The Histogenesis of Somaclones from Tomato (*Lycopersicon esculentum* Mill.) Cotyledons

B. Monacelli¹, M. M. Altamura¹, G. Pasqua¹, M. G. Biasini², and F. Sala³,*

¹ Dipartimento di Biologia Vegetale, Università “La Sapienza”, Roma, ² Dipartimento di Genetica e Microbiologia, Università di Pavia, ³ Istituto di Botanica, Università di Parma

Received July 20, 1987
Accepted September 28, 1987

**Summary**

A histological study of *in vitro* cultured cotyledonary explants of tomato (*Lycopersicon esculentum*) was performed in order to determine the site (differentiated tissue or developing callus) and the mode of plant regeneration. Results have shown that callus develops at the excision sites of cotyledonary explants and that shoots are formed exclusively within the unorganized callus: excision areas are the only morphogenetic sites and the proximal excision is the preferred site for plant regeneration. Shoots differentiate by organogenesis within the superficial region of the callus. Few neocambial cells cooperate in the neoformation. Origin from a single cell is highly unlikely since rarely observed single activated cells never developed into shoots. Regenerated plants may be chimeras if *in vitro* culture induces genetic diversity in the initial cells.

**Keywords:** Tomato; *Lycopersicon esculentum*; Somaclonal variation; Plant regeneration.

**Abbreviations:** IAA Indole-3-acetic acid; c callus; d vegetative dome; s shoot; ad adaxial; ab abaxial; t tracheid; p parenchyma; S sieve tube.

**1. Introduction**

Plants regenerated from *in vitro* cultures of somatic cells very frequently differ from the original phenotype. This phenomenon, which has been designated “somaclonal variation” (Larkin and Scowcroft 1981), is confined to plants regenerated from dedifferentiated cells. In fact, high genetic uniformity is obtained and often exploited for commercial purposes when plants develop from meristematic tissues. Progeny analysis has revealed that somaclonal variation is genetically based although the nature of the changes induced in somaclones DNA is still a matter of study. Thus, somaclonal variation has now been recognized as a powerful new tool to induce exploitable genetic variability in plants (Evans and Sharp 1986, Fox 1986, Sala and Biasini 1986).

Tomato has been frequently used for these studies, both because of the accumulated information on its genetics and the interest of the breeder for the selection of improved cultivars (Evans and Sharp 1983, Buatti et al. 1984, Gavazzi et al. 1987). We have already shown that regeneration from *in vitro* culture of tomato cotyledons not only produces more mutations than application of a chemical mutagen (ethyl metane sulphonate) to seeds, pollen or both, but also that the mutant types differ, with some mutant classes arising only from somaclonal variation. The latency of some mutant traits in the first generation, and their appearance in the homozygous state in the second and successive generations (Gavazzi et al. 1987) is also peculiar to regenerated plants.

In order to better understand this unusual genetic behaviour it was of interest to define the site (organized plant tissue or developing callus) and the mode of shoot
differentiation from tomato cotyledons cultured in vitro. Under different experimental conditions in different species plants have been shown to arise by embryogenesis or by organogenesis. Although conclusive evidence is lacking, embryogenesis is usually postulated as initiated from a single cell, while organogenesis should involve several initial cells. An important consequence of the latter is that mutations affecting a single cell may result in chimeral first generation plants.

Progeny analysis of regenerated tomato plants induced EVANS and SHARP (1983) to propose a unicellular origin, while BUIATTI et al. (1984) suggested a multicellular origin.

The result presented in this paper show that plant regeneration from tomato cotyledons always occurs through callus formation and that shoots differentiate through organogenesis from a few initial cells within the callus. These findings support the occasional occurrence of chimeral first generation plants (BUIATTI et al. 1984).

2. Materials and Methods

2.1. Materials

Seeds of the commercial variety (L50) of Lycopersicon esculentum Mill. were kindly supplied by Oris, Milano. Indole-3-acetic acid (IAA) and zeatin riboside were obtained from Sigma, agar from Difco and paraffin from Merck.

2.2. Seedling Growth

Seeds were sterilized in a 1% sodium hypochlorite solution with 0.04% Teepol 610 for 20 minutes under vacuum, washed 4 times in sterile water and sown in 1/2 MS medium (MURASHIGE and SKOOG 1962) containing 10 g/l sucrose and 0.8% agar (pH 5.8). Growth was at 26°C and 4,000 lux (fluorescent light, Philips TLD 58 W/83).

2.3. Induction of Callus Growth and Differentiation

Somaclones were produced as described by GAVAZZI et al. (1987). Cotyledons (16–18 × 3–4 mm in size) were excised on the 10th day of seedling growth, sectioned as shown in Fig. 1 and plated on MS medium containing 30 g/l sucrose, 0.8% agar, 2 × 10⁻⁴M IAA and 2 × 10⁻⁶M zeatin riboside (pH 5.8). Incubation was at 26°C and 10,000 lux. Callus was produced from the injured regions and shoots differentiated from this, as shown in Fig. 1.

2.4. Histological Analysis

Each day fragments of the apical, median and basal cotyledon explants were fixed in F.A.A. (70% ethanol: glacial acetic acid: formalin = 18:1:1; by volume), dehydrated, embedded in paraffin (melting point: 52–54°C), sectioned at 10 μm and stained with eosin and Carazzi’s haemalum (MAZZI 1977).

3. Results

3.1. Time Course of Morphogenesis

Basal, median and apical regions of tomato cotyledons were excised, plated and cultured as described above.

Fig. 1. Outline of the developmental events during callus (c) induction and morphogenesis of vegetative domes (d) and shoots (s), on the basal, median and apical explants of tomato cotyledonary laminae during in vitro culture. The figure shows the adaxial side. Swelling of midrib area at the proximal excision site of the basal (on the 2nd day), median and apical (on the 3rd–4th day) explants was followed by callus formation protruding in the same areas from the centre to the margin (5th day). At day 5 calli had also started to develop at the distal excision sites (same pattern). On day 7 most explants showed calli, although of different size. A basipetal gradient of callus development was evident, with the proximal region of the basal explant showing the most abundant callus growth. Small vegetative domes (d) were visible on the largest calli from day 7. Relative abundance of shoots (s) at day 11 of culture is shown. Shoots continued to be formed on the developing callus over the following 2–3 weeks with a similar relative frequency to that depicted for day 11. Meristematic centres and shoots were mostly visible near the midrib on callus formed at the proximal excision sites. After day 11, small calli were noticed on the adaxial surface by lateral veins. Soon afterwards sporadic callus formation occurred at adaxial marginal regions. At this time the cotyledonal laminae had rolled towards the abaxial side.

The development of callus and morphogenesis was followed at 24 hours intervals in several replicas. The essential events are shown in Fig. 1. Callus growth occurs exclusively at the sites of excision and shoots differentiate from these (Figs. 1–3). In over one hundred tested cotyledonal explants, callus formation and subsequent shoot differentiation showed polarity, being consistently more abundant at the proximal excision site of each explant compared to the distal site. The