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Initiation of direct and indirect cell suspension cultures of *L. chilense* Dun. was confined to the origin of auxin in the culture medium. Direct cell suspensions were initiated in MSPI culture medium containing NAA while indirect suspensions were initiated in culture media UM and UMI containing 2,4-D. The growth of direct and indirect cell suspensions was distinguished by the growth rate and phenolic production. Direct suspensions have shown a continuous growth in growth curve studies (8 day) and recorded a significantly higher cell growth in comparison to two other indirect cell suspensions. In indirect suspensions, 2,4-D toxicity arose after 5 months of culture and as a consequence loss of vigour followed by phenolic browning occurred. Direct cell suspensions proved to be long-term cultures without any adverse effects of NAA on the growth rate and were genetically stable. Higher protoplast yields were obtained from the direct cell suspension cultures which were associated with the increased growth rate. A productive protoplast-to-plant system was developed.

Keywords: *Lycopersicon chilense*, cell suspension cultures, protoplasts, plant regeneration

Introduction

In the genus *Lycopersicon*, the species of *peruvianum* group are sexually separated from the *esculentum* group and consists of two species, *L. peruvianum* Mill. and *L. chilense* Dun. (Rick, 1979). *L. chilense* possesses useful genes for tomato improvement such as resistance to fungal and viral diseases (Stevens & Rick, 1985; Sotirova & Rodeva, 1990), drought and salinity tolerance (Jones, 1987) and low temperature tolerance (Stevens & Rick, 1985). In somatic hybridization of tomato (*L. esculentum*) with members of *peruvianum* group, *L. chilense* was a later addition (Bonnema & O’Connell, 1992) to the earlier success with *L. peruvianum* (Kinsara *et al*, 1986). The major constraint in *L.

chilense* protoplast based genetic manipulation was the difficulties in isolation of protoplasts from explants. Alternatively, efforts were made to initiate cell suspension culture of *L. chilense*.

With the cell suspension culture studies, Greer & Tabaeizadeh (1991) reported plant regeneration from callus culture. Latif *et al* (1993) demonstrated protoplast-to-plant system while Bonnema & O’Connell (1992) reported somatic hybridization. However, in spite of these attempts, the regular source for protoplasts of *L. chilense* remained an unresolved problem. In preliminary studies, these three protocols were verified particularly for long-term cultures but all attempted cultures became necrotic after certain subculturings. It was interesting to note that in all these protocols 2.0 mg l⁻¹ 2,4-D was the common auxin source and these cultures were indirectly initiated from the cultured friable callus. Therefore, in the present investigation, the effects of three culture media and two initiation methods are evaluated on development of cell suspension culture of *L. chilense* and assessed further for protoplast culture.

Material and Methods

Plant Material and Growth Conditions

The seeds of *L. chilense* (accession CA 2930) were obtained from Tomato Genetic Center, University of
California, Davis, USA and axenic seedlings were developed at 25°C with 16 hrs photoperiod (40 μmol m⁻² s⁻¹).

Callus Induction from in vitro Grown Plant-explants

Stem explants (0.5 cm in length), taken from 28-day-old in vitro grown plants, were used for callus induction on agar-solidified MSP1 medium (MS medium with 2.0 mg l⁻¹ NAA and 0.5 mg l⁻¹ BAP) with the same environmental conditions as described for the axenic shoot cultures. After three subculturings (21 day) on agar-solidified MSP1 medium, embryogenic calli were taken for cell suspension culture initiation studies.

Initiation of cell suspension cultures. Three liquid culture media, MSP1, UM [a modified MS medium by Uchimiya & Murashige (1974) containing 2.0 mg l⁻¹ 2,4-D and 0.25 mg l⁻¹ Kinetin] and UMI (UM medium supplemented with 0.1 mg l⁻¹ IAA; Latif et al, 1993) were used to initiate both direct and indirect cell suspension cultures.

Indirect cell suspension cultures. Indirect cell suspension cultures were initiated by transferring 1.0 g f wt of callus to 50 ml liquid medium in 250 ml Erlenmeyer flasks. Suspension cultures were incubated on an orbital shaker (120 rpm) under the similar environmental conditions as that of axenic shoot cultures. Four successive (7 day) subcultures were carried out by the replacement of 40 ml of spent medium per flask with an equal volume of fresh medium, after allowing the cells to settle down for 15-20 min. Large cell clumps were removed at each subculture, using a 500 μm pore size nylon sieve, whereupon fine cells were subcultured on every 7th day. Thereafter, a 3.0 ml packed cell volume (PCV) of fine-loose aggregates with 7 ml of cultured medium was transferred to 40 ml of fresh medium.

