Organogenesis in Carnation

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Abstract. Shoot regeneration in carnation (Dianthus caryophyllus L.) was influenced by genotype, explant source, and plant growth regulator balance. Plants were regenerated from petals, calyxes, nodes, internodes, and leaves, but only petals, calyxes, and nodes were regenerative from all three cultivars examined (‘Scania’, ‘Improved White Sire’, ‘Sandra’). Maximum proliferation was achieved with petals on Murashige and Skoog medium supplemented with 0.05 µM TDZ and 0.5 µM NAA. Shoot initiation originated from cells near vascular regions and perhaps from epidermal cells in petals and via organogenic callus from other explants. There was no evidence of chimeral separation from petals or callus, but somaclonal variants (3.3%) were observed involving petal hue and plant dwarfness. Unstable color patterns were observed in tissue-cultured regenerants of ‘Scania’ and ‘Improved White Sire’ similar in type and frequency to propagules derived from cuttings; none were observed for tissue-cultured or cutting-derived plants of ‘Sandra’. Chemical names used: N-phenyl-N’-l,2,3 -thiadiazol-5-ylurea [thidiazuron (TDZ)]; 1-napthaleneacetic acid (NAA).
Table 4. Effects of cultivar, explant source, NAA, and TDZ concentration on shoot formation from carnation explants.

| NAA (µM) | TDZ (µM) | Shoot formation (%) | Petals | Calyces | Nodes | Internodes | Leaves |
|----------|----------|---------------------|--------|---------|-------|------------|--------|
| 0.0      | 0.0      | 0                   | 0      | 0       | 0     | 0          | 0      |
| 0.5      | 0.0      | 0                   | 0      | 0       | 0     | 0          | 0      |
| 0.5      | 0.0      | 86                  | 0      | 0       | 0     | 0          | 0      |
| 0.5      | 5.0      | 77                  | 33     | 0       | 0     | 0          | 0      |
| 5.0      | 0.0      | 85                  | 0      | 10      | 0     | 0          | 0      |
| 5.0      | 0.5      | 93                  | 0      | 0       | 0     | 0          | 0      |
| 5.0      | 5.0      | 92                  | 33     | 10      | 0     | 67         | 0      |

Improved White Sim

| NAA (µM) | TDZ (µM) | Shoot formation (%) | Petals | Calyces | Nodes | Internodes | Leaves |
|----------|----------|---------------------|--------|---------|-------|------------|--------|
| 0.0      | 0.0      | 0                   | 0      | 0       | 0     | 0          | 0      |
| 0.5      | 0.0      | 73                  | 0      | 10      | 0     | 0          | 0      |
| 0.5      | 5.0      | 72                  | 0      | 0       | 0     | 0          | 0      |
| 5.0      | 0.0      | 58                  | 0      | 10      | 0     | 0          | 0      |
| 5.0      | 0.5      | 76                  | 0      | 0       | 0     | 0          | 0      |
| 5.0      | 5.0      | 87                  | 0      | 0       | 0     | 0          | 0      |
| 5.0      | 0.5      | 46                  | 0      | 10      | 0     | 0          | 0      |
| 5.0      | 5.0      | 70                  | 67     | 10      | 13    | 0          | 0      |
| 5.0      | 5.0      | 92                  | 0      | 0       | 0     | 0          | 0      |

Sandra

| NAA (µM) | TDZ (µM) | Shoot formation (%) | Petals | Calyces | Nodes | Internodes | Leaves |
|----------|----------|---------------------|--------|---------|-------|------------|--------|
| 0.0      | 0.0      | 0                   | 0      | 0       | 0     | 0          | 0      |
| 0.5      | 0.0      | 23                  | 0      | 10      | 0     | 0          | 0      |
| 0.5      | 5.0      | 35                  | 0      | 0       | 0     | 0          | 0      |
| 0.5      | 0.5      | 30                  | 0      | 0       | 0     | 0          | 0      |
| 5.0      | 0.5      | 56                  | 0      | 0       | 0     | 0          | 0      |
| 5.0      | 5.0      | 0                   | 0      | 20      | 0     | 0          | 0      |
| 0.5      | 5.0      | 23                  | 33     | 0       | 0     | 0          | 53     |
| 5.0      | 5.0      | 31                  | 0      | 0       | 0     | 0          | 0      |

'Shoot regeneration differences between cultivars and explant types are significantly different at \( P = 0.01 \) based on \( x^2 \) analysis of presence or absence of shoots.

'Sixteen days in culture, trt \( n = 10-19 \).

'Eighty days in culture, trt \( n = 3 \).

'Forty-Wo days in culture, trt \( n = 10 \).

Eighty days in culture, trt \( n = 15 \).

'Fifty days in culture, trt \( n = 15 \).

