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BRIEF COMMUNICATION

Comparative performance of micropropagated and seed-grown tomato plants

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Abstract

Morphological, physiological, fruit yield and quality related traits were compared between the seed-grown and tissue-cultured plants of tomato (*Lycopersicon esculentum* Mill.) cv. Red Coat in a greenhouse. No significant differences were observed for any of the traits studied except for the number of leaves and branches, which were higher in the seed-grown plants than in tissue-cultured plants at the later stages of growth. No phenotypic abnormality of the tissue-cultured plants was observed suggesting 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.

Additional key words: explant, genetic stability, *Lycopersicon esculentum*, morphogenesis, multiplication, photosynthetic rate, rooting, stomatal conductance, respiration rate.

Tissue culture is an important tool of biotechnology, which can be used to improve productivity of a crop *via* rapid availability of superior planting stock. However, cultures can progressively lose their characteristics with subculturing (Henke *et al*. 1978, Bajaj and Dhanju 1981). Sometimes total loss can occur after two subcultures (Wu and Antonovics 1978). On the other hand, undiminished regeneration can occur for up to 15 subcultures in certain crops (Chen *et al*. 1977). 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). Many plants transferred from tissue culture may show reduced photosynthetic rate due to a sudden shortage of nutrients in the substrate. However, *in vitro* cultured plants adjust to *ex vitro* conditions when switched from heterotrophic to autotrophic conditions (Donnelly and Vidaver 1984, Kozai 1991). Pospíšilová *et al*. (1992) reported that the net photosynthetic rate increased from 5.34 to 6.48 μmol(CO_2) $m^{-2}s^{-1}$ after 3 weeks of acclimatisation of *in vitro* grown tobacco plants. Hronková *et al*. (2003) reported a significant increase in free abscisic acid content at the first and second day after plant transfer from *in vitro* to *ex vitro* conditions. 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).

In the current study, a number of morphological, physiological, fruit yield and quality traits were studied simultaneously with the view to identifying phenotypic abnormality or somaclonal variation if any, in micropropagated plants and to compare the over-all performance of tissue-cultured plants over seed-grown plants.

Tissue-cultured plants were obtained as per the procedures developed by Bhatia (2003). In summary, cotyledonary explants that were obtained from aseptically grown one-week-old seedlings were inoculated on to

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Abbreviations: IAA - indole-3-acetic acid, IBA - indole-3-butyric acid, B_5 medium - Gamborg *et al*. (1968) medium, MS medium - Murashige and Skoog's medium.

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regeneration medium consisting of Gamborg's basal medium (B_5) supplemented with 15 μM zeatin and 1.5 % sucrose. After 4 weeks, regenerated shoots were transferred to a multiplication medium of Murashige and Skoog (MS) supplemented with 30 μM kinetin + 5 μM indole acetic acid (IAA). 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 supplemented with 10 μM indole butyric acid (IBA). 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). These *in vitro* cultured plants were acclimatized for 1-week in a misthouse (maintained at *ca*. 90 % relative humidity) before being transferred to plastic pots (250 mm diameter) containing the same substrate.

The experiment was carried out in the greenhouse at the Central Queensland University, Rockhampton. During the experiment, the temperature, relative humidity and photosynthetically active radiation (max.) in the greenhouse ranged between 12 - 43 °C, 27 - 81 % and 1200 - 1600 μmol m^{-2} s^{-1}, respectively. Twenty pots each of tissue-cultured and seed-grown plants were arranged on benches according to a completely randomised design, and were re-randomised four times during the course of the experiment. The seedlings were irrigated via bottom irrigation system, where the rectangular trough in which the pots were kept was filled up to *ca*. 10 cm with the full strength *Manutec*® hydroponic mix (*Manutec Pvt. Ltd.*, Cavan, Australia) (bottom irrigation). The solution was replaced once every week and the pH adjusted to 6.5.