Direct cell suspension cultures. Direct cell suspension cultures were initiated by culturing single cells in liquid media. To obtain a single cell population, 1.5 g f wt callus was gently chopped with a sharp blade and the fine pieces were placed into 50 ml of liquid media in 250 ml Erlenmeyer flasks. Cultures were incubated on an orbital shaker (90 rpm) with cultural conditions of 16 hrs photoperiod (20 μmol m⁻² s⁻¹) and 25±2°C temperature. Seven day cultures were filtered through a fine nylon sieve (125 μm mesh size) and the filtrate examined under a microscope for the presence of homogenous single cell populations. Thereafter, 8 successive subcultures (on every 7th day) were performed by pipetting 10 ml of cell cultures to 50 ml fresh liquid medium with the same culture conditions as described earlier. At each subculture, suspensions were microscopically examined for cell shape, size and division. After forming a loose aggregate of 10-20 cytoplasmic globular cells (8 subculture period), cell cultures were subcultured on every 7th day by pipetting 3 ml of PCV with 7 ml of cultured medium to 40 ml of fresh medium (total volume 50 ml).

Growth assessment for cell suspension cultures. The growth of cell suspensions was determined by measuring the mean PCV under 5 replicates. To determine the PCV, 10 ml of samples were pipetted 3 times from well dispersed suspension cultures and centrifuged in 15 ml graduated centrifuged tubes at 80 x g for 5 min and measured under percentage of volume of packed cells over total volume.

Growth curve of the cell suspensions was studied by using 4 and 8 month old cultures and measuring the PCV daily for 8 days (Fig. 1). The growth of long-term cell suspension cultures was checked by measuring PCV on 8th day after subculture (Table 1). While growth rate of cell suspension cultures was determined as the "ratio of difference between initial (day 0) and final (day 8) PCV to the initial PCV (Y-X/X; Table 1). In this case, the value of initial PCV (X) was constant as 0.6 ml/10 ml medium (cell suspensions were subcultured with 3.0 ml of PCV/50 ml of medium). Observations were also recorded on colour of cell suspension and phenolic browning in the culture.

Protoplast Isolation, Culture and Plant Regeneration

After 4 months of culture, well established cell suspension cultures were used for protoplast isolation. The peak rise in the growth of the cultures (4th day) was selected for the enzymatic cell wall digestion. Cell suspension cultures were initially filtered through 250 μm pore size nylon sieve to obtain homogeneous fine cell aggregates (5 g f wt) and plasmolyzed in 20 ml CPW salt solution (Frearson et al, 1973) with 9% (w/v) mannitol at pH 5.8 in 14 cm Petri dishes (1 hr dark at 25°C). The plasmolysis solution was replaced by 20 ml of a filter sterilised enzyme mixture, which consisted of 0.3% (w/v) Cellulase RS (Yakult Honsha Co Ltd, Hyogo, Japan) combined with 0.03% (w/v) Pectolyase Y23 (Seishin Pharmaceutical Co Ltd, Tokyo, Japan), 5 mM MES, CPW salts, 8.4% mannitol at pH 5.6. Petri dishes were sealed with Nescofilm (Brando Chemical Ind. Ltd, Kobe, Japan).
and cells were incubated in the dark for 10-14 hrs at 25°C on slow rotary shaker (40 rpm). Released protoplasts were filtered through a 64 μm pore size nylon sieve and centrifuged (80 × g, 5 min). The pellets of protoplasts were washed in 10 ml of W5 washing solution (Menczel et al, 1981) by resuspension and centrifugation (80 × g, 5 min). The pellets were then resuspended in 10 ml of Flotation solution (Wijbrandi et al, 1990) and gently topped with 2.0 ml of W5 solution, prior to centrifugation (100 × g, 10 min). The protoplasts obtained at the Flotation/W5 solution interface were removed and resuspended again in 10 ml of W5 solution. Protoplasts were counted by using a haemocytometer and the viability was measured with fluorescein diacetate (Larkin, 1976).

