue culture.

Fragments of leaf blades were taken from plants raised in greenhouse conditions and placed on a modified MS medium containing 0.3 mg/l IAA and 3.0 mg/l BAP solidified with 1% agar. The explants were transferred every 4-5 weeks on fresh medium of the same composition. It was shown that all the three primitive tomato species revealed much higher multiplication coefficients than the two cultivars. Appropriate values were: 11 – for *L. glandulosum*, 8 – for *L. peruvianum*, 7 – for *L. peruvianum* var. *dentatum*, 4 – for *L. esculentum* cv. Potentat and 2 – cv. Rutgers. Completely regenerated plants were obtained from all the tested species, but organogenesis occurred almost two weeks earlier in wild tomatoes than in the cultivated varieties of *L. esculentum*.

KEY WORDS: Tomato, regeneration, tissue culture.

INTRODUCTION

Several primitive species of the genus *Lycopersicon*, especially *L. peruvianum*, *L. hirsutum* and *L. glandulosum*, represent an important source of genes, conferring resistance to various diseases and pests of cultivated tomatoes (Lukyanenko 1991). However introduction of those genes to commercial varieties of *L. esculentum* by conventional breeding techniques encounters often serious difficulties due to high incompatibility barriers by hybridisation (Kaul 1991). To overcome this problem certain modern approaches of gene manipulation are recommended, which as a rule require regeneration of plants in tissue cultures starting from single cells, protoplasts or small tissue fragments. Standardization of conditions for the regeneration process in vitro seems therefore to be of primary importance for that purpose. In previous experiments (Lech et al. 1993, 1994) the authors tested various nutrient media for their effectiveness to regenerate plants from leaf fragments of several wild species of tomato, which are known to confer resistance to tomato mosaic virus (ToMV), and tomato spotted wilt virus (TSWV) (Alexander 1963, Czuber and Miczyński 1981), and of two tomato cultivars. As result one universal medium was selected, and its suitability for regeneration of the tested tomatoes was evaluated according to the observed rate of organogenesis in particular species, and calculation of their multiplication coefficients in tissue culture.

MATERIAL AND METHODS

Investigations were carried out with 3 accessions of wild tomato species kindly supplied by the Plant Introduction Station

in Ames, USA: *Lycopersicon peruvianum* (L.) Mill., line PI 128650, *L. peruvianum* var. *dentatum* Dun., line PI 128655, *L. glandulosum* C.H. Mull., line 731163, and with 2 cultivars of *L. esculentum*: Potentat and Rutgers highly susceptible to ToMV and TSWV.

Leaf pieces 0.25 sq. cm, containing a fragment of the main vein were taken out from 6-8 week old plants raised in the greenhouse. They were dipped for 1-2 minutes in 70% ethanol, followed by 0.1 HgCl₂ and washed 5 times with sterile water. Next they were placed on 1 per cent Difco agar supplied with the MS basal medium (Murashige and Skoog 1962), to which 0.3 mg/l IAA and 3.0 mg/l BAP was added. The cultures were incubated at 24±2°C under white fluorescent light (60 µmol. m⁻²s⁻¹) with 16h photoperiod. The explants were transferred every 4-5 weeks onto fresh medium of the same composition, except when vitrification of the cultures occurred. In such cases the tissues were transferred onto 1% agar containing a reduced amount of all other compounds to two thirds of the full strength medium applied previously. Average numbers of regenerating shoots per explant (micropropagation coefficients) were calculated for every tested species at each subsequent transfer.

RESULTS AND DISCUSSION

Completely regenerated plantlets showing no morphological abnormalities were obtained by direct regeneration of the explants from all the tested tomatoes (Figs 1-4. The photos present 3 stages of regeneration – from left to right – taken after 4, 8, and 12 weeks of culture of explants). The modified MS medium appeared to be suitable for cultivation of *L. peruvia-*

Figs 1-3. Three stages of regeneration of explants of wild tomato species after 4, 8, and 12 (from left to right) weeks of culture:
1 – *L. peruvianum* PI 128650, 2 – *L. peruvianum* var. *dentatum* PI 128655, 3 – *L. glandulosum*.

Fig. 4. Potentat after 12 weeks of culture.