Seeds of cv. Red Coat 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. To compensate for the difference in the age of tissue-cultured and seed-grown plants, the seed-grown plants were harvested two weeks after harvesting the tissue-cultured plants. The whole experiment was harvested at two stages. The first harvest was carried out 14 weeks after transplanting of tissue-cultured plants and 16 weeks after sowing the seed-grown plants. The second harvest was carried out after 18 weeks of transplanting of tissue-cultured plants and 20 weeks after sowing of seed-grown plants. At each stage of harvest, ten plants each of tissue-cultured and seed-grown plants were harvested.

Stomatal conductance, photosynthetic rate, and transpiration rate were measured on the 25th and 53rd day of transplanting using a leaf chamber *PLC4* with portable *LCA4* infrared gas analyser (*Analytical Development Company*, Hoddesdon, England) on the first and second fully expanded leaves for the first measurement and on the mature leaves of the mid canopy for the second measurement.

Randomly selected ten fruits at visually similar ripening stage from the tissue-cultured and seed-grown plants were analysed for fruit quality characteristics. Firmness was measured by Quick Measure Penetrometer System (*HortPlus*, Cambridge, New Zealand) using a 7 mm plunger as per the manufacturer's instructions. Following measurements of firmness, fruits were cut into halves (along the longitudinal axis); the first halves were weighed and placed in a drying oven at 70 °C for 4 d to determine total solids. The other halves of each fruit was homogenised using *Ultra Turrax*® homogeniser (*BDH Merck Pty Ltd*., Berthold, Australia) and centrifuged at 700 *g* for 10 min to separate the clear juice from the pulp. An aliquot of clear juice was used to measure total soluble solids (TSS) using a refractometer (*Leica AR 200*, *Leica Microsystems*, Sydney, Australia). The same juice was used to measure pH and citric acid concentration. Citric acid, is a dominant acid in tomato, and was measured by titrating the juice with sodium hydroxide.

The data were analysed using *GenStat*® statistical analysis software. Unpaired *t*-test was used to compare means of the studied traits of two types of plants.

A markedly high survival (95 %) of tissue-cultured plants was noticed after one-week of acclimatisation in the mist room. No phenotypic abnormalities in vegetative, flowering or fruit-related characteristics were observed amongst the tissue-cultured plants. At first harvest, no significant differences were observed for any of the studied morphological traits (Table 1). At the second harvest, all traits except number of branches and number of leaves were significantly higher in seed-grown plants than in tissue-cultured plants. At the later stages of growth, the seed-grown plants produced larger number of very small sized leaves and numerous tiny branches. However, in tissue-cultured plants, both the number of leaves and branches were fewer, but the leaves were broader than those found in seed-grown plants (data not recorded). The tissue-cultured plants flowered and fruited *ca*. 4 weeks earlier than seed-grown plants. No significant differences between the seed-grown and tissue-cultured plants were found for stomatal conductance (g_s), transpiration rate (E) and net photosynthetic rate (P_N) on both the 25th and 53rd day observations (Table 2), but g_s, E and P_N were higher in both types of plants on the 25th day than on the 53rd day (Table 2). None of the fruit quality characteristics of tissue-cultured plants was significantly ($P < 0.05$) different from those of seed-grown plant (Table 3).

High survival rate (95 %) of *in vitro* raised plants in the current experiment implies the proper functioning of stomata, photosynthetic apparatus and other leaf tissues of tissue-cultured plants. Donnelly *et al*. (1985) also reported that the new leaves that were developed from the tissue-cultured plants during acclimatisation resembled those developed in the greenhouse or field. Diettrich *et al*. (1992) also suggested that the leaves from *in vitro*

Table 1. Morphological traits of tissue-cultured (TC) and seed-grown plants (SG) at the first (14 - 16 weeks after transplanting) and second (18 - 20 weeks after transplanting) harvest. Values are means ± SE, $n = 10$.