After centrifugation (80 × g, 5 min) the protoplast pellet was resuspended and cultured in TMP liquid medium (Wijbrandi et al, 1990) at 1×10^5 ml^(-1) plating density (3 ml/5 cm Petri dish; Nunc, Denmark) at 25±1°C in the dark. By day 4 and after the first mitotic division, culture dishes were diluted every 3rd day by the addition of 3.0 ml of TMd liquid medium (Wijbrandi et al, 1990) and exposed to low light (20 μmol m^(-2)s^(-1), 16 hrs photoperiod). Plating efficiency of 8 day cultures was recorded as the percentage of dividing cells. After 12 days, 1-2 mm fast growing P-derived tissues from the culture were transferred onto agar-solidified TMc medium (Wijbrandi et al, 1990) and chlorophyllous P-derived calli (21 days) were recovered.

P-derived calli were transferred to a regeneration medium, consisted of MS salts, 2.0% (w/v) sucrose, 1.8 % (w/v) mannitol, 0.8% (w/v) agar (Sigma), 0.1 mg l^-1 IAA and 2.0 mg l^-1 zeatin, pH 5.8 (3 calli/175 ml capacity glass jar with 50 ml medium) and kept at 25±1°C and 16 hrs photoperiod (40 μmol m^(-2)s^(-1)). However, for actual shoot regeneration, 2-4 more subculturings (21 days) were required on this medium but containing no mannitol.

**Results**

**Callus and Suspension Culture Initiation**

The hypocotyl-derived callus of *L. chilense* on agar-solidified MSP1 medium was characterised as being chlorophyllous, fast growing and nodular callus. However, for the initiation of direct and indirect suspension cultures, the marked effects of culture media were noticed particularly between the media containing auxins such as NAA and 2,4-D.

For initiation of the indirect suspension cultures, the liquid media containing 2,4-D [UM and UMI] were the most effective. Within the first 4 subculturings these media gave rise loose, friable cell
Table 1—Effect of culture media containing various auxins on long-term cell suspension cultures of *L. chilense* in respect of packed cell volume (PCV), growth rate and protoplast culture

<table>
<thead>
<tr>
<th>Cell suspension medium</th>
<th>PCV (%) (Growth rate) 8 day post subculture</th>
<th>Protoplast yield (×10⁶ ml⁻¹) (4 day post subculture)</th>
<th>Protoplast viability (%)</th>
<th>Planting efficiency (×10⁵ ml⁻¹; 8 day) (%)</th>
<th>Caulogenic response of P-derived calli (%)</th>
<th>P-derived calli giving one or more shoots</th>
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<tbody>
<tr>
<td>4-month-old cultures</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>UM</td>
<td>24.8 (3.13)</td>
<td>5.66</td>
<td>83.6</td>
<td>48.4</td>
<td>61.4</td>
<td>36.3</td>
</tr>
<tr>
<td>UMI</td>
<td>27.7 (3.61)</td>
<td>6.86</td>
<td>89.8</td>
<td>51.8</td>
<td>64.1</td>
<td>39.2</td>
</tr>
<tr>
<td>MSP1</td>
<td>37.8 (5.30)</td>
<td>8.84</td>
<td>94.2</td>
<td>58.6</td>
<td>75.6</td>
<td>46.9</td>
</tr>
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<td>5-month-old cultures</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>UM</td>
<td>14.2 (1.36)</td>
<td>2.75</td>
<td>53.2</td>
<td>32.5</td>
<td>53.7</td>
<td>25.2</td>
</tr>
<tr>
<td>UMI</td>
<td>18.6 (2.10)</td>
<td>3.63</td>
<td>64.9</td>
<td>39.7</td>
<td>55.0</td>
<td>28.7</td>
</tr>
<tr>
<td>MSP1</td>
<td>38.7 (5.45)</td>
<td>8.95</td>
<td>92.6</td>
<td>60.8</td>
<td>78.5</td>
<td>52.4</td>
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<td></td>
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<tr>
<td>MSP1</td>
<td>43.0 (6.16)</td>
<td>13.56</td>
<td>93.8</td>
<td>64.1</td>
<td>80.2</td>
<td>50.6</td>
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<tr>
<td>16-months-old cultures</td>
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<tr>
<td>MSP1</td>
<td>44.7 (6.45)</td>
<td>14.02</td>
<td>90.9</td>
<td>62.7</td>
<td>77.3</td>
<td>54.7</td>
</tr>
<tr>
<td>SED ±</td>
<td>0.7 (0.16)</td>
<td>0.60</td>
<td>3.8</td>
<td>5.6</td>
<td>5.1</td>
<td>4.7</td>
</tr>
<tr>
<td>LSD 5%</td>
<td>1.8 (0.41)</td>
<td>1.42</td>
<td>7.9</td>
<td>11.5</td>
<td>10.9</td>
<td>9.8</td>
</tr>
</tbody>
</table>

