Observation of High-frequency Occurrence of Chimeral Adventitious Shoots in Tissue Culture from the Chimeral Tissues of *Pelargonium zonale*

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Abstract. In *Pelargonium*, the plastid mutation in three independent cell layers L1, L2, and L3, can produce plastid chimeras with visible shoot colour difference such as GWG (green-white-green) and GGW (green-green-white). Chimera can be used to trace the relationship between the cell layers of different genotypes during shoot development and the effect of the mutated genes on shoot development. In this study, we have obtained different adventitious shoots with GGG, GWG, GGW, and WWW combinations of cell layers through tissue culture of petioles and internodes from GWG and GGW chimeras of *Pelargonium zonale* ‘Mrs Pollock’. Much higher percentage (14.9%) of chimeral adventitious shoots was obtained from GGW tissues than from GWG tissues (4.2%). Of the 10.8% chimeral adventitious shoots regenerated in this experiment, 8.6% are different from the original type of explants. This result indicated that cells at least in both L2 and L3 of the explants were involved in the regeneration of the adventitious shoots. The number of shoot types regenerated is likely dependent on the number and the type of cells that were in direct contact with the culture medium. It is suggested that the mixed cells can be used to produce the chimera by tissue culture. Three possible ways to form the chimeras in vitro culture were discussed. Chemical names used: TDZ = 1-phenyl-3-(1,2,3-thiadiazol-5-yl)urea (Thidiazuron); IAA = Indole-3-acetic acid; PVP = polyvinylpyrrolidone.

*Pelargonium* is among the most popular ornamental plants over the world. The meristem in shoot apex is consisted of three independent cell layers (Baur, 1909). The cells in the two outer layers (L1 and L2) of the meristem divide anticlinally (i.e. the new cell walls are perpendicular to the meristem surface), resulting in the daughter cells remaining in the same layer. This ensures that the cell lineages of L1 and L2 are kept separate from each other. The cells in inner layer (L3) divide periclinally in any plane; occasionally divisions can result in daughter cells invading a different layer (Carpenter and Coen, 1995). The L1 gives rise to the epidermis, the L2 gives rise to the subepidermal mesophyll and the germ cells of the reproductive organs, and the L3 forms most of the internal and vascular tissues of the plant (Hantke et al., 1995).

Leaves always arise in association with a shoot meristem. The leaf epidermis is derived solely from the L1. The L2 gives rise to first palisade parenchyma and the under layer of the spongy parenchyma on the leaf margins. The L3 gives rise to the upper and middle layers of the spongy parenchyma, but makes no contribution to the blade margins (Burk et al., 1964).

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Materials and Methods

The plant used in this experiment was a plastid-mutated chimera *Pelargonium zonale* ‘Mrs Pollock’ with a GWG pattern that has genetically green L1, white L2, and green L3, and its variant in type GGW that has green L1, green L2 and white L3. The position and the size of the L2-derived and L3-derived tissue were variable. Some mixed cells (cells that contain both wild green plastids and the mutated white ones) were found in leaf section of variegated plants. In this experiment, 20 petioles with the leaf attached and 20 segments (about 1 cm) of internodes of the 2 typical chimeras, GGW and GWG, were used as explants for tissue culture. The explants were rinsed in distilled water and sterilised in 70% ethanol for 1 min, then shaken continuously in a sterilizing solution containing 0.25% Wofasteril E 400 (Kesla, Germany) and Tween 20 (2 drops/100 mL solution) for 5 min. The explants were then rinsed 3 times in sterile distilled water, placed upright in a 50-mL glass jar containing 10 mL liquid medium with Perlite, and incubated in dark for a week. Each glass jar contained one petiole with the leaf attached or two (segments of internodes) explants. The culture medium contained ½ macro elements and whole concentration of other elements of MS (Murashige and Skoog, 1962) with B5 (Gamborg et al., 1968) vitamins, 30 g L⁻¹ sugar, 100 mg L⁻¹ myo-inositol, and 22 µM TDZ. After induction of callus, the explants were transferred on to the surface of 20 mL solid medium containing 0.4% agar and 500 mg mL⁻¹ PVP in a 100 mL glass at 22 to 24 °C.
in a culture room providing 12 h d⁻¹ of cool white fluorescent light. The types of adventitious shoots and numbers of each type were recorded at the end of shoot induction. For the regeneration of roots, the adventitious shoots were isolated individually and transferred to a basal medium with 0.3 mg IAA/L and 50 µg L⁻¹ Adenine instead of TDZ.

Results and Discussion

In this experiment, the different types of adventitious shoots were regenerated through the callus from explants of chimeral types in vitro culture (Table 1), of which most adventitious shoots were homogeneous green (GGG) and white (WWW). The white shoots died during isolation culture. This indicated that plant chimeral tissue could be dissected into their component genotypes by production of adventitious shoots. In addition, three chimeral histological combinations of the adventitious shoot and variegates were regenerated through the callus from explants of chimeral types GWG and GGW (Fig. 1). These results revealed that the various chimeras could be produced from calluses of the chimeral tissue of Pelargonium zonale ‘Mrs Pollock’. The chimeras from chimeral explants, however, did not necessarily possess the same histogenic arrangement as the original explant, disagreeing with the investigation of Pelargonium zonale ‘Madame Salleron’ by Bergann and Bergann (1959). This further substantiated the idea that cells and cell types and chimeras in adventitiously formed chimeral shoots, in which 4.2% chimeral shoots were from GWG explants and 14.9% from GGW explants (Table 1).