Trait	First harvest TC	SG	*P* value	Second harvest TC	SG	*P* value
Plant height [cm]	209.7 ± 4.28	216.4 ± 2.44	0.191	226.8 ± 5.10	221.3 ± 6.33	0.53
Number of branches	8.8 ± 0.62	10.2 ± 0.33	0.064	24.7 ± 1.40	30.0 ± 0.92	0.005
Number of leaves	124.5 ± 8.08	125.6 ± 8.38	0.926	277.3 ± 16.91	438.7 ± 25.89	<0.001
Shoot fresh mass [g]	692.6 ± 57.38	637.3 ± 46.64	0.465	879.6 ± 55.11	949.9 ± 61.49	0.409
Leaf fresh mass [g]	948.1 ± 68.04	761.5 ± 57.43	0.051	1077.0 ± 68.89	955.1 ± 54.30	0.181
Root fresh mass [g]	245.9 ± 19.70	272.3 ± 16.38	0.317	879.6 ± 26.81	949.4 ± 38.16	0.409
Shoot dry mass [g]	116.0 ± 8.47	115.5 ± 7.17	0.967	166.2 ± 11.44	161.3 ± 10.92	0.760
Leaf dry mass [g]	117.7 ± 10.57	124.3 ± 6.68	0.605	157.4 ± 2.41	155.2 ± 10.19	0.889
Root dry mass [g]	58.0 ± 9.53	55.3 ± 7.06	0.821	63.5 ± 9.99	126.7 ± 28.30	0.059
Flowering peduncles	4.4 ± 1.39	7.0 ± 1.48	0.217	12.3 ± 2.31	19.4 ± 3.75	0.124
Fruiting peduncles	14.7 ± 1.12	13.6 ± 0.90	0.452	28.4 ± 2.77	26.7 ± 2.61	0.661
Number of fruits [plant^{-1}]	53.0 ± 7.57	44.7 ± 5.87	0.396	84.8 ± 7.82	95.2 ± 8.79	0.388
Average fruit diameter [mm]	39.3 ± 3.62	41.6 ± 2.86	0.612	33.9 ± 3.28	37.1 ± 1.64	0.394
Total fruit yield [g plant^{-1}]	2137.0 ± 458.5	1832.0 ± 242.2	0.581	3701.0 ± 315.5	3884.0 ± 364.3	0.706
Harvest index (fruit/shoot mass)	2.9 ± 0.64	3.0 ± 0.36	0.854	4.2 ± 0.29	3.8 ± 0.24	0.349

Table 2. Physiological characteristics of the tissue-cultured (TC) and seed-grown (SG) plants. Values are means ± SE, $n = 10$. Observations were recorded on 25th and 53rd days after transplanting.

Trait	25th day TC	SG	*P* value	53rd day TC	SG	*P* value
Photosynthetic rate [μmol(CO_2) m^{-2} s^{-1}]	17.750 ± 0.952	15.480 ± 1.042	0.125	8.983 ± 1.374	7.136 ± 0.859	0.269
Transpiration rate [mmol(H_2O) m^{-2} s^{-1}]	2.213 ± 0.103	2.090 ± 0.187	0.371	1.627 ± 0.152	1.425 ± 0.158	0.370
Stomatal conductance [mmol mol^{-2} s^{-1}]	0.098 ± 0.007	0.081 ± 0.008	0.142	0.067 ± 0.009	0.055 ± 0.008	0.336

cultured plants were unable to develop further in *ex vitro* conditions, and after a few weeks they were replaced by the newly formed normal leaves possessing stomata that functioned similar to those of seed-grown plants. By contrast, Grout and Aston (1978) for cauliflower and Wetzstein and Sommer (1982) for sweet gum reported that the *in vitro* grown plants failed to develop a clearly defined palisade layer.

In the present study, enhancement of the vigour of the tissue culture raised plants was not observed. However, Nikolić *et al*. (1997) stated that the tissue-cultured plants showed improved vigour as these are unintentionally selected for vigour during multiplication and rooting.

Table 3. Fruit quality traits of tissue-cultured (TC) and seed-grown plants (SG). Values are means ± SE, $n = 10$.

