A genetic analysis of cell culture traits in tomato

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Received March 7, 1987; Accepted March 20, 1987
Communicated by I. Potrykus

Summary. Tomato genotypes superior in regenerating plants from protoplast and callus cultures were obtained by transferring regeneration capacity from *Lycopersicon peruvianum* into *L. esculentum* by classical breeding. The genetics of regeneration and callus growth have been studied in selfed and backcross progenies of a selected plant (MsK93) which has 25% *L. peruvianum* in its ancestry. Segregation data showed that the favourable cell culture traits of *L. peruvianum* are dominant. Regeneration capacity from established callus cultures was controlled by two dominant genes. Callus growth on primary explants, callus growth of established cultures and shoot regeneration from explants had high heritabilities (0.47, 0.78, 0.87, respectively). Callus growth and regeneration capacity were not correlated within the populations studied.

Key words: *Lycopersicon* – Protoplasts – Regeneration – Callus growth

Introduction

The behaviour of plants in cell and tissue culture is determined to a large extent by medium composition and culture conditions. However, the plant species used and even the genotype within the species are also important additional factors. Intraspecific differences have often been described and analysed for particular tissue culture systems only. Examples of such genetic studies are the induction of callus on maize explants (Nesticky et al. 1983; Tomes and Smith 1985), the morphogenic response of explants in cauliflower (Buatti et al. 1974), the response to anther culture in potato (Jacobsen and Sopory 1978) and the regeneration of shootbuds from dedifferentiated callus tissue in *Medicago* (Reisch and Bingham 1980). The interaction of genetic differences with plant hormone composition in the media has been studied in *Petunia* (Izhar and Power 1977; Skvirsky et al. 1984) and *Phaseolus* (Mok et al. 1980). For tomato (*Lycopersicon esculentum* Mill.), differences between cultivars and mutants in shoot and callus induction on primary explants have been described by many authors (Padmanabhan et al. 1974; Behki and Lesley 1976; Tal et al. 1977; Ohki et al. 1978; Frankenberger et al. 1981a; Morgan and Cocking 1982; Kurtz and Lineberger 1983; Zelcer et al. 1984). Only in two cases (Ohki et al. 1978; Frankenberger et al. 1981b) were the cultivar differences analysed by comparing hybrids with their parents. Differences between cultivars for the regeneration of shoots from subcultured callus, which in general is difficult in tomato (Locky 1983), were reported by Meredith (1979) and Tatchell and Binns (1986).

Compared to *L. esculentum*, the related species *L. peruvianum* is much easier to regenerate from long-term callus cultures without preorganized areas (Morgan and Cocking 1982; Locky 1983) and from protoplasts (Zapata et al. 1977; Mühlbach 1980; Thomas and Pratt 1981a). Also, callus growth is more abundant in *L. peruvianum*. Thomas and Pratt (1981a) suggested transferring these favourable cell culture traits from this species into *L. esculentum*. Easy regenerating tomato genotypes allow the efficient application of cell biological techniques for both basic research in a plant species that genetically is well characterized and for the genetic improvement of this important crop species. In a programme designed to obtain these genotypes (Koornneef et al. 1986), callus growth characteristics and the ability of both primary explants and subcultured callus were analysed in segregating populations derived from hybrids of *L. esculentum* and *L. peruvianum*.
Materials and methods

Plant materials

An F₃ hybrid population of *L. peruvianum × L. esculentum* (IVT741505) and an *L. peruvianum* strain (PI 128650) were donated by Dr. Hogenboom of the Institute of Horticultural Plant Breeding (IVT), Wageningen, The Netherlands. The pedigree of this material is described in Koornneef et al. (1986). The F₃ population was screened for plants with the ability to regenerate shoots on callus that had been in culture for at least 1 year. Upon crossing such plants with the male sterile mutant *ms-lO* in *L. esculentum* cultivar VFI1, one plant (K93) gave a single offspring. This backcross hybrid plant (F₁ MsK93) was selfed to give F₂ MsK93, and was also backcrossed to VFI1. The F₃ population was screened for plants that were easy to regenerate from subcultured callus and that were crossable with VFI1. One F₂ plant (F₂ MsK93-19) fulfilled both criteria and from its backcross to VFI1, two plants were selected for further analysis (MsK8 and MsK9). The pedigree of the material analysed genetically for cell culture traits is shown in Fig. 1.

The male sterile mutant, *ms-lO* in VFI1 background was a gift from Prof. Rick, Davis, USA and was maintained by seed propagation; the cultivar Bellina was a gift from the Rijk Zwaan Seed Company and the cultivar Moneymaker from Nunhems Seed Company, both in The Netherlands.

Plants of K93, MsK93, MsK8 and MsK9 were propagated by cuttings both in vitro and in vivo. Vegetative propagation was facilitated by the apparent resistance to tobacco mosaic virus of this material.

Tissue culture and protoplast techniques

Leaf discs (5 mm) were punched from surface-sterilized leaves of plants grown in a greenhouse. For shoot induction, these explants were placed on 2Z medium (Thomas and Pratt 1981b). Callus was induced on similar explants on R3B medium (Meredith 1979) with vitamins (T) as in Tewes et al. (1984). This medium was used for all callus cultures. Plant regeneration on callus was achieved by repeated transfers to 2Z medium. All cultures were grown at 25 °C and at 16 hour light (approx. 2000 lx) and transferred to new plates every 4 weeks. In all experiments 9 cm plastic Greiner Petri dishes were used.

Several cell-culture traits were assayed per individual donor plant according to the scheme of Fig. 2. Shoot-like structures were counted on the 3 best looking (out of 6) explants placed on 2Z medium for 4 weeks. At the same time, callus weight (plus original explant tissue) was determined for the three (out of six) best looking R3B explants. Six approx. 15 mg calli from the remaining pieces were transferred to new R3B plates. From these cultures, 6 calli were transferred again to R3B medium after 4 weeks; thereafter this “established” callus was used to assay the relative growth rate (RGR) by determining the initial weight and that after 4 weeks. From the same source of callus material used for this RGR assay, 10 pieces were placed on 2Z medium. After 4 weeks, the best looking (green or shoot primordia containing) parts were transferred from each of these 10 calli. If after another 4 weeks on 2Z medium at least 1 of these calli showed a clearly visible shoot bud, the original plant was said to be regenerable from an established callus culture.

For protoplast isolation, plants were grown sterile in glass containers with Murashige Skoog (MS) salts, vitamins as in Tewes et al. (1984) and 1% sucrose. The plants were kept in the dark 1 day prior to harvesting of leaflets. These leaflets were floated in the dark at 4 °C on a preincubation medium: ½ strength MS salts, T vitamins, 0.5 mg l⁻¹ benzylaminopurine (BA) and 1 mg l⁻¹ 2,4-dichlorophenoxyacetic acid (2,4-D). The leaflets were subsequently incubated for 16 h in an enzyme solution: 0.6% Cellulase RS and 0.2% Macerozym dissolved in CPW salts (Zapata 1981), and 73 g l⁻¹ mannitol.

![Pedigree of MsK93 and its progeny](image_url)

Fig. 1. Pedigree of MsK93 and its progeny. ♘: selfing