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Somaclonal variation in *Chrysanthemum morifolium* generated through petal cultures

(Penghasilan variasi somaklon melalui kultur kelopak bunga *Chrysanthemum morifolium*)

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Key words: *Chrysanthemum morifolium,* somaclonal variation, in vitro, petal cultures

Abstrak

Kelopak lima varieti *Chrysanthemum morifolium* dikultur dan somaklon dinilai pada pokok-pokok yang terhasil. Anak-anak cambah menunjukkan variasi dalam pertumbuhan vegetatif dan peringkat pembungaan manakala variasi yang terhad didapati pada ciri-ciri bunga. Teknik mengkultur kelopak bunga ini boleh digunakan untuk menyelamatkan mutasi semula jadi. Ini telah dibuktikan apabila satu kelopak berwarna kuning diasingkan, kemudian dikultur dan pokok berbunga kuning dihasilkan daripada teknik kultur tisu ini. Mutasi seperti ini tidak boleh diasingkan melalui teknik tampang yang biasa.

Abstract

Ray florets of five varieties of *Chrysanthemum morifolium* were cultured in vitro and the regenerants were evaluated for somaclonal variation. The regenerated shoots exhibited variation in vegetative growth and flowering but limited floral variation. The technique used here could be used to secure naturally occurring colour mutants or sports. This was demonstrated when a yellow coloured floret was successfully isolated and regenerated to exhibit a solid mutant. Such mutants are impossible to isolate vegetatively due to its location and their limited extent.

Introduction

Somaclonal variation offers an opportunity to uncover the natural variability in plants and to use this genetic variability for new product development (Evans and Sharp 1986). Somaclonal variation generated through tissue culture has been described for many ornamental plants. Variations in *Chrysanthemum* have been obtained from shoot-tip callus (Bush et al. 1976), petals (Stewart and Dermen 1970; Khalid et al. 1989) or young capitula and stems (Miyazaki and Tashiro 1978).

Morphological variations have been obtained from regenerants of petal and leaf callus origins (Sutter and Langhans 1981), thus for this study petals were used as explants to generate variation. Chrysanthemums are chimeras with leaves consisting of L1, L2 and L3 layers and floret L1 and L2 layers (Stewart and Dermen 1970; Bush et al. 1976). The L1 and L2 layers determine flower colour while the L2 layer determines floral shape.

In this study, the petals of five commercial varieties were peeled and both the epidermal peel and the peeled petals were cultured in vitro, establishing an efficient plant regeneration procedure from petal cultures. An assessment was made of

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the somaclonal variants produced from these petal cultures.

Materials and methods

Plant material

Chrysanthemum morifolium varieties Weldon (white), Impala (off white), Pink Impala (white with pinkish tinge), Daymark (white) and White Palaver (white) were obtained as potted plants from MARDI, Cameron Highlands. These varieties were picked for their flower colour which ranged from white to light pink. These stock plants were grown under glasshouse conditions in MARDI, Serdang and maintained by vegetative propagation.

Production of plants from ray florets

Fully opened flowers were used. Flowers were washed thoroughly under running water, after which the ray florets were removed from the inflorescence and surface sterilised in 15% (v/v) domestic chlorox bleach solution (5.25% sodium hypoclorite) for 20 min followed by 6 washes of sterile distilled water. The lower epidermis of the ray florets was peeled and both the peel (L1) and the peeled petal (L2) were cultured with the abaxial surface of the peeled petal touching the surface of the medium. Medium used was MS based (Murashige and Skoog 1962) and supplemented with various combinations of plant growth regulators (PGR) namely 6-benzylaminopurine (BAP) and 1-naphthalene acetic acid (NAA), kinetin and NAA, 3% (w/v) sucrose and 0.8% (w/v) Difco Bacto agar. In both PGR combinations, concentrations used ranged from 1–4 mg/L. The pH of the media was adjusted to 5.8 using 1M KOH.

The L1 and L2 layers were cultured separately on 10 mL of medium in 9 cm Petri dishes (4 explants/Petri dish), sealed with parafilm and incubated under 16/8 h light/dark regime at 25 °C for 3–4 weeks before they were subcultured onto the same fresh medium. Plantlets regenerated were transferred to MS medium with no plant growth regulators (MSO) solidified with 0.7% Difco Bacto agar for elongation, rooting and incubated under the same conditions as those used for explant cultures. About 4–6 weeks later the rooted plants were potted in polybags containing compost with a plastic bag over it for acclimatisation. Plants of the parent cultivars and floret-derived plants were grown side by side for assessment of morphological and floral changes in the regenerated plants. At least 50 shoots from tissue culture derived plants and 50 control plants of each cultivar were grown to maturity.