Trait	TC	SG	*P* value
Total soluble solids	7.16 ± 0.42	7.20 ± 0.30	0.93
Total solids [%]	8.45 ± 0.42	8.18 ± 0.24	0.58
Firmness [kg]	6.67 ± 0.61	5.44 ± 0.26	0.09
pH	4.08 ± 0.03	4.07 ± 0.04	0.92
Citric acid [g dm^{-3}]	0.46 ± 0.05	0.48 ± 0.03	0.64

Tissue cultured plants are particularly prone to the induction of profuse branching that may affect field performance (Zimmerman 1988). However, in the current experiment a higher number of branches in tissue-cultured plants was not observed, probably because the plantlets were not maintained in the multiplication medium for long time (only for four weeks) and hence did not have carry over effects of the cytokinin that usually occurs in commercial tissue culture laboratories where several cycles of multiplication is undertaken before the plantlets are potted out.

No significant differences in the fresh or dry mass were observed between the tissue-cultured and seed-grown plants, probably due to similarities in the photosynthetic rates of the two plant types. These results contradict those reported for mulberry by Zaman *et al*. (1997). They found that leaf mass of the micropropagated plants was much higher than that obtained for their seed or cutting counterparts. This increase in mass was attributed to increase in protein, mineral content, total sugar, reducing sugar, starch and soluble solids. Pandey and Singh (1988) reported an increase in total and reducing sugars in *in vitro* raised papaya plants, a result similar to

that reported by Swartz *et al*. (1981) for strawberry.

In the current experiment, tissue-cultured plants did not show any phenotypic abnormality for either vegetative or reproductive traits reflecting a non-occurrence of any genetic or epigenetic changes. These results are consistent with the observations of Venkatachalam *et al*. (2000) who found no genetic variation in *in vitro* raised tomato plants. Tyagi *et al.* (2004) also reported that the tissue culture raised plants of *Curcuma* spp. were genetically similar to their parent plants. However, in contrast to the current findings, Somasunder and Gostimsky (1992) recorded 20.6 % of genetically variable plants for agronomically important traits such as early flowering and fruit colour for tomato plants derived from the long-term callus cultures of tomato. In the current experiment the only noticeably significant morphological difference between tissue-cultured and seed-grown plants was the production of higher numbers of leaves and branches by the seed-grown plants at later stage of plant growth. It is presumed that the seed-grown plants remained juvenile for a longer period than the tissue cultured plants and hence they kept on producing shoots and leaves unlike the tissue-cultured plants, and the advanced physiological maturity of tissue cultured plants would have allowed them to divert most of the fixed carbon to reproductive than to vegetative growth.

Precautions were implemented during the development of this propagation protocol. Concentrations and the types of plant growth regulators that induce larger-sized calli were avoided and the cultures were not subcultured for many cycles. These are major factors that possibly assisted in maintaining genetic fidelity of tissue-cultured plants. There is however a possibility that the plants raised from tissue culture could show morphological and genetic variations, if different type of plant growth regulators are used, and most importantly, if subcultured frequently on the multiplication medium. Nevertheless, this study demonstrates that if the protocol is followed as such, the performance of resulting tissue-cultured plants would be comparable with those raised from seeds.

References

Bajaj, Y.P.S., Dhanju, M.S.: Regeneration of plants from callus cultures of Napier grass (*Pennisetum purpureum*). - Plant Sci. Lett. **20**: 343-345, 1981.

Bhatia, P.: Regeneration, Micropropagation and Somatic Embryogenesis in Tomato. (*Lycopersicon esculentum* Mill.). - PhD Thesis. Primary Industries Research Centre, Central Queesland University, Rockhampton 2003.

Chen, C.H., Stenberg, N.E., Ross, J.G.: Clonal propagation of big bluestem by tissue culture. - Crop Sci. **17**: 847-850, 1977.

Deng, L.P., Guo, Y.H., Jia, Y.K., Sheng, J.H.: Preliminary report on cultures of shoot tips of tomato and the yields of plants from culture. - Hereditas **10**: 9-10, 1988.

