MORPHOGENESIS AND TISSUE CULTURE STUDIES OF *Gerbera jamesonii* BOLUS Ex. Hook F.

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Field of Study: **(Science) Plant Biotechnology**

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ABSTRACT

In vitro regeneration of *Gerbera jamesonii* Bolus ex. Hook f. was successfully carried out in this study. Petiole and leaf explants from 8-week-old aseptic seedlings were used as source of explants. Different response was observed when different explants were cultured on the culture media. MS (Murashige and Skoog, 1962) supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA was identified as the optimum medium for shoot regeneration, while petiole explant was identified as the most responsive explant in this study. Petiole explants regenerated shoots (94.3 ± 2.5 %) and produced 9.3 ± 0.6 shoots per explant. MS basal medium was the optimum medium for plantlet root induction. Various concentrations of auxin such as NAA, IAA, IBA and 2,4-D at a range of 0.1-2.0 mg/l and cytokinin such as BAP, Kinetin, Zeatin and 2iP at a range of 0.1-3.0 mg/l were used in order to achieve *in vitro* regeneration of *G. jamesonii*.

Callus was also induced from *G. jamesonii* petiole and leaf explants. Optimum callus induction was obtained when petiole explant was cultured on MS medium supplemented with 2.0 mg/l 2,4-D and 1.0 mg/l BAP. Green compact callus were induced. Through screening of secondary metabolites in *Gerbera* callus, flavonoid, terpenoid and conjugated chain compounds were found in *Gerbera* callus.

Studies on indirect somatic embryogenesis of *G. jamesonii* were carried out using leaf explant. Induction of embryogenic callus was obtained when petiole explants were cultured on MS media supplemented with 0.01-2.0 mg/l 2,4-D. White friable callus was induced. Embryogenic callus was distinguished from non-embryogenic callus through

double staining method. Embryogenic callus obtained were transferred to MS liquid medium fortified with 0.1-2.0 mg/l 2,4-D added with 0.1 or 1.0 mg/l NAA. Somatic embryos produced from embryogenic callus cultured in liquid medium were then transferred to MS medium supplemented with 1.0 mg/l BAP and 0.1 mg/l NAA added with 50 mM L-Proline. A total of 29.8 ± 1.2 embryos per explant were obtained.

Synthetic seeds of *G. jamesonii* were successfully produced when micro shoots, globular and cotyledonary phase somatic embryos were encapsulated with encapsulation matrix composed of 3.0% sodium alginate dissolved in Ca-free MS liquid medium supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA. Germination rates obtained from encapsulated micro shoots was 74.5 ± 2.6%, 34.8 ± 1.2% for encapsulated globular somatic embryos and 54.2 ± 1.3% for encapsulated cotyledonary phase somatic embryos. Synthetic seeds from encapsulated micro shoots could be stored at 4 ± 1 ^{o}C for 180 days while synthetic seeds from encapsulated globular and cotyledonary phase somatic embryos could be stored at 4 ± 1 ^{o}C up to 90 days only before being germinated.

Generally, *in vitro* regeneration from petiole explants were reduced when explants were exposed to gamma irradiation (10-60 Gy). As the irradiation dose increased, *in vitro* shoots regeneration was declined and abnormal shoots were formed. *In vitro* plantlets exposed to gamma irradiation also showed height reduction as the irradiation dose was increased. Effects of gamma irradiation were also observed on callus tissues. Growth of *G. jamesonii* callus tissues was also reduced with the increase of the irradiation dose. Reduction of chlorophyll content in callus tissues was observed when *Gerbera* callus

were exposed to gamma irradiation. Soluble protein content in callus tissues were also reduced with the increase of irradiation dose.

