Turkish Journal of Botany

Volume 23 | Number 5 Article 7

1-1-1999

Regeneration and Agrobacterium-mediated TransformationStudies in Tomato (Lycopersicon esculentum Miller)

HÜSEYİN AVNİ ÖKTEM

YETER BÜLBÜL

EMRE ÖKTEM

MERAL YÜCEL

Tr. J. of Botany
23 (1999) 345-348

Research Note

Regeneration and *Agrobacterium*-mediated Transformation Studies in Tomato (*Lycopersicon esculentum* Miller)

Hüseyin Avni ÖKTEM, Yeter BÜLBÜL, Emre ÖKTEM, Meral YÜCEL
Department of Biology, Middle East Technical University, 06531 Ankara-TURKEY

Received: 03.03.1997
Accepted: 22.04.1999

Abstract: An optimized regeneration protocol and a suitable transformation technique is a necessity in obtaining transgenic plants in a given plant species. This work summarizes a simple culture method to obtain rooted plants from leaf pieces of two tomato cultivars (ES58 and WC156) within 3-4 months. Here, we also present our preliminary results on transformation experiments carried out with 8-day-old cotyledon explants and *Agrobacterium tumefaciens* EHA105 strain.

Key Words: Tomato, regeneration, *Agrobacterium*, transformation, *Lycopersicon*

Domatesde (*Lycopersicon esculentum*) Rejenerasyon ve *Agrobacterium* Aracılığı ile Transformasyon Çalışmaları

Özet: Bir bitki türünde transgenik bitkilerin eldesi için optimize edilmiş bir rejenerasyon yöntemi ve uygun bir gen aktarım sistemi en önemli öğelerdir. Sunulan çalışmada iki farklı domates çeşidinde (ES58 ve WC156) yaprak parçacıklarından 3-4 aylık bir süreçte köklenmiş bitkilerin eldesini olası kılan basit bir kültür yöntemi özetlenmektedir. Ayrıca bu raporda, 8 günlük kotiledon dokusunda ve *Agrobacteriumtumefaciens* EHA105 suşu ile yürütülen transformasyon çalışmalarından elde ettiğimiz ön sonuçlarda sunulmaktadır.

Anahtar Sözcükler: Domates, rejenerasyon, *Agrobacterium,* transformasyon, *Lycopersicon*

Introduction

In different plant species different gene transfer protocols are applicable (1). In such studies, transformation frequency is one of the most important limiting factors in obtaining transgenic plants. In addition, a reliable regeneration protocol for the target plant material is another important parameter. Therefore, prior to transformation work, culture conditions for plant regeneration have to be optimized for a given plant species and type of explant.

For the tomato (*Lycopersicon esculentum* Miller), plant regeneration from different explants, such as leaf disks (2), cotyledon (3) and protoplasts (4), is possible. In such studies, it has been clearly demonstrated that regeneration capacity and transformation frequencies show variation among cultivars.

Currently, in our laboratory we have been trying to optimize conditions for regeneration and *Agrobacterium*-mediated transformation in the tomato in order to develop transgenic plants. In this respect, we have studied regeneration conditions for leaf pieces of two tomato cultivars (ES58 and WC156). This report presents the data obtained from regeneration experiments together with preliminary data on transformation work.

Materials and Methods

Fully expanded leaves of soil-grown, 2- to 3-month-old plants were surface sterilized in 2% sodium hypochloride for 10 minutes and rinsed three times with sterile distilled water. After removal of the midribs, the remaining parts of the leaves were sectioned into 1-2 cm2 random pieces. Hereafter, these explants will be referred to as leaf pieces.

For callus and shoot formation, leaf pieces were cultured (upside-down) on a Murashige and Skoog-based (MS) medium composed of MS salts (5), Gamborg's (6) vitamins, 2.5 mg/l benzylamino purine (BAP), 0.2 mg/l indole acetic acid (IAA), 3% sucrose and 0.7% (w/v) agar (MSA medium). pH was adjusted to 5.7 with NaOH (before adding agar) and the medium was sterilized by autoclaving at 121°C for 15 minutes at 1 kg/cm^2

pressure. Culture plates were incubated at 25˚C, 16 hours light cycle (white fluorescent tubes-2000 lux) and subcultured every 3 weeks. After callus and shoot development, the shoots were excised from the callus and transferred to MSC medium (MSA medium without growth regulators) without (MSC-) or with IAA (MSC+) for root formation and further development of the shoots.

