Studii şi Cercetări	Martie 2010	Biologie	18	51-54	Universitatea"Vasile Alecsandri" din Bacău

A COMPARATIVE STUDY REGARDING SOME PHENOTYPIC AND GENETIC FEATURES OF *IN VITRO* MICROPROPAGATED AND SEED-BORNE TOMATO (*LYCOPERSICON ESCULENTUM* MILL) PLANTS

Tina Oana Cristea, Maria Prisecaru, Maria Călin

***Key words*:** *genetic, stability, multiplication, vitro*

INTRODUCTION

Micropropagation in vitro at tomatoes (*Lycopersicon esculentum* Mill.) represents a viable option when a large number of rare genotypes (such as interspecific hybrids) is required. As in other crops (Baroncelli *et al*., 1973; Bayliss and Dunn, 1979; Pence *et al.*, 1979; Jarret *et al.*, 1980), different responses (callus production, regeneration of whole plants, roots and pseudo-fruit differentiation) have been reported in tomato, depending on the genotypes, explants, culture media and incubation conditions (Kartha *et al.*, 1976; Tal *et al.*, 1977; Kut and Evans, 1982; Kurtz and Lieneberger, 1983; Locky, 1983; Zorzoli *et al.*, 1993a, b). Somaclonal variation can pose a severe threat to the genomic integrity of regenerated plants, which is particularly required during the genetic transformation experiments and to achieve genetic uniformity of the propagules. Somaclonal variation can either bring the changes at the DNA level or it may induce changes in chromosome numbers. However, for most of the micropropagated crops 5 % somaclonal variation is permitted (Leela *et al.* 2003). Although reports are available for propagation of tomato *via* tissue culture, relatively few results are available on the performance of tissue-cultured tomatoes in field or greenhouse conditions (Deng *et al*. 1988, Somasunder and Gostimsky 1992, Venkatachalam *et al*. 2000).

Many studies of classical and vitro breeding were accomplished for the tomato (*Lycopersicon esculentum* Mill.; 2n = 2x = 24), due to its unmatched culinary uses, is the most produced vegetable crop around the world (Nonecke 1989). Tomato is rich in vitamins A and C and fibre, and is also cholesterol free (Hobson and Davies, 1971). An average sized tomato (148 g) boasts only 35 calories. Tomato contains approximately 20–50mg of lycopene/100 g. of fruit weight (Kalloo, 1991).

Lycopene is part of the family of pigments known as carotenoids which are natural compounds that create colors of fruits and vegetables. Lycopene is the most powerful antioxidant in the carotenoid family and it protects humans from free radicals that degrade many parts of the body; lycopene is also known to prevent cancer (Block et al., 1992; Gerster, 1997; Rao and Agarwal, 2000). At present, tomatoes are consumed at a higher rate in the developed countries than in the developing countries and hence it may be referred to as a luxury crop.

In the present study we focused on a number of phenotypic and genetic features in order to establish if there are any changes in micropropagated plants comparing with seed grown plants.

The phenotypic features analyzed in the present study are: plant's height, number of leaves, number of inflorescences, number of flowers, number of fruits, percent of setting out the fruits, production.

The genetic analyses refer to mitotic activity (mitotic index and percentages of cells with chromosomal aberrations). In the analysis accomplished in the present study we differentiated the micropropagated plants depending on the originated explant (from which the plant were regenerated).

MATERIAL AND METHODS

The experiments were carried out in the Laboratory of Tissue Culture and in greenhouse at Vegetable Research and Development Station Bacau, Romania.

Tissue-cultured plants were obtained from explants (apexes, hypocotyls) that were obtained from aseptically grown one-week-old seedlings and buds harvested from mother plants grown in greenhouse conditions.

The explants were inoculated on a regeneration medium consisting of Murashige Skoog basal medium (B5) supplemented with KIN – 1,5 mg/l^{-1}, IAA – 0,5 mg/l^{-1} and 3 % sucrose. After 4 weeks, regenerated shoots were transferred to a multiplication medium of Murashige and Skoog (MS) supplemented only with 2 mg/l^{-1} kinetin. Cultures remained on the multiplication medium for 4 weeks before the shoots were separated and transferred to the rooting medium consisted of MS basal medium without growth regulators.

After another 4 weeks of growth on the rooting medium, rooted shoots were removed from the tissue culture tubes, thoroughly washed to remove any traces of agar and then transferred to potting trays containing vermiculite, peat moss and sand (2:1:1; v/v).

In the greenhouse, twenty plants each of tissue-cultured and seed-grown plants were planted according to a completely randomised design.

The seedlings were irrigated via drop irrigation system. Seeds of L24 S and L27S plants were sown at the same time as the tissue-cultured plants were transplanted. The seeds germinated in two weeks and the seedlings attain the similar height as tissue-cultured plants had at the time of planting within two weeks time.

The cytogenetic studies were accomplished in meristematic root cells, stained in Carnoy fixing solution for 24 hours at 4^{0}C then hydrolyzed with HCl for 7 minutes and colored with the basic coloring solution Carr. The root meristems were displayed using squash technique and for each genotype and variant 2000 cells were counted.

