ABSTRACT

Cellular behaviour in primary roots of standard length 11.15 ± 0.33 mm from four-day-old seedlings of *Dianthus caryophyllus* Linn. cv. Grenadin were studied *in vivo* and *in vitro*. Parameters such as mitotic index (MI), chromosome count, the ploidy level, the mean cell and nuclear areas and the cell doubling time (Cdt) were determined from 2 mm segments of root tips.

The MI value of the root segment decreased when transferred from *in vivo* to Murashige and Skoog (MS) medium supplemented with 2.0 mg/l NAA. The mean chromosome count were generally stable during 6 months in culture period with 2n=2x=30 within the range of 25-33 chromosomes per cell. The ploidy level increased with high percentage of polyploid cells during 6 months in culture. Most of the cells also accumulated in G2-phase of the cell cycle. The mean cell and nuclear areas were stable for the initial 2 weeks and then dropped after 4 weeks until 6 months in culture. The cell doubling time increased to 96.07 h in 3 weeks culture while Cdt *in vivo* was only 66.11 h.

In tissue culture technique, nodal stem and shoot were the most responsive explants to regenerate multiple shoots compared to stem, leaf and root explants. Combination of 0.5 mg/l NAA and 1.5 mg/l BAP, pH 5.8, 30 g/l sucrose, 8 g/l agar, temperature 25 ± 1 °C and photoperiod of 16 h light and 8 h darkness were suitable for carnation regeneration. Multiple shoots were also produced when explants from shoot and nodal stem were cultured in liquid medium, 1/3 MS

medium supplemented with 30 g/l sucrose. The concentration of 30 g/l sucrose could prevent vitrification problems which often happened in carnation *in vitro*. Multiple shoots were able to regenerate better on MS medium compared to Nistch and Nistch (NN) medium. The presence of NAA hormone was more capable of inducing roots compared with IAA and 2, 4-D. BAP hormone obviously could enhance the frequency of shoot regenerations than 2iP, kinetin and zeatin. Acclimatization was successfully done and the plantlets obtained could survive in the glasshouse.

Shoot and nodal stem explants on MS supplemented with 2.0 mg/l NAA could produce multiple shoots. Cytological studies on these regenerative tissues had been done and compared with non-regenerative tissues from root, leaf and stem explants. The MI value in roots of both tissues were high, i.e., 36-43%. But then, the MI decreased during culture. The mean chromosome was more or less 28.3 chromosomes per cell. The differences between regenerative and non-regenerative tissues were the percentage of S-phase and G2-phase of the cell cycle. In non-regenerative tissues, the percentage of S-phase and G-2 phase were higher. The percentage of polyploid cells in regenerative and non-regenerative tissues were obviously lower than *in vivo* cells. Nevertheless, the regenerative tissues had more polyploid cells compared to non-regenerative tissues. The mean cell and nuclear areas of regenerative tissues obtained were larger than non-regenerative. The Cdt of regenerative tissues, e.g., shoot was only 62.8 h and non-regenerative tissues , e.g., stem was 155.8 h. Perhaps, the ability of regenerative

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tissues to divide faster than non-regenerative explained their ability to produced multiple shoots.

The interesting fact about regeneration of *Dianthus caryophyllus* Linn. cv. Grenadin is that although high ploidy level was detected in regenerative cells, the tissues could still regenerate into complete plants. It shows that this species maintained the polyploid cells from *in vivo* to *in vitro* plantlets. The regenerants were successfully transferred to the soil.

ABSTRAK

Kelakuan sel akar primer carnation, *Dianthus caryophyllus* Linn. cv. Grenadin yang berusia 4 hari dengan purata panjang akar 11.15 ± 0.33 mm dan ditanam secara *in vivo* dan *in vitro* telah dikaji. Parameter seperti indeks mitosis (MI), bilangan kromosom, tahap ploidi, luas sel, luas nukleus dan masa penggandaan sel (Cdt) bagi segmen hujung akar berukuran 2 mm telah dilakukan.