Diettrich, B., Mertinat, H., Luckner, M.: Reduction of water loss during *ex vitro* acclimatization of micropropagated *Digitalis lanata* clone plants. - Biochem. Physiol. Pflanz. **188**: 23-31, 1992.

Donnelly, D.J., Vidaver, W.E.: Pigment content and gas exchange of red raspberry *in vitro* and *ex vitro*. - J. amer. Soc. hort. Sci. **109**: 177-181, 1984.

Donnelly, D.J., Vidaver, W.E., Lee, K.Y.: The anatomy of tissue cultured red raspberry prior to and after transfer to soil. - Plant Cell Tissue Organ Cult. **4**: 43-50, 1985.

Grout, B.W.W., Aston, M.J.: Modified leaf anatomy of cauliflower plantlets regenerated from meristem culture. - Ann. Bot. **42**: 993-995, 1978.

Henke, R.R., Mansur, M.A., Constantin, M.J.: Organogenesis and plantlet formation from organ- and seedling-derived calli of rice (*Oryza sativa*). - Physiol. Plant. **44**: 11-14, 1978.

Hronková, M., Zahradníčková, H., Šimková, M., Šimek, P., Heydová, A.: The role of abscisic acid in acclimation of plants cultivated *in vitro* to *ex vitro* conditions. - Biol. Plant. **46**: 535-541, 2003.

Kozai, T. Acclimatization of micropropagated plants. - In: Bajaj, Y.P.S. (ed.): Biotechnology in Agriculture and Forestry 17. High-tech and Micropropagation I. Pp. 127-141. Springer-Verlag, Berlin 1991.

Leela, S., Soneji, J.R., Bollamma, K.T.: Analyzing somaclonal variation in micropropagated bananas (*Musa* spp.). - *In Vitro* cell. dev. Biol. Plant **39**: 551-556, 2003.

Nikolić, R., Mitić, N., Nešković, M.: Evaluation of agronomic traits in tissue culture-derived progeny of birdsfoot trefoil. - Plant Cell Tissue Organ Cult. **48**: 67-69, 1997.

Pandey, R.M., Singh, S.P.: Field performance of *in vitro* raised papaya plants. - Indian J. Hort. **45**: 1-2, 1988.

Pospíšilová, J., Solárová, J., Čatský, J.: Photosynthetic responses to stresses during *in vitro* cultivation. - Photosynthetica **26**: 3-18, 1992.

Somasundar, M., Gostimsky, S.A.: Somaclonal variation in tomato tissue culture. - Crop Improvement **19**: 92-96, 1992.

Swartz, H.J., Galletta, G.J., Zimmerman, R.H.: Field performance and phenotypic stability of tissue culture-propagated strawberries. - J. amer. Soc. hort. Sci. **106**: 667-673, 1981.

Tyagi, R.K., Yusuf, A., Dua, P., Agrawal, A.: *In vitro* plant regeneration and genotype conservation of eight wild species of curcuma. - Biol. Plant. **48**: 129-132, 2004.

Venkatachalam, P., Geetha, N., Priya, P., Rajaseger, G., Jayabalan, N.: High frequency plantlet regeneration from hypocotyl explants of tomato (*Lycopersicon esculentum* Mill.) via organogenesis. - Plant Cell Biol. mol. Biol. **1**: 95-100, 2000.

Wetzstein, H.Y., Sommer, H.E.: Leaf anatomy of tissue-cultured *Liquidambar styraciflua* (Hamamelidaceae) during acclimatization. - Amer. J. Bot. **69**: 1579-1586, 1982.

Wu, L., Antonovics, J.: Zinc and copper tolerance of *Agrostis stolonifera* L. in tissue culture. - Amer. J. Bot. **65**: 268-271, 1978.

Zaman, A., Islam, R., Joarder, O.I.: Field performance and biochemical evaluation of micropropagated mulberry plants. - Plant Cell Tissue Organ Cult. **51**: 61-64, 1997.

Zimmerman, R.H.: Micropropagation of woody plants: post tissue culture aspects. - Acta Hort. **227**: 489-499, 1988.