To explain the formation of the chimeras in vitro culture, Geier (1989) suggested that the mutations during the development of the adventitious shoots could produce the chimeral shoot. Because the cell mutation often appeared during the shoot developing process, both the mutated and the wild type cells can maintain as one entity and grow together to produce a chimeral shoot that has at least two genotypes. Plastid mutation, leading to chimerism, has been chemically induced in Saintpaulia (Polhheim, 1974). In this way, a chimeral shoot was not developed from a single origin cell.

Several studies based on the investigation of chimeral plants suggested that chimeral adventitious shoots had multicellular origin of cells with different genetic makeup (Dulieu, 1967). Multicellular theory was also supported by histological investigation of Bergann and Bergann (1959) by Pelargonium zonale ‘Madame Salleron’ and by experiments of chimeral synthesis with artificially mixed calluses in vitro culture (Marcotrigiano and Gouin, 1984), in which interspecific chimeral calluses of Nicotiana tabacum L. were used and 0.3% chimeral plants were obtained.

The results of this study were similar with the observation by Vaughn (1983), in which tissue culture of variegated leaf with periclinal chimeras gave a high percentage of variegated plantlets. In this study, some new types of chimera arose with a constitution different from the original chimeral explants, agreeing with Dulieu’s experiment (Dulieu et al., 1967). This further substantiated the idea that cells from several tissue layers must be involved in apex formation. Therefore, it supports the notion that at least some chimeral adventitious shoots had a multicellular origin of cells with different genetic makeup.

The single cell origin theory precludes the formation of large numbers of multiple cell types and chimeras in adventitiously regenerated plants (Prell, 1986). However, as each plant cell contains many of plastids, it is possible to produce a mixed cell when some of these plastids were mutated while the others remained normal. Tilney-Bassett (1963) found that a chimeral shoot could be produced from a mixed cell with various genotypes if the normal and mutant plastids are similar in number and survival rate in a mixed cell. Hagemann and Scholze (1974) obtained chimeral shoots from mixed zygotes of a cross between a plastid chimeral ‘Mrs. Pollock’ and a green ‘Frautlieb’

This result suggests that the competence of cells for regeneration from the explants depends on cell genotype and cellular location.

Cell origin of the chimeral shoot formation in tissue culture. Of the six possible periclinal arrangements of the chimeral genotypes with G and W markers, the two periclinal chimeras (GWG, GGW) and sectorial chimera in adventitious shoots were observed in this study by tissue culture of the ‘Mrs. Pollock’. The average percentage of the chimeral shoots in this experiment is 10.8% (there were 8.6% new chimeral adventitious shoots), in which 4.2% chimeral shoots were from GWG explants and 14.9% from GGW explants (Table 1).

Fig. 1. Adventitious shoots from the chimeral tissues of Pelargonium zonale ‘Mrs Pollock’ in vitro culture. (A) Callus induction; (B) somatic embryo-like structure induction; (C) white shoot in WWW type; (D) chimeral shoot in GWG type; (E) chimeral shoot in sandwiched GWG type; (F) sectorial variegate.

Table 1. Regenerated shoots from the different chimeral explants in vitro; G = green, W = white.

| Explants | Total shoots | GGG (%) | WWW (%) | GWG (%) | GGW (%) | Sectorial (%) |
|----------|--------------|---------|---------|---------|---------|--------------|
| GWG      | 71           | 61 (85.9) | 7 (9.9) | 1 (1.4) | 1 (1.4) | 1 (1.4)      |
| GGW      | 114          | 49 (43.0) | 48 (42.1) | 5 (4.4) | 3 (2.6) | 9 (7.9)      |
| Total    | 185          | 110 (59.5) | 55 (29.7) | 6 (3.2) | 4 (2.2) | 10 (5.4)     |

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in *Pelargonium zonale*. They concluded that the green and white plastids in *Pelargonium zonale* ‘Mrs Pollock’ had similar growth chance in practice. Seeni and Gnanam (1981) observed about 10% to 15% regenerated chimeral shoots from the callus of hypocotyl and cotyledonary explants of tomato seedling heterozygous for the semidominant × *anthophyllic-2* mutant, but no chimeras occurred in shoots derived from the callus of homozygous plants. In addition, Pohlheim (1974) observed mixed cells in variegated leaves of variegated shoots produced by applying N-Nitroso-N-Methyurea in *Saintpaulia*. Therefore, it is possible that the plastid chimeral plants could be regenerated from a mixed cell, in which some plastids are mutated and others are normal. The production of chimeras from mixed cells is similar to cell fusion by Philip (1990), in which the chimeral adventitious shoots could be produced via fusion of two or three protoplasts of different genotypes. However, as a mixed cell is a single cell, the mixed cells may have a better chance to regenerate chimeras than fused cells in tissue culture. The mixed cell origin is a specific single cell origin, in which the mutant and normal plastids exists in one cell. The formation of the green, white, sectorial, mericlinal and periclinal adventitious shoots from the mixed cells as described by Tilney-Bassett (1986) depends on mass, distribution and the propagation ability of the white and the green chloroplasts in the mixed cells.

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