7. On day 3, the ‘Scania’ and ‘Sandra’ petals, which were white in the bud, turned red and pink, respectively; ‘Improved White Sire’ petals remained white. On day 5, petal bases of all cultivars were green. Shoot formation was first visible from ‘Improved White Sire’ petals on day 9, from ‘Scania’ petals on day 12, and from ‘Sandra’ petals on day 35. Shoot proliferation and growth in ‘Improved White Sire’ and ‘Scania’ continued as the petals slowly senesced and turned brown. All petals appeared limp and necrotic by day 28.

Shoot regeneration from petals depended on cultivar and TDZ-concentration.
petals is regenerative (Alzate et al., 1990; Gimelli et al., 1982, 1983, 1984a, 1984b; Kakehi, 1979; Leshem, 1986).

Light and scanning electron microscopy (Figs. 3 and 4) indicated that the shoot primordium developed near the petal vascular region, including bundle sheath cells, or closely related mesophyll cells (Fig. 4A), and perhaps from epidermal cells on the perimeter of the petal base (Fig. 4B). Shoots were not formed from preformed meristematic cells. The first observed event was the formation of an “amorphous layer” on the basal edge (Fig. 4A), followed by the formation of proliferating callus (Figs. 3B and 4A, B), and finally shoot initiation (Figs. 3C, D, and 4C, D).

Callus formed on the cut surfaces of all explant types from days 8 to 11. Shoot regeneration depended on genotype and TDZ-NAA balance (Table 1). All cultivars developed shoots from calyxes and nodes, but only ‘Improved White Sire’ developed shoots from internodes and only ‘Scania’ and ‘Sandra’ developed shoots from leaves.

Although organogenesis was obtained from all explants studied, only petals proved to be a constant, dependable, and prolific source of shoots for all cultivars (Table 1). Regeneration from petals tended to be optimum from medium supplemented with a combination of 0.5 µM TDZ and 0.5 µM NAA. Shoot formation appeared to arise directly from petal tissue, but SEM suggested that a transient callus phase was involved. Organogenesis was sporadic in all other explants (i.e., calyxes, nodes, internodes, and leaves) and only occurred directly from callus.

Several techniques improved shoot regeneration from petals. The most shoots were obtained by culturing petals at 30°C on medium containing 60 g sucrose and 1 g casein hydrolysate/liter, 0.05 µM TDZ, 0.5 µM NAA, and that was solidified with 8 to 10 g agar/liter (data not presented). However, many treatments that maximized shoot proliferation also increased vitrification. Taking both proliferation and vitrification into consideration, the optimum protocol used to maximize unvitrified shoot production from petals was as follows: Temperature,
Table 4. Effect of propagation method on carnation flower phenotypes.

| Floral phenotype | Frequency (% of plants) | Stability (% of flowers/variant plant) |
|------------------|------------------------|---------------------------------------|
|                  | Tissue cultured |       | Petal | Callus | Cuttings |
|                  | n = 81 | n = 8  | n = 7 |       |          |
| All white        | 0.0   | 0.0   | 0.0   |       |          |
| All red          | 0.0   | 12.5  | 0.0   |       |          |
| Unstable red     | 100   | 87.5  | 100   |       |          |
| Hue              | 3.7   | 0.0   | 0.0   |       |          |
| Tinged           | 100   | 87.5  | 100   |       |          |
| Flecked          | 69.1  | 37.5  | 57.1  |       |          |
| Blotched         | 4.9   | 0.0   | 14.3  |       |          |
| Sectored         | 17.3  | 12.5  | 14.3  |       |          |
| Striped          | 7.4   | 12.5  | 28.6  |       |          |
| Malformed        | 3.7   | 12.5  | 0.0   |       |          |

|                  | Scania |       |       |       |          |
|                  | n = 1444 | n = 103 | n = 174 |
| Hue              |       |       |       |       |          |
| Tinged           | 64    | 15.6  | 64    | 15.6  | 64    | 15.6  |
| Flecked          | 1153  | 10.3  | 1153  | 10.3  | 1153  | 10.3  |
| Blotched         | 158   | 3.2   | 158   | 3.2   | 158   | 3.2   |
| Sectored         | 287   | 5.9   | 287   | 5.9   | 287   | 5.9   |
| Striped          | 169   | 3.6   | 169   | 3.6   | 169   | 3.6   |
| Malformed        | 79    | 35.4  | 79    | 35.4  | 79    | 35.4  |

|                  | Improved White Sire |       |       |       |          |
|                  | n = 659 | n = 301 | n = 136 |
| Hue              |       |       |       |       |          |
| Tinged           | 342   | 14.6  | 342   | 14.6  | 342   | 14.6  |
| Flecked          | 26    | 3.8   | 26    | 3.8   | 26    | 3.8   |
| Blotched         |       |       |       |       |          |
| Sectored         |       |       |       |       |          |
| Striped          | 7     | 14.3  | 7     | 14.3  | 7     | 14.3  |
| Malformed        |       |       |       |       |          |

|                  | Sandra |       |       |       |          |
|                  | n = 153 | n = 56  | n = 100 |
| Hue              |       |       |       |       |          |
| Tinged           |       |       |       |       |          |
| Flecked          |       |       |       |       |          |
| Blotched         |       |       |       |       |          |
| Sectored         |       |       |       |       |          |
| Striped          |       |       |       |       |          |
| Malformed        |       |       |       |       |          |

Includes plants from leaves, internodes, nodes, or calyx.