TABLE 1. Results of the experiment.

species/cultivar	number of primary explants	Micropropagation coefficient calculated after			number of regenerated explants
		4 weeks	8 weeks	12 weeks	
<i>L. glandulosum</i>	50	11	11	11	5500
<i>L. peruvianum</i>	50	8	8	8	4000
<i>L. peruvianum</i> var. <i>dentatum</i>	50	7	7	7	3500
Potentat	50	2.5	4	4	172
Rutgers	50	0.5	2	2	93

num, *L. glandulosum* and both cultivars: Potentat and Rutgers during the whole period of regeneration. In one month old cultures of *L. peruvianum* var. *dentatum* vitrification occurred which could be eliminated after transfer on media with reduced nutrient contents.

Table 1 summarises results of the experiment in which the tested explants were cultivated in vitro for 12 weeks. As seen, the total number of plantlets, regenerated during this period was over 30 times higher for the primitive tomato species as compared with the cultivars Potentat or Rutgers. The highest regeneration potential revealed here *L. glandulosum* which amplified the starting material 110 times compared with only 1.9 times in case of the cultivar Rutgers. All the three primitive tomato species showed also much higher multiplication coefficients during the whole period of cultivation, than the cultivars. Appropriate values at the final evaluation were: 11 – for *L. glandulosum*, 8 – for *L. peruvianum*, 7 – for *L. peruvianum* var. *dentatum*, 4 – for cv. Potentat, and only 2 – for cv. Rutgers. Organogenesis occurred also ca. two weeks earlier in explants of the primitive tomatoes, than in those of the cultivars. Similar results were obtained by de Langhe and de Bruijne (1976) who found micropropagation coefficients for *L. peruvianum* 2-3 times higher, than for *L. esculentum*, whereas Górecka and Krzyżanowska (1990), working with other cultivars of tomatoes, calculated their multiplication coefficients for 3,3-5,6. Much higher morphogenetic potential of *L. peruvianum*, as compared with several cultivars of *L. esculentum*, was reported also by Natkaniec (1991), and a very low

ability for regeneration of the cv. Rutgers was stated earlier by Kantapradija (1989), and Koblitz (1991).

The medium used in our experiments induced differentiation of both shoots and roots on explants of all the tested tomatoes, without necessity to cultivate them on separate shoot inducing and rooting media. This complies only partially with the findings of the majority of other authors, who usually used for regeneration of *L. esculentum* different media for induction of shoots and roots containing IAA and BAP in concentrations of 0.2-4.0 mg/l (Selwi and Khader 1993, Villiers et al. 1993, Natkaniec 1991, Górecka i Krzyżanowska 1991). However, rooting was also obtained after transfer of the cultures on media without any growth hormones (Kantapradija 1989) or containing only kinetin (Santana and Ramirez 1989). These data clearly show how variable are the requirements of different cultivars of tomato as to the concentrations of particular growth hormones indispensable for regeneration. The comparatively high demand for kinins in that process is interpreted by de Langhe and de Bruijne (1976) as result of the high level of endogenous auxins in tomato tissues.

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PORÓWNIANIE ZDOLNOŚCI DO REGENERACJI DZIKICH I UPRAWNYCH GATUNKÓW POMIDORA (*LYCOPERSICON* SP.)

STRESZCZENIE

Zbadano zdolność do regeneracji w kulturach *in vitro* dwie odmiany pomidorów: Potentat i Rutgers oraz trzy linie dzikich gatunków pomidora: *L. peruvianum* PI 128650, *L. peruvianum* var. *dentatum* PI 128655 i *L. glandulosum*, stosując uniwersalną pożywkę, przydatną do regeneracji wszystkich wymienionych odmian z kawałków liści.

Fragmenty blaszki liściowej pobierano z roślin rosnących w szklarni i umieszczano je na zmodyfikowanej pożywce agarowej wg. Murshige Skooga, zawierającej 0.3 mg/l IAA + 3.0 BAP. Eksplantaty przenoszono co 4-5 tygodni na nową pożywkę o tym samym składzie. Stwierdzono, że wszystkie 3 dzikie gatunki pomidora wykazały o wiele wyższe współczynniki namnażania w porównaniu z dwoma odmianami uprawnymi. Odpowiednie wartości wynosiły: 11 – dla *L. glandulosum*, 8 – dla *L. peruvianum*, 7 – dla *L. peruvianum* var. *dentatum*, 4 – dla odmiany Potentat i 2 – dla odmiany Rutgers.

Z tkanek wszystkich badanych pomidorów otrzymano całkowicie zregenerowane rośliny, ale organogeneza (wytwarzanie pąków pędowych i korzeni) zachodziła u gatunków dzikich ok. 2 tyg. wcześniej aniżeli u odmian uprawnych.

SŁOWA KLUCZOWE: pomidor, regeneracja, kultury tkankowe.