Chromosome slides were then observed microscopically. Numbers of dividing cells at different levels of mitosis were recorded.

Mitotic data were subjected to statistical analysis by calculating the mitotic index (% cells in division per total number of examined cells and percentages of cells with chromosomal aberrations).

RESULTS AND DISCUSSIONS

The results obtained in the present study prove that tissue culture derived plants maintain the agronomic properties of mother plants.

No phenotypic abnormalities in vegetative, flowering or fruit-related characteristics were observed amongst the tissue-cultured plants.

As it is illustrated in tables 1 and 2 the values of the main morpho-physiologic indices of tissue culture plants are similar with the values obtained for seed-growing plants.

Table 1. The value of some morpho-physiologic features of tomato plants cultivated in greenhouse - genotype L24S

Origin	Plant's height (cm)	No of leaves	No of inflorescences	No of flowers	No of fruits	% fruits	Production kg/m^2
V1	217	48,4	9,7	46,8	37,2	79,5	8,60
V2	212	46,3	8,8	42,1	31,6	75,0	8,36
V3	180	38,1	7,4	38,4	27,4	71,5	7,36
V4	220	50,2	9,6	46,2	36,8	79,7	8,51

V1 – apex, V2 – bud, V3 – hypocotyl, V4 – seed-borne plants

Fig. 1. Tomato plants in greenhouse conditions genotype L24S

No significant differences between the seed-grown and tissue-cultured plants were found for genotype L27S. All the studied parameters of tomato plants regenerated via in vitro culture have the same values with the seed-borne plants.

Table 2.Thealue of some morpho-physiologic features of tomato plants cultivated in greenhouse - genotype L27S

Origin	Plant's height (cm)	No of leaves	No. of inflorescences	No. of flowers	No. of fruits	% fruits	Production kg/m^2
V1	227	40,6	10,2	47,8	39,8	83,3	10,96
V2	219	38,2	9,1	46,3	38,1	82,3	9,95
V3	201	36,5	8,3	45,1	35,9	79,6	9,02
V4	228	41,1	10,0	48,1	40,4	83,9	11,20

V1 – apex, V2 – bud, V3 – hypocotyl, V4 – seed-borne plants

For both genotypes lower results were obtained at plants regenerated from hypocotyls. The other two types of explants produced plants that have no alteration of phenotypic traits.

Regarding the genetic analyses, mitotic data were subjected to statistical analysis by calculating the mitotic index (% cells in division per total number of examined cells) and percentages of cells with chromosomal aberrations.

The results obtained showed that for both genotypes the mitotic index was similar or slightly higher at plants obtained from apex, at plants regenerated *in vitro* and at seed-borne plants.

In tables 3 and 4 are presented the values obtained for each genotype.

Table 3. Results of genetic investigations realized at seed-borne plants and plants regenerated in vitro at L24S genotype

Origin	Total analysed cells	Cells in interphase	No of dividing cells	Mitotic index	% aberra tions
V1	6175	5470	699	11.32	0.203
V2	6038	5427	611	10.12	0.189
V3	6392	5760	632	9.89	1.1
V4	6190	5530	657	10.61	0.192

V1 – apex, V2 – bud, V3 – hypocotyl, V4 – seed-borne plants

The main types of abnormalities in the root cells of tomatoes seed-borne plants and vitroplants are ana-telophases with inter-chromatin bridges, metaphases with lagging chromosomes, expelled chromosomes, ring chromosomes, retard chromosomes multipolar ana-telophases, as well as binucleate cells and interphases with micro-nucleuses.

Table 4. Results of genetic investigations realized at seed-borne plants and plants regenerated in vitro at L27S genotype

Origin	Total analysed cells	Inter-phase	No of dividing cells	Mitotic index	% aberra-tions
V1	6525	5681	844	12.94	0.59
V2	6293	5502	787	12.51	0.62
V3	6315	5612	703	11.14	0.88
V4	6333	5520	811	12.81	0.66