Results

Although the peeled petals of all the varieties responded on medium supplemented with NAA and BAP at 4 mg/L each, there was a visible genotype medium interaction *(Table 1* and *Table 3)*. Response of the explants on media with NAA and kinetin was not as effective as with BAP as the cytokinin *(Table 3)*. The cultured florets produced callus after 10–14 days and subcultured every month. The regenerability of shoots from callus of ray florets varied with variety. This was similarly found to be true by Kaul et al. (1990) and Malaure et al. (1991) who found varietal differences in shoot regeneration frequency of *Chrysanthemum*. All the varieties exhibited greater regenerative capacity from the L2 layer when compared to the L1 layer *(Table 1* to *Table 3)*.

Extent and type of somaclonal variation

The percentage of regenerants that exhibited any form of variation was low when compared to the control plants but there was a morphological reduction in size. Most of the regenerants in general produced inflorescence that was reduced in size and number of petals per inflorescence. This was also accompanied by reduction in plant height *(Table 4)*. In one of the varieties, White Palaver, there was a marked increase in length of pedicel *(Plate 1* and *Plate 2)*. Weldon (peeled) produced flowers that were

Table 1. Response of peeled petals (L2) of *Chrysanthemum* varieties on MS medium with BAP and NAA at various concentrations

Variety	NAA (1 mg/L) BAP (1 mg/L)	NAA (2 mg/L) BAP (2 mg/L)	NAA (3 mg/L) BAP (3 mg/L)	NAA (4 mg/L) BAP (4 mg/L)
Weldon	+	+	++	+++
Impala	–	+	+++	+++
Pink Impala	+	+	++	+++
Daymark	–	+	+++	++++
White Palaver	–	–	+	++

\+ = callus produced around the edges of the explant
++ = callus produced over the surface of the explant with plant regeneration <5 plantlets/explant
+++ = callus produced over the surface of the explant with plant regeneration >5 but <12/explant
++++ = callus produced over the surface of the explant with plant regeneration >12/explant
– = no response

Table 2. Response of petal peel (L1) of *Chrysanthemum* varieties on MS medium with BAP and NAA at various concentrations

Variety	NAA (1 mg/L) BAP (1 mg/L)	NAA (2 mg/L) BAP (2 mg/L)	NAA (3 mg/L) BAP (3 mg/L)	NAA (4 mg/L) BAP (4 mg/L)
Weldon	–	+	++	++
Impala	–	–	+	++
Pink Impala	–	–	+	+
Daymark	–	+	++	+++
White Palaver	–	–	+	++

\+ = callus produced around the edges of the explant
++ = callus produced over the surface of the explant with plant regeneration <3 plantlets/explant
+++ = callus produced over the surface of the explant with plant regeneration >3 but <8/explant
++++ = callus produced over the surface of the explant with plant regeneration >8/explant
– = no response

Table 3. Response of petal peel (L1) and peeled petals (L2) of *Chrysanthemum* varieties on MS medium with Kinetin and NAA at various concentrations

Variety	1 mg/L	2 mg/L	3 mg/L	4 mg/L
Petal peel (L1)				
Weldon	–	–	–	–
Impala	–	–	+	+
Pink Impala	–	–	–	+
Daymark	–	+	+	+
White Palaver	–	–	–	–
Peeled Petals (L2)				
Weldon	–	–	–	+
Impala	–	–	+	+
Pink Impala	–	–	+	+
Daymark	–	+	+	++
White Palaver	–	–	–	++

\+ = hard nodular callus produced around the explant
++ = hard nodular callus with plant regeneration at <5/explant
– = no response

Table 4. Floral and vegetative characteristics forming the somaclonal variation in *Chrysanthemum* varieties

Variety	Characteristics of floral variants
Weldon	Reduction in plant height at flowering, flower disk reduced by 80%, diameter of flower reduced, plant architecture changed to cascading type
Impala	Overall reduction in plant height, single yellow petal which was isolated and cultured to produce all yellow flowers
Daymark	Reduction in flower size and number of florets
White Palaver	Increase in length of flower pedicel, reduction in plant height at flowering