Gerbera jamesonii plantlets obtained from tissue culture system through direct regeneration, somatic embryogenesis, synthetic seeds and also irradiated plantlets were successfully transferred and acclimatized to the new environment. Garden soil and vermiculite were used as sowing media. Growth and development of plantlets were optimum when plantlets obtained from regeneration of petiole explants were acclimatized in garden soil (combination of black soil and red soil at 2:1) with 86.0 ± 0.9% survival. *Ex-vitro* flowering was achieved after 6 months. *Gerbera jamesonii* plants obtained from *in vitro* regeneration showed similar morphological characters to the mother plant.

ABSTRAK

Regenerasi lengkap bagi tumbuhan *Gerbera jamesonii* Bolus ex. Hook f. daripada sistem kultur tisu telah berjaya diperoleh. Eksplan petiol dan daun daripada anak benih aseptik yang berumur 8 minggu telah digunakan untuk tujuan ini. Respons yang berbeza telah diperoleh apabila eksplan yang berlainan dikultur di atas media kultur. Medium MS (Murashige and Skoog, 1962) ditambah dengan kombinasi hormon 2.0 mg/l BAP dan 0.5 mg/l NAA telah dikenalpasti sebagai medium optima bagi regenerasi pucuk manakala eksplan petiol pula telah dikenalpasti sebagai eskplan yang terbaik dalam kajian ini. Eksplan petiol telah berjaya menghasilkan pucuk *in vitro* (94.3 ± 2.5%) dengan 9.3 ± 0.6 pucuk dihasilkan bagi setiap eksplan. Medium MS tanpa hormon merupakan medium yang optima untuk penginduksian akar. Pelbagai kombinasi auksin seperti NAA, IAA, IBA dan 2,4-D dalam julat antara 0.1-2.0 mg/l dan sitokinin seperti BAP, Kinetin, Zeatin dan 2iP pada julat 0.1-3.0 mg/l telah digunakan untuk tujuan regenerasi lengkap tumbuhan *G. jamesonii* secara *in vitro*.

Induksi kalus juga telah dijalankan ke atas eksplan petiol dan daun tumbuhan *G. jamesonii*. Penginduksian kalus optima telah diperoleh apabila eksplan petiol dikultur di atas media MS dengan 2.0 mg/l 2,4-D dan 1.0 mg/l BAP. Kalus yang dihasilkan bewarna hijau dan mempunyai struktur yang padat. Kajian penskrinan bahan metabolit sekunder ke atas kalus yang dihasilkan mendapati kalus daripada tumbuhan *Gerbera* mengandungi flavonoid, terpenoid dan juga sebatian rantai berkonjugat.

Kajian embriogenesis somatik secara tidak langsung telah dijalankan ke atas eksplan daun. Pengaruhan kalus embriogenik telah diperoleh apabila eksplan petiol dikultur di atas medium MS ditambah dengan 0.01-2.0 mg/l 2,4-D. Kalus yang dihasilkan bewarna keputihan yang mempunyai struktur yang lembut dan rapuh. Kalus embriogenik telah dibezakan dengan kalus bukan embriogenik dengan menggunakan kaedah pewarnaan berganda. Kalus embriogenik yang dihasilkan kemudian dipindahkan ke dalam media cecair MS yang mengandungi 0.1-2.0 mg/l 2,4-D yang ditambah dengan 0.1 atau 1.0 mg/l NAA. Embrio somatik dihasilkan apabila kalus embriogenik daripada kultur ampaian dipindahkan ke atas media MS dengan 1.0 mg/l BAP dan 0.1 mg/l NAA dan ditambah dengan 50 mM L-Proline dengan penghasilan embrio sebanyak 29.8 ± 1.2 embrio bagi setiap eksplan yang telah digunakan.

