MICROPROPAGATION OF *Etlingera coccinea* (ZINGIBERACEAE)

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SCHOOL OF SCIENCE AND TECHNOLOGY
UNIVERSITI MALAYSIA SABAH
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DEGREE **: MASTER OF SCIENCE (BIOTECHNOLOGY)**

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ABSTRACT

MICROPROPAGATION OF *Etlingera coccinea* (ZINGIBERACEAE)

An effective protocol was developed for *in vitro* regeneration of valuable food additive plant *Etlingera coccinea* (zingiberaceae) through different methods of plant tissue culture. Micropropagation was possible via shoot regeneration from rhizome bud explants, callus induction and proliferation derived from leaf explants, plantlets regeneration from callus culture and shoot multiplication through pseudo stem explants. This study was involved the effect of Plant Growth Regulators (PGRs), types and strengths of basal media, and types and concentrations of carbon sources. The results obtained showed that plant growth factors manipulation as stated markedly influence shoot regeneration, callus induction and proliferation, plantlets regeneration from callus culture, and shoot multiplication of *Etlingera coccinea*. Rhizome buds were sterilized and cultured on Murashige and Skoog (MS) basal medium containing 0.1-2.0 µM of thidiazuron (TDZ), benzylaminopurine (BAP) or kinetin (KIN) supplied with 3% (w/v) of sucrose. High percentage of shoot regeneration (100%) was achieved by (~1±0.5 cm) of the whole bud cultured on MS medium containing 1.0 µM TDZ after 20 weeks of culture. Shoots were transferred to medium containing 5.0 µM BAP for shoot elongation and multiplication. High frequency of rooting (90%) was observed on medium supplemented with 1.0 µM IBA. The maximum percentage (100%) of callus formation was obtained after 10 weeks of culture from *in vitro* leaf explants cultured on half strength MS basal medium supplemented with 5.0 µM 2,4-dichlorophenoxyacetic acid (2,4-D) and 10 µM Benzylaminopurine (BAP). In media selection, half strength MS medium was most suitable for callus induction while 3% (w/v) of sucrose was the best carbon source among other sugar types tested. Callus cell proliferation was rapidly increased on MS basal medium supplemented with 2.5 µM 2,4-D within 5 weeks of culture. Combination of 2.5 µM 2,4-D and 5.0 µM BAP improved callus proliferation. 3% (w/v) of sucrose was most effective for callus proliferation and long term maintenance of callus culture. Plantlets regeneration with highest frequency of shoot formation (88%) from callus culture were obtained on MS medium containing 2.5 µM BAP and 0.1 µM IAA with a mean of 5.5 and 7.0 shoots and roots per explant after 6 weeks of culture. The most responsive explants were observed on full strength MS basal medium while 3% (w/v) of sucrose was the best source of carbon among other carbon sources tested. Roots regeneration were observed along with the shoot formation. 2.5 µM BAP was superior to KIN and ZEA on shoot multiplication from pseudo stem explants. Combination of 2.5 µM BAP and 0.7 µM NAA enhance shoots regeneration percentage up to 100% over a 6 weeks period of culture and produced 12.0 shoots per explant with 100% of rooting percentage. Full strength of MS basal medium and 3% (w/v) of sucrose were the best supplements for shoot multiplication via pseudo stem explants. Micropropagated plantlets were grown healthy after transplanted to the pots containing a mixture of 3:1 (w/w) soil: sand with 85% of survival explants after 6 month of transplantation. The procedure reported here offers a potential system for micropropagation of *Etlingera coccinea* and expected to be useful in improvement and conservation of this species in the future.