Medium for cell suspension culture: **Indirect** cell suspensions were initiated in UM and UMI culture media containing auxin, 2 mg l⁻¹ 2,4-D and 2 mg l⁻¹ 2,4-D + 0.1 mg l⁻¹ IAA respectively, while **direct** cell suspensions were initiated in MSP1 culture medium containing auxin, 2.0 mg l⁻¹ NAA.

Growth rate of cell suspension = \( \frac{PCV \text{ at day 8 (Final PCV)} - PCV \text{ at day 0 (Initial PCV)}}{\text{Initial PCV}} \)

aggregates from cultured callus, which subsequently easily dissociated in the liquid medium and formed a fine creamy coloured cell suspension. While medium containing NAA (MSP1) was unresponsive to the initiation of indirect suspension cultures as it accelerated fast growing large compact green cell masses which did not dissociate in the liquid medium.

As compared to the indirect suspension cultures, the initiation response for direct suspension cultures was totally different with these three culture media. The MSP1 liquid medium was the only medium to encourage the culture of a single cell population. Within the first 2 subculturings, a great proliferation of single cells was noticed. Initially cells were vacuolated, which underwent abnormal cell division and cell elongation which formed thread like structures. But with 3-4 subsequent subculturings, cells slowly changed towards a globular structure, which were cytoplasmically rich containing starch grains. Such cell cultures began to form small cell aggregates, which consisted of 10-20 actively dividing cells. These direct cultures were characterised by a light green colour and rapid growth.

However, with UM and UMI liquid media, an early 2,4-D toxicity was observed with the initiation of direct cell cultures. As at the first subculture, the finely chopped callus showed some degree of phenolic production in the culture. Following separation of single cells from the callus cell division was not promoted. After 3-4 subculturings in these media, cell growth was completely arrested and suspension were found to be necrotic. Thus, the initiation responses of cell cultures of *L. chilense* were found to be auxin-specific. Initiation of indirect cell suspension was restricted with culture media containing 2,4-D, while direct cell suspension cultures were initiated in the culture medium containing NAA.

**Growth Curve Studies of 4-Month-Old Suspension Cultures (16th Subculture)**

The data from Fig. 1 show that the initial growth pattern of all cell cultures was similar for the first 4 days. Upto 3 days of culture, a steady growth was observed, which was followed by a sudden rise on day 4. Thereafter, the growth of two indirect cell suspensions initiated in UM and UMI media remained constant, but the growth of direct cell suspensions in
MSP1 medium was continuously increased up to the 8 days of culture. At the end of 8 days, a significantly higher cell growth (37.8% PCV, Table 1) was observed in 4-month-old direct cell suspensions in MSP1 liquid medium in comparison to the 4-month-old two other indirect cell suspensions in UM and UMI liquid media (i.e. 24.8 and 27.7%, respectively, Table 1).

**Cell Growth and Vigour of 5-Month-Old Cultures (20th Subculture)**

After 4 months of culture, especially in both indirect suspensions (cultured in UM and UMI media), a decline of growth was noticed. Therefore, at 20th subculture, growth of all cell suspension cultures was again checked. Drastic growth reduction was observed in both indirect cell suspensions. The mean percentage PCV value for cell suspensions initiated in UM and UMI media was 14.2 and 18.6, respectively (Table 1). Thus, in comparison with 4-month-old cultures, a reduction in growth rate of 5-month-old indirect cell suspension cultures was 56.5% in UM medium (as growth rate reduced from 3.13 to 1.36) and 41.8% in UMI medium (as growth rate reduced from 3.61 to 2.1). However, growth rate of 5-month-old direct cell suspension cultures in MSP1 medium found to be normal and unaffected (38.7% PCV with 5.45 growth rate; Table 1).