Biji benih tiruan bagi tumbuhan *G. jamesonii* telah berjaya diperoleh apabila pucuk mikro, embrio somatik peringkat globul dan kotiledon disalut menggunakan matriks pengkapsulan yang dihasilkan daripada 3.0% larutan sodium alginat yang dilarutkan di dalam media cecair MS tanpa kalsium dan ditambah dengan 2.0 mg/l BAP dan 0.5 mg/l NAA. Peratus percambahan biji benih tiruan daripada eksplan pucuk mikro adalah 74.5 ± 2.6%, 34.8 ± 1.2% bagi embrio somatik peringkat globul dan 54.2 ± 1.3% bagi embrio somatik peringkat kotiledon. Biji benih tiruan yang dihasilkan daripada pucuk mikro dapat disimpan pada suhu 4 ± 1 ^{o}C sehingga 180 hari manakala biji benih tiruan yang dihasilkan daripada pengkapsulan eksplan somatik embrio peringkat globul dan kotiledon hanya dapat disimpan pada suhu 4 ± 1 ^{o}C sehingga 90 hari sebelum dicambahkan.

Penghasilan pucuk *in vitro* daripada eksplan petiol telah didapati merosot apabila eksplan didedahkan kepada sinaran gamma (10-60 Gy). Semakin tinggi dos pendedahan kepada sinaran gamma, penghasilan pucuk *in vitro* semakin berkurangan dan pucuk abnormal mula terhasil. Plantlet *in vitro* yang didedahkan kepada sinaran gamma juga mengalami kesan radiasi di mana ketinggian plantlet semakin berkurang apabila kadar radiasi bertambah. Kesan sinaran radiasi gamma juga dapat diperhatikan ke atas tisu kalus. Kadar perkembangan tisu kalus tumbuhan *Gerbera* semakin berkurang dengan pertambahan kadar sinaran radiasi yang didedahkan kepada tisu kalus tersebut. Analisa ke atas kandungan klorofil di dalam tisu kalus mendapati bahawa kandungan klorofil semakin berkurangan dengan pertambahan dos sinaran gamma yang didedahkan. Kandungan protein terlarut di dalam tisu kalus juga berkurangan dengan pertambahan dos sinaran gamma yang didedahkan.

Plantlet tumbuhan *G. jamesonii* yang berjaya dihasilkan daripada sistem kultur tisu samada daripada regenerasi secara langsung, embriogenesis somatik, biji benih tiruan dan juga plantlet yang dihasilkan melalui regenerasi *in vitro* yang telah didedahkan kepada sinaran radiasi gamma telah berjaya dipindahkan ke persekitaran luar dan menjalankan proses aklimatisasi. Tanah kebun dan juga vermiculite telah digunakan sebagai substrat pertumbuhan. Pertumbuhan dan perkembangan plantlet yang diaklimatisasi didapati optima apabila plantlet yang dihasilkan daripada regenerasi eksplan petiol dipindahkan ke tanah kebun (campuran tanah hitam dan tanah merah pada nisbah 2:1) dengan peratus keterushidupan sebanyak 86.0 ± 0.9%. Pembungaan *ex-vitro* telah dapat diperhatikan selepas 6 bulan. Perbandingan dari segi morfologi mendapati bahawa tumbuhan

G. jamesonii yang diregenerasi daripada sistem kultur tisu mempunyai ciri-ciri morfologi yang serupa dengan tumbuhan induk.

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BAP	Benzylaminopurine
$CaCl_2.2H_20$	Calcium chloride dehydrate
2,4-D	2,4- Dichlorophenoxyacetic acid
HCL	Hydrochloric acid
IAA	Indole-3-acetic acid
IBA	Indolebutyric acid
2-iP	2-isopentenylaminopurine
Kinetin	6-furfurylaminopurine
kPa	Kilo Pasca
mg/l	Milligram per liter
min	minute
MS	Murashige and Skoog
$MgCO_3$	Magnesium carbonate
NAA	Naphthalene acetic acid
NaOH	Sodium hydroxide
$NaC_6H_7O_6$	Sodium alginate
Rpm	Rotation per minute
SEM	Scanning electron microscope
TDZ	Thidiazuron
Tween 20	Polyoxyethylene sorbitan monolaurate
v/v	Volume per volume
w/v	Weight per volume