ABSTRAK

Kaedah terbaik regenerasi secara in vitro bagi tumbuhan berkepentingan makanan Etlingera coccinea (zingiberaceae) telah dikaji melalui pelbagai kaedah kultur tisu tumbuhan. Mikropropagasi telah dilakukan melalui regenerasi pucuk daripada eksplan tunas rizom, induksi dan proliferasi kalus daripada eksplan daun, regenerasi plantlet daripada kultur kalus, dan penambahbilangan pucuk daripada eksplan menyerupai batang. Kajian ini melibatkan kesan pengawalaturan tumbuhan, jenis dan kepekatan medium, serta jenis dan kepekatan sumber karbon. Hasil kajian yang diperolehi menunjukkan manipulasi faktor pertumbuhan yang dinyatakan mempengaruhi regenerasi pucuk, induksi dan proliferasi kalus, regenerasi plantlet daripada kultur kalus, dan penambahbilangan pucuk Etlingera coccinea. Tunas rizom disterilkan dan dikulturkan ke dalam medium MS yang mengandingi 0.1-2.0 µM TDZ, BAP atau KIN secara berasingan dan dibekalkan dengan 3% (w/v) sukrosa. Peratus tertinggi regenerasi pucuk (100%) diperolehi daripada tunas rizom bersaiz (~1±0.5 cm) tanpa pembelahan, selepas 20 minggu dikulturkan dalam medium MS yang mengandungi 1.0 µM TDZ. Mikropucuk dipindahkan ke dalam medium yang mengandungi 5.0 µM BAP untuk pemanjangan dan proliferasi pucuk. Frekuensi tertinggi pertumbuhan akar (90%) didapati dalam medium yang dibekalkan 1.0 µM IBA. Peratus maksimum (100%) pembentukan kalus diperolehi daripada eksplan daun in vitro selepas 10 minggu dikulturkan dalam ½ kepekatan medium MS yang mengandungi 5.0 µM asid diklorofenoksiasetik (2,4-D) dan 10 µM benzilaminopurina (BAP). Dalam pemilihan media, ½ kepekatan medium MS didapati paling sesuai untuk induksi kalus manakala 3% (w/v) kepekatan sukrosa membekalkan sumber karbon yang terbaik. Proliferasi sel kalus meningkat dengan cepat dalam tempoh 5 minggu dalam medium MS yang dibekalkan 2.5 µM 2,4-D. Kombinasi 2.5 µM 2,4-D dan 5.0 µM BAP menambah peningkatan proliferasi kalus. 3% (w/v) sukrosa adalah paling efektif untuk proliferasi kalus dan pengekalan kultur kalus dalam jangka masa panjang. Regenerasi plantlet dengan frekuensi tertinggi pembentukkan pucuk (88%) daripada kultur kalus diperolehi dalam medium MS yang mengandungi 2.5 µM BAP dan 0.1 µM IAA dengan min 5.5 dan 7.7 pucuk dan akar per eksplan selepas 6 minggu dikulturkan. Tindakbalas eksplan yang terbaik didapati dalam medium MS pada kepekatan penuh manakala 3% (w/v) sukrosa membekalkan sumber karbon yang terbaik. Regenerasi akar terbentuk bersamaan dengan pembentukkan pucuk. 2.5 µM BAP mengatasi KIN dan ZEA dalam penambahbilangan pucuk daripada eksplan menyerupai batang. Kombinasi 2.5 µM BAP dan 0.7 µM NAA meningkatkan peratus regenerasi pucuk kepada 100% selama 6 minggu pengkulturan dan menghasilkan 12.0 pucuk per eksplan dengan 100% pembentukkan akar. Medium MS pada kepekatan penuh dan 3% (w/v) sukrosa adalah suplemen terbaik untuk penambahbilangan pucuk daripada eksplan menyerupai batang. Plantlet yang dipropagasikan bertumbuh dengan baik selepas dipindahkan ke dalam pasu yang mengandungi 3:1 (w/w) tanah:pasir dengan 85% eksplan berkembang selepas 6 bulan pemindahan. Kaedah yang dijelaskan ini menawarkan sistem mikropropagasi yang berpotensi untuk perkembangan dan pemuliharaan spesies Etlingera coccinea pada masa hadapan.

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