**Initiation of Phenolic Browning in Indirect Cell Suspension Cultures**

Loss of vigour of indirect cell suspensions (cultured in UM and UMI media) was accompanied with subsequent phenolic browning as noticed between 21st and 24th subculturing of UM and UMI cultured cell suspensions. To overcome this problem, the UM medium was either diluted to half strength with liquid MSO medium (UM0 medium; containing 1.0 mg l⁻¹ 2,4-D) or mixed with liquid MSP1 medium in 1:1 ratio (UMP1 medium; containing 1.0 mg l⁻¹ 2,4-D and 1.0 mg l⁻¹ NAA). These modifications delayed the browning for 2-3 weeks but could not prevent the production of phenolic compounds and ultimate necrosis. On the contrary, MSP1 cultured direct cell suspensions were found totally free from phenolic browning.

**Growth of Direct Cell Suspensions after 8 and 16 Months Culture: Development of Long-term Cultures**

A significantly higher growth rate (6.16; Table 1) was recorded for 8-month-old MSP1 initiated direct suspension cultures over the 4- and 5-month-old culture. These direct cultures were again assessed after 16 month for growth rate as a long-term culture. However, no significant variation was observed for growth (44.7% PCV) in long-term cultures and a rapid growth rate (6.45) was maintained.

**Protoplast Isolation, Culture and Plant Regeneration from P-derived Calli**

With the initiation of cell suspension cultures higher protoplast yields were obtained (5.66-14.02 x 10⁶ g⁻¹ f wt, Table 1). Nevertheless, like growth of cell suspensions, the protoplast yields were also influenced by the age and culture medium of the cell suspensions. At age of 4 months, a significantly higher protoplast yields (8.84 x 10⁶) were obtained from the direct cell suspensions, initiated in MSP1 medium over the two indirect cell suspensions cultured in UM and UMI media (5.66 and 6.86 x 10⁶, respectively). At 5 months culture, a loss of vigour was observed in indirect cell suspensions, which had also affected the protoplast yield and viability. A significant reduction was recorded in the yields and viabilities of indirect suspension-derived protoplasts (Table 1). However, no noticeable effect was observed on protoplasts of direct suspensions. The peak growth observed in long-term cultures of direct cell suspensions at 8-month culture (43% PCV) and 16-month culture (44.7% PCV) also reflected in protoplast yields. Estimable protoplast yields were obtained from 8- and 16-month-old direct suspension cultures (13.56 and 14.02 x 10⁶ g⁻¹ f wt, respectively) together with higher protoplast viability values (>90%; Table 1). Thus, long-term direct cultures proved to be a superior and regular source for protoplasts.

With the present protocol, satisfactory results were obtained for protoplast culture. Higher values were recorded for protoplast viability (85-94%) and for plating efficiency (48-64%). Recovery of P-derived calli was more than 90% on TMc medium. Although more calli differentiated into leaflet like structures (61-80%) on the regeneration medium, a comparatively lower actual shoot regeneration (25-54%) was observed. Regenerated callus produced on an average 4-5 shoots. Efficient rooting of regenerated shoots was only observed when agar-solidified MS medium was supplemented with 0.2 mg l⁻¹ IBA. Comparatively higher shoot regeneration was recorded from protoplasts isolated from direct suspension cultures than those from indirect.
suspension cultures. Moreover, aging of direct suspension cultures (4,5,8,16-month-old) had no significant effect on the plant regeneration capacity. The important stages of cell suspension cultures and protoplast to plant system are shown in Fig. 2.

Assessment of Growth of Protoplast Regenerated Plants under Glasshouse

All regenerated plants grew normally and bore flowers. However, like the seed-derived plants (control), all regenerants proved to be self-incompatible with no fruit set. Also no noticeable morphological variation was observed in them. However, the ploidy level of the regenerated population was variable in comparison to the control (Unpublished data). The protoclonal population produced 7.5±4.3% tetraploid (2n=4x=48) plants and 2.5±4.5% hexaploid (2n=6x=72) plants. Long-term cultures of direct suspensions were genetically stable as a considerably higher percentage (90±7.0) of diploid (2n=2x=24) plants were regenerated.

Discussion

The results of the present investigation have demonstrated the unique effects of auxins on the initiation of cell suspension cultures and their subsequent growth. The two culture media, UM and UMI, which contained 2.0 mg l⁻¹ 2,4-D were succeeded to initiate indirect cell suspensions. Thus, an effect of 2,4-D on the production of friable callus (which is responsible for formation of loose cell aggregates) became evident. In contrast, MSPI medium containing 2.0 mg l⁻¹ NAA produced fast growing hard-compact callus, which failed to initiate indirect cell suspensions. However, for the initiation of direct cell suspension the response of auxins was the completely opposite. NAA favoured the culture of single cells while 2,4-D showed early toxicity to their cultures.