No. flowers that showed floral variation.

15 to 20°C; agar, 8 to 10 g·liter⁻¹ sucrose, 30 g·liter⁻¹ casein hydrolysate, 0 to 0.5 g·liter⁻¹ cytokinin, 0.05 µM TDZ; auxin, 0.5 µM NAA; culture vessel, baby food jars or Magenta GA7.

Somaclonal variation. A total of 182 tissue culture-derived plants that produced 2716 flowers were evaluated for plant growth habit and flower morphology (Table 4) for 1 year. One plant derived from a 'Scania' petal was variant in growth habit with a compact, bushy form and shiny, nonglaucous leaves.

Individual flowers were examined for variations in hue, color pattern, and form in plants derived from tissue culture and cuttings (controls). Color pattern variants were visually classified as tinged, a discoloration of petals, flecked, small off-color irregular spots on the petals; sectored, a clearly defined petal sector of a variant color; striped, short streaks of a variant color traversing the petals; or blotched, similar to flecking except that flowers had larger off-color irregular patches dispersed across the petals. Some flowers from 'Scania' had one cream and 20 white flowers, whereas the nodal variant had two cream and 20 white flowers. None of the 'Scania' or 'Improved White Sire' controls were variant for pigment hue.

Stability differences in flower patterns were noted between the three cultivars: 'Sandra' was very stable, and no variants were observed in either tissue-cultured or control plants, whereas 'Scania' and 'Improved White Sire' were unstable for tissue culture-derived and control plants. There was no convincing evidence that variant phenotypes were more frequent in tissue culture-derived plants than in vegetatively propagated plants from cuttings (Table 4). Many of the floral instabilities observed in tissue-cultured 'Scania' and 'Improved White Sire' plants also were noted in parental controls at similar frequencies.

There were two classes of malformed flowers from tissue culture-derived plants: those from flowers initiated in vitro, and those from flowers initiated ex vitro. Buds that were induced in vitro had malformed flowers, whereas those that were induced ex vitro did not.

There were two types of floral instabilities observed in tissue culture-derived 'Scania' and 'Improved White Sire' plants: those from flowers initiated in vitro, and those from flowers initiated ex vitro. Buds that were induced in vitro had malformed flowers, whereas those that were induced ex vitro did not.
respect to predominant flower color, i.e., tissue-cultured ‘Scania’ were red with some small white spots, ‘Improved White Sire’ were white with small red spots, and ‘Sandra’ were all pink. No tissue culture-derived plants were obtained in which all flowers were of a variant color. The instability of petal color in ‘Scania’ and ‘Improved White Sire’ was similar for petal-derived, callus-derived, and vegetatively propagated plants. ‘White Sire’ is a periclinal chimera of ‘William Sire’ (Johnson, 1980; Pereau-Leroy, 1974; Sagawa and Mehlquist, 1957), and although ‘Improved White Sire’ is derived from ‘William Sire’, there are no reports confirming that it also is a periclinal chimera. However, an occasional completely red flower was noted in ‘Improved White Sire’ stock plants derived from cuttings. Assuming that ‘Improved White Sire’ is a periclinal chimera with L₁ being white and L₂ and L₃ being red, the expected phenotypes from tissue culture could be either 1) all-white flowers derived from L₁, 2) white flowers with red spots derived from a combination of L₁ and L₃, or 3) all-red flowers derived from L₂ or L₃. In our study, there was no evidence of complete chimeral separation in ‘Improved White Sire’ from either petals or callus as would have been expected from a periclinal chimera. Our observations of red flower reversion and lack of chimeral separation from organogenesis may be reconciled with one of the following assumptions: 1) ‘Improved White Sire’ is not a periclinal chimera and the red flowers produced by cuttings were due to a mutation. The floral patterns of ‘Improved White Sire’ may be a result of genetic instabilities, perhaps transposons or highly mutable genes; 2) ‘Improved White Sire’ is a periclinal chimera, but the L₁ and L₃ layers are genetically white and only the L₂ layer is genetically red. The lack of chimeral separation in ‘Improved White Sire’ may be explained if L₂ tissue is not involved in organogenesis. The red flowers observed for some ‘Improved White Sire’ cuttings could be due to adventitious buds derived from L₂.

Further studies will be needed to determine if petal regeneration could be used for a propagation system or if any of these organogenic systems can be used for genetic transformation or recovery of interesting genetic variants from different histogenic layers.

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