V1 – apex, V2 – bud, V3 – hypocotyl, V4 – seed-borne plants

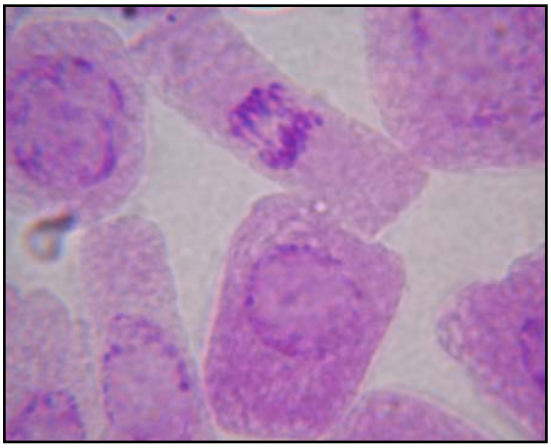

Fig. 2. Ana-telophase with inter-chromatin bridges at L24S genotype

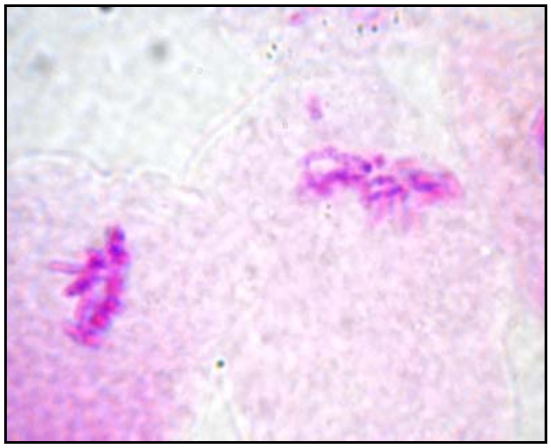

Fig. 4. Metaphases with expelled chromosomes

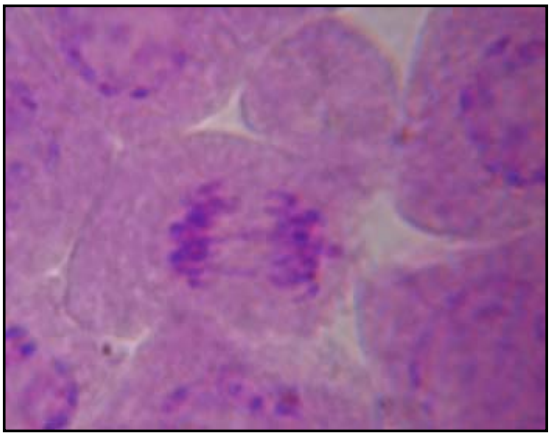

Fig. 3. Ana-telophase with bridges and delayed chromosome at L24S genotype

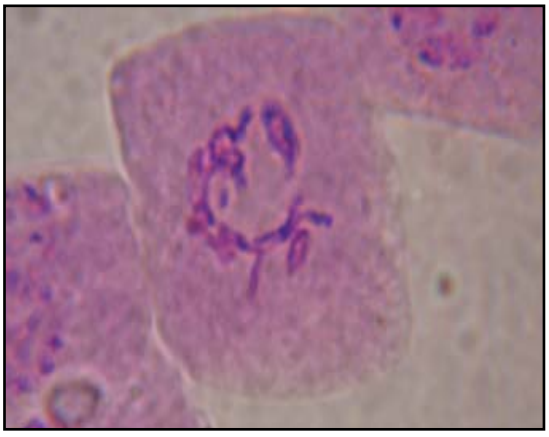

Fig. 5. Prophase with ring chromosome

For all the plants studied the highest incidence was observed in ana-telophases.

The most common abnormalities were ana-telophases with simple or multiple bridges, expelled or late chromosomes and multipolar ana-telophases – figure 2 and 3.

The second phase which presented a higher percentage of abnormalities is metaphases that were abnormally organized, with ring chromosomes, minutes, expelled chromosomes, fragment, etc – figure 4.

In a smaller number we detected prophases that presented different types of chromosomal aberrations like late prophases, with ring chromosomes – figure 5, expelled chromosomes etc.

CONCLUSIONS

The results obtained in the present study demonstrated that tissue-cultured plants did not show any phenotypic abnormality or any genetic changes.

These results are similar with the results presented in the literature for tomatoes and other species (Venkatachalam et al., 2000, Tyagi et al., 2004) who found no genetic variation in *in vitro* regenerated plants.

This study demonstrates that if the *in vitro* protocol is proper (certain types of hormones, low number of subcultures, the utilization of a certain type of explant), the performance of resulting tissue-cultured plants would be comparable with those raised from seeds.

ABSTRACT

Tomato (*Lycopersicon esculentum* Mill.) is a major vegetable crop that has a tremendous popularity, being cultivated in almost every country of the world either for fresh market or for processing.

Due to its economic worldwide present and future potential of improving the crop through molecular techniques, improvement in the efficiency of regeneration is expected to have a positive impact on transformation results.

The present study aimed at the accomplishment of a comparative study regarding the main agronomic features of tomatoes plants regenerated *in vitro* and seed-grown tomato plants. Morphological, fruit yield and genetic features were compared between the seed-grown and tissue cultured plants of two genotypes of tomato (*Lycopersicon esculentum* Mill) L24S and L27S in greenhouse conditions at V.R.D.S. Bacau. The results obtained in the present study showed that there were no phenotypic or genetic abnormalities at tissue-cultured plants compared with the seed-borne plants.

This study proves that genetic fidelity of tissue cultured plants can be maintained if appropriate plant growth regulators are used with fewer member of subcultures in the multiplication medium.

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AUTHOR'S ADDRESS

CRISTEA TINA OANA, CĂLIN MARIA - Vegetable Research and Development Station Bacau, Calea Barladului, No. 220, code 600388, Bacau, Romania
e-mail: tinaoana@yahoo.com

PRISECARU MARIA - University „Vasile Alecsandri, Faculty of Biology, Marasesti Street, no. 157, Bacau, Romania
e-mail prisecaru_maria@yahoo.com