## Abstract

Establishment of protoplasts system provides a useful platform for cloning and genetic manipulation of ginger plants. In this study, an efficient protocol for developing protoplast isolation and culture for *Boesenbergia rotunda* has been established. *B. rotunda* embryogenic cell suspension cultures showed good growth rate ($\mu = 0.1125$) when cultured in plant growth regulator (PGR)-free liquid Murashige and Skoog (MS) basal medium supplemented with 3 % sucrose, where no promotive effect were observed in the presence of any concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) and sonication treatment. This suspension culture was subsequently used as a source to isolate protoplast using enzyme cocktails. A total number of 1 - $5 \times 10^5$ per mL protoplasts were isolated using 0.25 % macerozyme and 1 % cellulase incubated for 24 h under continuous shaking condition of 50 rpm in dark condition. Of the isolated protoplasts, 54.93 % were viable according to fluorescein diacetate staining test. About 7.61 ± 1.65 % cultured protoplasts successfully formed micro-colonies when cultured in liquid MS basal medium supplemented with 9 g/L mannitol, 2 mg/L 1-naphthaleacetic acid (NAA), 0.5 mg/L benzylaminopurine (BAP) and incubated at 25 ± 2 ℃ in dark condition for 4 weeks. The osmoticum of the culture media were reduced weekly during the protoplast culture period from 9 to 5 % followed by 1 %. These colonies were subsequently transferred to solid MS medium supplemented with 0.5 mg/L BAP for callus initiation. The callus was formed after 5 weeks of culture.

## Abstrak

Sistem penghasilan protoplas menyediakan satu platfom yang berguna untuk genetik manipulasi dan teknik pengklonan bagi tumbuhan halia. Dalam kajian ini, satu protokol yang berkesan untuk penghasilan dan pengkulturan protoplas untuk *Boesenbergia rotunda* telah dibangunkan. Kultur ampaian embriogenik sel *B. rotunda* menunjukkan kadar pertumbuhan yang baik ($\mu = 0.1125$) apabila dikultur dalam cecair Murashige dan Skoog (MS) medium tanpa zat pengatur tumbuhan (ZPT) dengan 3 % sukrosa, di mana tiada kesan penggalakan diperhatikan dalam kehadiran pelbagai kepekatan dengan asid 2,4-dichlorophenoxyacetic (2,4-D) dan rawatan sonikasi. Kultur ampaian ini kemudiannya digunakan sebagai sumber untuk menghasilkan protoplas menggunakan koktel enzim. Sebanyak $1 - 5 \times 10^5$ per mL protoplas telah diasingkan mengguna 0.25 % maserozim dan 1 % selulase diinkubasi selama 24 jam dengan rotasi berterusan sebanyak 50 rpm dalam keadaan gelap. Daripada protoplas yang dihasilkan, 54.93 % menunjukkan daya kehidupan berdasarkan ujian pewarnaan fluorescein diaceta. Anggaran $7.61 \pm 1.65$ % protoplas yang dikulturkan berjaya menghasilkan mikro-koloni apabila dikulturkan dalam cecair MS medium dengan 9 g/L mannitol, 2 mg/L asid 1-naphthaleacetic (NAA), 0.5 mg/L benzilaminopurina (BAP) dan diinkubasi pada $25 \pm 2$ ℃ dalam keadaan gelap selama 4 minggu. Tekanan osmotik media kultur dikurangkan setiap minggu sepanjang tempoh pangkulturan protoplas dari 9 ke 5 % sehingga 1 %. Koloni ini kemudian dipindahkan ke medium MS pepejal dengan 0.5 mg/L BAP untuk inisiasi kalus. Kalus berjaya dihasilkan selepas 5 minggu pengkulturan.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| 2,4-D | 2,4-dichlorophenoxyacetic acid |
| ANOVA | analysis of variance |
| ave. | average |
| BAP | 6-benzylaminopurine |
| $CaCl_2.2H_2O$ | calcium chloride dihydrat reinst |
| $CoCl_2.6H_2O$ | cobalt (II) chloride 6-hydrate |
| CPW | cell and protoplast washing solution |
| CPW13M | cell and protoplast washing solution with 13% mannitol |
| CPW21S | cell and protoplast washing solution with 21% sucrose |
| $CuSO_4.5H_2O$ | copper (II) sulphate 5-hydrate |
| EDTA | ethylenediaminetetraacetic acid |
| *et al.* | Latin: et alii or English: and others |
| FeSO4.7H2O | iron (II) suphate |
| *g* | gravity force |
| $H_3BO_3$ | boric acid |
| HCl | hydrochloric acid |
| $KH_2PO_4$ | monopotassium phosphate |
| KI | potassium iodide |
| $KNO_3$ | potassium nitrate |
| $MgSO_2.7H_2O$ | magnesium sulphate |
| $MnSO_4.4H_2O$ | mangan (II) sulphate |
| MS | Murashige and Skoog, (1962) medium |
| MSP1 | MS protoplast culture medium |
| MSP1 9M | MS protoplast culture medium with 9% mannitol |
| Na2EDTA.2H2O | ethylenediaminetetraacetic acid-disodium dihydrate |
| $Na_2MoO_4.2H_2O$ | sodium molybdate dihydrate |

| | |
|---|---|
| NAA | 1-naphthaleacetic acid |
| $NH_4NO_3$ | ammonium nitrate |
| PGR | plant growth regulator |
| pH | the negative logarithm of the hydrogen ion concentration |
| Rep. | replicate |
| SD | standard deviation |
| SE | standard error |
| uv | ultra violet |
| w/v | weight per volume |
| $ZnSO_4.7H_2O$ | zinc sulphate |

## LIST OF SYMBOLS AND UNITS

| | |
|---|---|
| % | percentage |
| / | per |
| > | more than |
| ± | more less |
| × | times |
| ℃ | degree Celcius |
| µ | specific growth rate |
| h | hour |
| L | litre |
| mg | miligram |
| mL | millilitre |
| rpm | revolution per minute |
| s | second |
| SCV | settle cell volume |

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