After initiating two types of cell suspensions (direct and indirect) in two independent culture media (containing NAA and 2,4-D as an auxin-source), the growth of each culture remained distinguished. The direct cell suspensions produced chlorophyllous cells which had a rapid growth rate, while indirect cell suspensions produced colourless cells with a slower growth rate. The differences between the two suspensions became more clear with the increased age of culture. Growth curve studies after 4 month culture showed that unlike indirect suspensions, the direct suspensions exhibited continuous growth during 8 days of culture and a significantly higher growth was recorded over indirect suspensions. It indicates the beneficial effect of NAA on the cell growth of Lycopersicon. After 5 months culture, 2,4-D proved toxic to both indirect suspensions cultured in UM and UMI media. The cultures initially appeared to lose vigour, which was followed by phenolic browning. Tewes et al (1984) also recorded similar observations with L. esculentum cell suspension cultures. They reported that several subcultures with culture medium containing 2 mg l⁻¹ 2,4-D resulted in a decline of cell growth and plasmolysis of the cells, which was mainly due to the gradual increase in the absolute concentration of 2,4-D per cell. Tewes et al (1984), however, succeeded in controlling the phenolic browning due to 2,4-D toxicity by adopting the strict culture regime of a lowered 2,4-D concentration of the medium to 0.1 mg l⁻¹, shortening the subculture time (3-4 day) and using less inoculum (0.25-0.50 mg dry wt per ml) at subculture. In the present investigations, modifications such as addition of 0.1 mg l⁻¹ IAA to the UM medium (UMI medium), lowering the 2,4-D concentration of medium to 1.0 mg l⁻¹ (UMO medium) or the combination of 1.0 mg l⁻¹ 2,4-D and 1.0 mg l⁻¹ NAA (UMPI medium) did not prevent phenolic browning, which resulted in the death of indirect cell suspension cultures. Westcott and Henshaw (1976) also demonstrated that phenolic metabolism in suspension cultures of Acer sp. was influenced by levels of sucrose, nitrogen and 2,4-D in the culture medium. Khatun (1993) also noticed that 2.0 mg l⁻¹ 2,4-D concentration in culture medium enhanced phenolic browning in the cell suspension cultures of Corchorus sp. Whilst studying the organogenetic potential of Lycopersicon, Garcia-Reina & Luque (1988) observed the harmful effect of 2,4-D on L. esculentum tissues that produced friable, colourless and non-morphogenic callus, which later on subjected to the phenolic browning.

On the contrary, direct suspensions initiated in MSPI medium containing the auxin, NAA proved to be phenolic-free long-term cultures. It is worthy to note that these cultures maintained their fast growth-rate throughout the culture period and were genetically stable. These direct cultures also served as a regular source for protoplast. Protoplast yields were directly correlated with the growth of cell suspension cultures. Such higher protoplast yields (>1×10⁶ g⁻¹ f wt) are a prerequisite for fusion studies. Information about the initiation of direct long-term cell suspension cultures and specific effects of various auxins on cell
Fig. 2—Initiation of cell suspension cultures of *L. chilense* (LA 2930) and development of protoplast to plant system. A. Cytoplasmic, globular cell aggregates in MSP1 liquid medium (2-month-old); B. A cell suspension culture (4 day post-subculture) in MSP1 medium (8-month-old); C. Isolated protoplasts from a 4 day post-subculture cell suspension culture (medium TMp; plating density $1.0 \times 10^5 \text{ ml}^{-1}$); D. Viable protoplasts (93.8%) with FDA staining under UV illumination; E. Protoplast-derived micro-calli of *L. chilense* in TMp+TMC medium (plating density $1 \times 10^5 \text{ ml}^{-1}$) as a final plating efficiency (FPE) at day 22; F. Callus development of P-derived micro-calli of *L. chilense* on callus-inducing TMc medium (day 28); G. Differentiation of P-derived callus of *L. chilense* on MS3 medium (day 14).
growth was first-time discussed for the genus *Lycopersicon*. With the development of an efficient protoplast-to-plant system, *L. chilense* can safely be used for protoplast based genetic manipulation studies. Furthermore, P-derived embryogenic nodular callus can be employed as a distinct selectable marker in characterisation of somatic hybrids.

**References**