Results and Discussion

The results presented are the average of 2 independent experiments with 25 explants in each trial. In both cultivars, callus formation was observed in more than 90% of the cultured leaf pieces in 15-25 days. Within 4-5 weeks after culturing, well-developed shoots appeared on leaf pieces of both cutivars. A representative result is given in Figure 1. After the 8th week of the of culture period, 70% of the leaf pieces in cv. ES58 developed shoots, whereas this value was around 50% in cv. WC156. Single-shoot formation was generally observed in cv. WC156, whereas multiple-shoot formation occurred in cv. ES58.

In cv. ES58, the presence of IAA in the rooting medium seemed to be important since no rooting was observed in the absence of IAA in the MSC medium (Figure 2). Preliminary work with cv. WC156 showed that 50% of the shoots obtained from this cultivar were able to develop roots in the absence of IAA (Figure 3). Currently, rooting response in the presence of IAA is also under investigation. In both cultivars, the first rooting response was observed around the 10th day of incubation on MSC medium, and after 4-6 weeks, the plants developed enough to be transferred to soil.

Currently, our experiments are focused on regeneration studies in hypocotyl and cotyledon explants together with *Agrobacterium*-mediated transformation trials on various explants. Our transformation work revealed that transformation frequency showed drastic variations according to explant type (cotyledon, hypocotyl or leaf pieces) and *Agrobacterium tumefaciens* strain (LBA4404 or EHA105). For example, transformation experiments with LBA4404 hypocotyl combination revealed no kanamycin-resistant calli (4 independent sets of experiments with 40 explants each). Similarly, experiment with LBA4404 leaf piece combination resulted with no success (2 independent sets of experiments with 30 explants each). Recently, by using 8-14 days old cotyledonary explants and pGUSINT (7) harboring supervirulent *Agrobacterium tumefaciens* EHA105 strains, we were able to obtain kanamycin-resistant callus and shoot formation in cv. WC156 (Figure 4). In view of