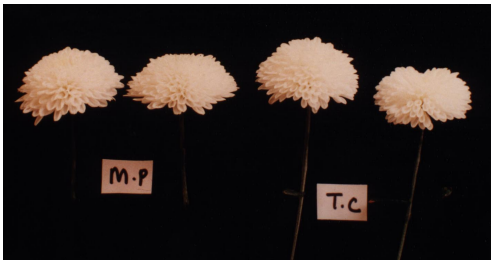

Plate 1. Tissue cultured peeled petal (L2) of ***Chrysanthemum*** *variety White Palaver exhibiting longer flower petiole*

Plate 2. Tissue cultured peeled petal (L2) of ***Chrysanthemum*** *variety White Palaver (left) exhibiting increased flower petiole and Weldon (right) showing cascading plant architecture*

Plate 3. Tissue cultured peeled petal (L2) of ***Chrysanthemum*** *variety Weldon (center and right) as compared to control (left)*

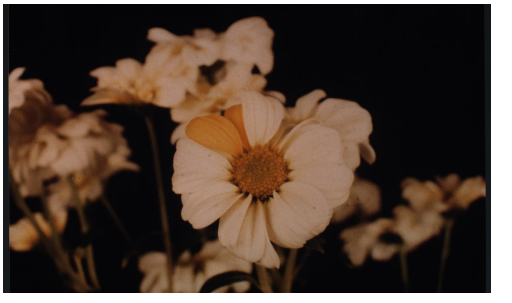

Plate 4. Tissue cultured petal peel (L1) of ***Chrysanthemum*** *variety Impala showing a single yellow petal*

reduced in size with the disk florets almost not visible *(Plate 3)*. The plant architecture was also changed producing florets that were not erect as compared to the stock plants *[Plate 2 (right)]*. The variety Impala produced flowers of uniform size but one of the florets of an inflorescence was yellow in colour *(Plate 4)*. This yellow sport (both the peel and the peeled) was then cultured in vitro and plants regenerated from peel and

Plate 5. Tissue cultured petal peel (L1) of single yellow petal of ***Chrysanthemum*** *variety Impala* ***(Plate 4)*** *showing yellow flower*

peeled petals produced flowers that were totally yellow in colour *(Plate 5)*.

Discussion

The PGR concentrations and combinations were important for shoot regeneration *(Table 1* to *Table 3)*. The combination of NAA and BAP at 4 mg/L each proved to be the best for all the varieties (both L1 and L2) used in this study based on the highest number of regenerants/explant for all the cultivars studied *(Table 1* and *Table 2)*. However, the shoot regeneration capacity varied with cultivar and also tissue layer (L1 or L2) as was seen by Malaure et al. (1991).

The percentage of regenerants that exhibited somaclonal variation was variety specific. Weldon produced the highest number of variants although it was not as responsive in culture as the other varieties. The peeling of the epidermis or L1 layer separated the chimeric layers of the petal but not sufficient to produce solid mutants as the L1 layer could not be removed completely. A vast majority of the regenerants that were produced had only reduction in inflorescence size compared to control plants. These plants arose from the L2 layer. The reduction in flower size *(Plate 3)* was not an epigenetic effect as the characteristic was maintained after several cycles of vegetative propagation.

Both L1 and L2 layers determine flower colour and carotenoid is present in both L1 and L2 layers. Culturing the separated layers L1 and L2 successfully isolated the yellow sport that was produced through tissue culture. The regenerants that were produced from these two layers were totally yellow but with a reduction in flower size. This confirmed the presence of the carotenoid pigments in both layers. A floret sport cannot be secured vegetatively as the mutation is often limited to a few florets and does not extend to the whole plant.

It can be concluded that tissue culture method is an efficient way to secure sports where the layer of the petal can be separated and cultured. As the regenerated shoots from in vitro cultured florets normally regenerate from only one or a few cells of the floret cell layers, the regenerants will have identical L1 and L2 layers, producing solid mutants. Regenerants from the petal explants where the layers were not separated were similar morphologically to the control plants. This was probably due to the fact that chimeral layers were not separated and according to Stewart and Dermen (1970) and Bush et al. (1976) the different layers control colour (L1 and L2), shape and size (L2) of flowers.

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