Apabila segmen akar primer carnation yang ditanam secara *in vivo* dipindahkan kepada keadaan *in vitro* di atas media Murashige dan Skoog (MS) yang ditambah dengan 2.0 mg/l NAA, purata nilai MInya didapati menjadi lebih rendah. Purata bilangan kromosom didapati stabil sepanjang 6 bulan dikultur dengan nilai 2n=2x=30 dan julat 25-33 kromosom per sel. Tahap ploidi meningkat dengan peratus sel poliploid yang tinggi sepanjang 6 bulan dikultur. Kebanyakan sel juga didapati berada pada fasa G2 kitaran sel. Purata luas sel dan nukleus adalah tinggi dan stabil pada 2 minggu pertama tetapi turun semula pada minggu ke 4 sehingga 6 bulan dikultur. Masa penggandaan sel bagi eksplan yang dikultur secara *in vitro* didapati lebih panjang (96.07 jam) berbanding sel-sel yang ditanam secara *in vivo* (66.11 jam).

Dalam kajian kultur tisu, eksplan batang bernod dan pucuk yang berusia 3 minggu didapati paling responsif untuk regenerasi pucuk berganda berbanding eksplan batang, daun dan akar. Media optimum untuk regenerasi pucuk adalah media MS yang ditambah dengan 0.5 mg/l NAA dan 1.5 mg/l BAP, pH 5.8, 30 g/l sukrosa, 8

g/l agar, suhu 25 ± 1 °C dan kalacahaya 16 jam cahaya serta 8 jam gelap. Pucuk berganda juga boleh dihasilkan apabila eksplan pucuk dan batang bernod dikultur di dalam media 1/3 MS yang ditambah dengan 30 g/l sukrosa. Sukrosa yang berkepekatan 30 g/l didapati dapat menghindar masalah vitrifikasi yang kerap berlaku pada carnation *in vitro*. Media Murashige dan Skoog didapati lebih sesuai digunakan untuk regenerasi pucuk berbanding media Nistch dan Nistch. Hormon NAA lebih baik daripada IAA dan 2, 4-D untuk menginduksi akar dan hormon BAP pula didapati lebih baik daripada 2iP, kinetin dan zeatin untuk regenerasi pucuk berganda. Aklimatisasi berjaya dilakukan dan pokok carnation hidup subur di rumah kaca.

Oleh kerana dari kajian kultur tisu menunjukkan cuma eksplan pucuk dan batang bernod sahaja yang berjaya membentuk pucuk berganda apabila dikultur di atas MS yang ditambah dengan 2.0 mg/l NAA, kajian sitologi telah dijalankan ke atas tisu eksplan regeneratif itu. Tisu yang tidak regeneratif di atas media MS yang ditambah dengan 2.0 mg/l NAA, iaitu, tisu eksplan akar, batang dan daun turut dikaji untuk perbandingan. Didapati nilai MI kedua-dua jenis tisu regeneratif dan tidak regeneratif adalah tinggi, iaitu antara 36-43%. Namun begitu, nilai MI semakin berkurangan apabila semakin lama di dalam kultur. Purata bilangan kromosom didapati hampir sama dengan purata 28.3 kromosom per sel. Perbezaan yang ditunjukkan adalah peratus sel pada fasa S dan G2 bagi tisu tidak regeneratif yang didapati lebih tinggi berbanding tisu regeneratif. Peratus sel poliploid bagi kedua-dua jenis tisu didapati lebih rendah daripada sel-sel akar yang ditanam secara *in vivo*. Namun begitu, tisu eksplan regeneratif mempunyai

peratus sel poliploid yang lebih tinggi daripada sel tisu tidak regeneratif. Luas sel dan luas nukleus tisu regeneratif juga didapati lebih besar berbanding tisu tidak regeneratif. Masa penggandaan sel eksplan regeneratif seperti pucuk didapati cuma 62.8 jam manakala bagi eksplan tidak regeneratif seperti batang adalah 155.8 jam. Keupayaan tisu regeneratif untuk membahagi dalam masa yang lebih singkat mungkin menjelaskan kemampuannya untuk regenerasi menghasilkan pucuk berganda.

Yang menarik tentang regenerasi carnation cv. Grenadin ini adalah walaupun eksplan regeneratifnya mempunyai banyak sel-sel poliploid dengan peratus yang tinggi, ia masih mampu meregenerasikan pucuk berganda yang banyak. Spesis ini didapati mengekalkan sifat poliploid sel induk *in vivo*nya apabila dikultur secara *in vitro*. Regeneran juga berjaya diaklimatisasikan dan tiada perbezaan fenotip diperhatikan.