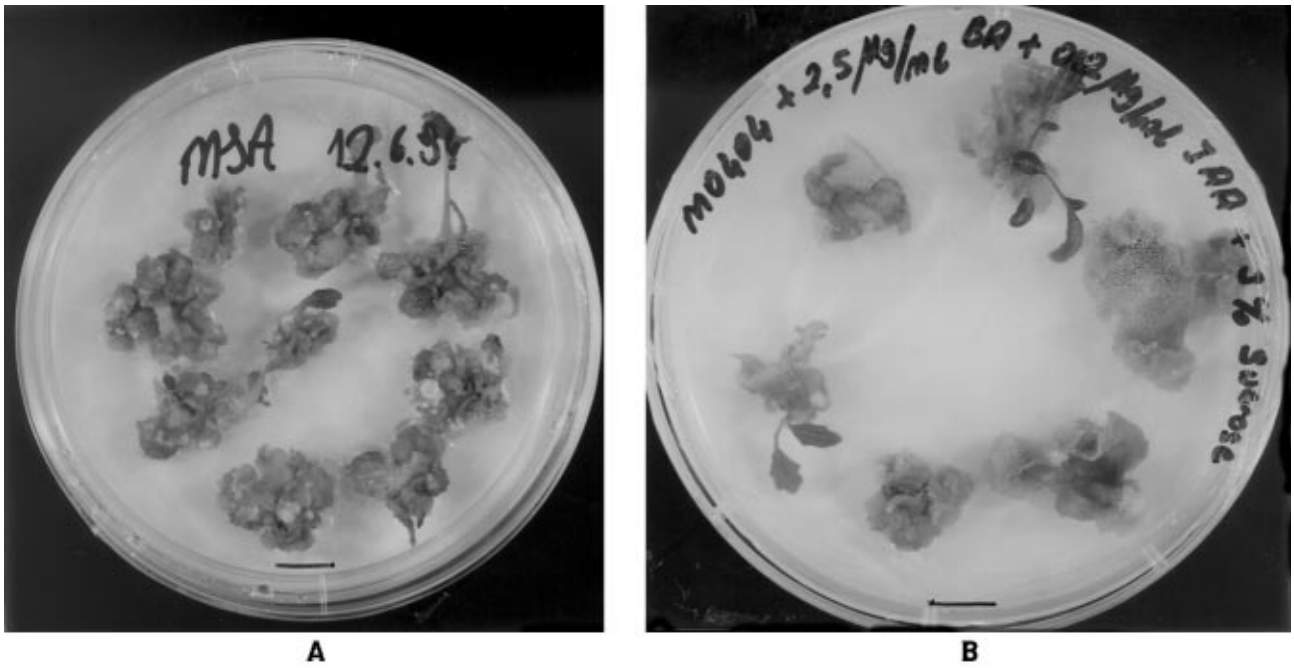

Figure 1. Callus and shoot development in leaf pieces of tomato. Five-week development of the leaf pieces prepared from 3-month-old tomato plants of cvs. ES58 (A) and WC156 (B).

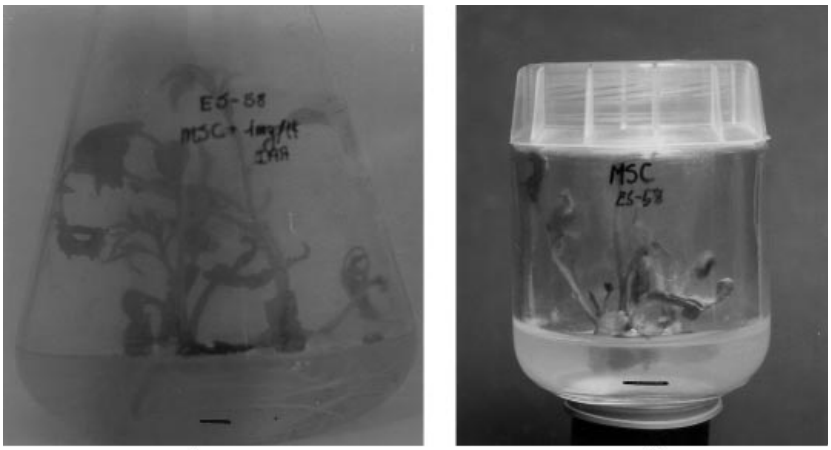

Figure 2. Root formation in cv. ES58. Five-week development of regenerated shoots of cv. ES58 in root inducing medium (MSC) with (A) and without (B) IAA.

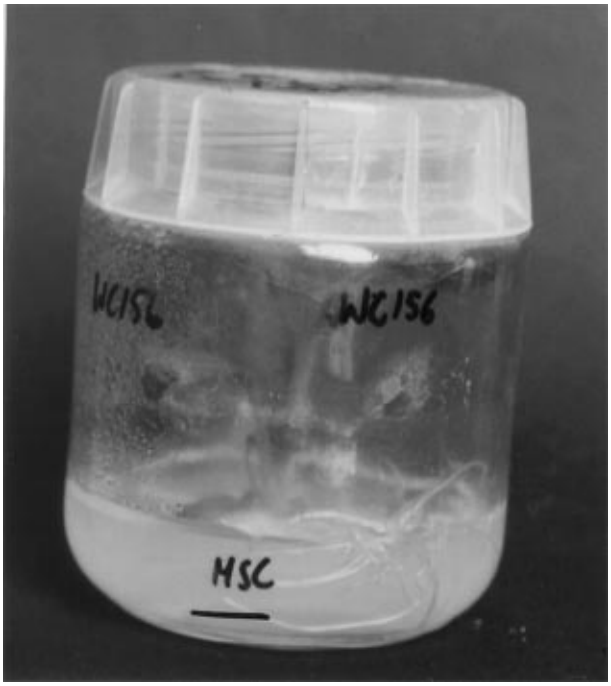

Figure 3. Root formation in cv. WC156. Four-weeks development of regenerated shoots of cv. WC156 in root inducing medium (MSC) without IAA.

these findings, the *Agrobacterium* strain seems to be the most critical factor in our experimental system. Currently, transformation work with EHA105 strain and leaf pieces is in progress.

Our future goal is to increase the transformation frequency and, as we have done in tobacco, to obtain herbicide resistant transgenic tomato plants via transfer of the *bar* gene (8).

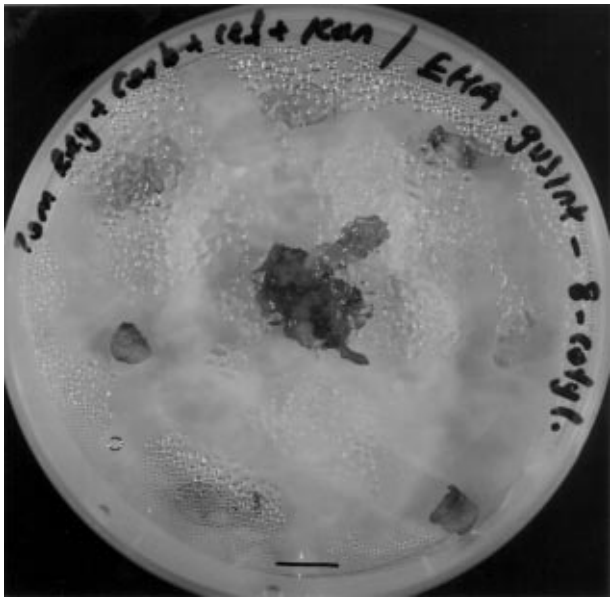

Figure 3. Callus and shoot initiation on the transformed cotyledonary explants in the presence of 50 mg/l kanamycin. Eight-days old cotyledons of cv. WC156 were transformed with *Agrobacterium tumefaciens* EHA105 strain harboring pGUSINT binary vector. A modified transformation procedure of van Roekel (3) was followed. The photograph demonstrates 8 weeks development after the transformation process.

Acknowledgements

Prof. Dr. Lothar Willmitzer (Inst. Genbiologische, Berlin, Germany) and Dr. Theo C. Verwoerd (Mogen International nv, Leiden, The Netherlands) are gratefully acknowledged for providing pGUSINT binary vector and *A. tumefaciens* EHA105 strain, respectively.

References

1. Potrykus I., Spangenberg G. (eds.), Gene transfer to plants, Springer Publications, The Netherlands (1995).
2. McCormic S., Niedermeyer J., Fry B., Barnason A., Horch R., Farley R., Leaf disk transformation of cultivated tomato (*L. esculentum*) using *Agrobacterium tumefaciens.* Plant Cell Rep., 5, 81-84 (1986).
3. van Roekel J.S.C., Damm B., Melchers L.S., Hoekema A., Factors influencing transformation frequency of tomato. Plant Cell Rep., 12, 644-647 (1993).
4. Roest S., Gilissen J.W., Plant regeneration from protoplasts: a literature review. Acta Bot. Neerl., 38, 1-23 (1989).
5. Murashige, T., Skoog, F., A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant, 15, 473-497 (1962).
6. Gamborg, O., Miller, R., Ojima, K., Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res., 50, 151-159 (1968).
7. Vancanneyt G., Schmidt R., O'Conor-Sanchez A., Willmitzer L., Rocha-Sosa M., Construction of an intron containing marker gene: Splicing of the intron in transgenic plants and its use in monitoring early events in *Agrobacterium*-mediated plant transformation. Mol. Gen. Genet., 220:245-250 (1990).
8. Öktem H.A.: "Development of Phosphinotricin Resistant Fertile Transgenic Tobacco Plants", ACTA Agronomica Hungarica, 46:97-104 (1998).