GERMINATION, REGENERATION AND PIGMENT DETECTION IN *Nelumbo nucifera* Gaertn. (PINK ASIAN LOTUS)

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***Nelumbo nucifera* Gaertn. (PINK ASIAN LOTUS)**

Field of Study: **PLANT BIOTECHNOLOGY**

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ABSTRACT

In vivo and *in vitro* germination of three types of seeds including matured (black), immature or fruits (green) and young (yellow) of *Nelumbo nucifera* Gaertn. (pink Asian lotus) were done. The black and green seeds both gave the highest responses to technique of scarified with medium-sand paper and germinated in liquid (tap water) and solid (MS basal media) substrates. The black seeds with germination rate of 67.01±0.28% *in vivo* and 78.35±0.61% *in vitro*, while, green seed with 75.12±0.16% *in vivo* and 100.00% *in vitro* germination. The highest shoot length in seeds without cotyledon for black and green seed with 28.00±0.55 mm and 26.00±0.16 mm, respectively. The highest shoot length was in media with pH 5.5 for both black seed and green seeds and with 64.03±0.02 mm and 32.55±0.04 mm, respectively. The maximum root length for both black and green seeds was 14 mm on the 14^{th} day with 3 green shoots and 6 white primary roots. Whilst, maximum root length for yellow seeds were on the 18^{th} day with 3 green shoots and 6 white primary roots. For storage purposes, the optimum concentration for the formation of encapsulation matrix was 3.0% sodium alginate ($NaC_6H_7O_6$). Encapsulated explants were soaked in 100 mM calcium chloride dehydrate ($CaCl_2.2H_2O$) solution for 30 minutes. Through sodium alginate encapsulated method (4±1°C), seeds germination reduced from 100% (day 1) to 53.3±1.2% (day 90), while, through frozen whole seeds method (-20±1°C), 100% germination rate until 60 days in storage. After 90 days in storage, the germination rate was still high with 93.3±0.6%.

Direct regeneration of *Nelumbo nucifera* Gaertn. were successfully achieved from green seed explants cultured on solid MS media supplemented with combinations of 1.5 mg/l BAP and 0.5 mg/l NAA with 10.33 ±0.23 shoots per explant (true-to-type), and with 3.67 ±0.31 roots per explant. Direct regeneration of *Nelumbo nucifera* Gaertn.

were successfully achieved from yellow seed explants cultured on solid MS media supplemented with combinations of 0.5 mg/l BAP and 1.5 mg/l NAA with 16.00±0.30 shoots per explant, with new characteristics of layered multiple shoots. Roots were formed on solid MS basal media. Some formation of abnormal shoots (pinkish, red and oval leaf) occurred from green seed explants on solid MS media supplemented with combinations of 1.0 mg/l BAP and 2.5 mg/l NAA, 2.5 mg/l BAP and 2.5 mg/l NAA, 1.5 mg/l BAP and 2.0 mg/l NAA and 1.5 mg/l BAP and 2.5 mg/l NAA. Furthermore, the highest shoots per explant for green seed explants was in 8.8mg/l MS powder (double strength) with mean 19.03±0.05 shoots per explant. The highest shoots per explants for yellow seed explants was on 4.4 mg/l MS powder (full strength) with mean 16.06±0.06 shoots per explant. Media with pH 5.5 resulted in the highest height of shoots for green and yellow seed explants with mean 12.04±0.7 mm and 16.03±0.30 mm, respectively. While, the highest height of shoots for green seed explants with 9.41±1.11 mm in 250.00 mm light distance. The highest height of shoots with 16.67±0.23 mm for yellow seed explants in 200.00 mm light distance. Solid MS basal media was optimum for root formation within 4 weeks for green seed explants and after 24 weeks for yellow seed explants on solid MS media supplemented with 0.5mg/l BAP and 1.5mg/l NAA. In double layer media, the highest number of shoots per explant was both in ratio liquid to solid 2:1 with mean 16.67±0.23 number of shoots per explant with formation of primary and secondary roots for explants from yellow seeds with formation of layered multiple shoots, while mean 9.00±0.15 number of shoots per explant for green seeds.

Extraction from lotus stamen was analyzed through HPLC system. Pigment detected was found to have the total carotenoid content of 526.96 ± 0.52 µg/g DW, whereas for individual carotenoid β-carotene (460 ± 10.28 µg/g DW) was found with a relatively high concentration and neoxanthin (39.26 ± 0.82 µg/g DW) was found in lower

concentrations. One unknown carotenoid also was detected. For coating experiments, at room temperature with acidic conditions (pH 1-6), yellow pigment changed to the darker colour, yellow-brown, while, at strong alkaline condition (pH 12-14), the colour change to yellow-green. The optimum yellow colour was at pH 8-10. The highest glossiness was in 1.0ml/30.0ml v/v of pigment-resin solution with mean of 74.67±0.33°. Yellow pigment in 20% PMMA (resin) was as stable as pigment-resin added with 1% tartaric acid, coated on glass slides. In contrast, the addition of 1% citric acid reduced the carotenoid color. Extraction for antimicrobial activity from *in vivo* and *in vitro* rolled leaves showed no inhibition zone to all tested bacteria *Staphylococcus aureus, Pseudomonas aeruginosa ,Bacillus cereus* and *E.coli*. However, the highest inhibition zone was from *in vivo* sample for both on fungi, *Fusarium* sp. and *Trichoderma* sp. with mean 9.0±0.1 mm and with 7.3±02 mm diameters, respectively. Inhibition zone for *in vitro* samples were 8 times lower than *in vivo* with mean of 2.0±0.4 mm on *Fusarium sp.* and 1.1±0.3 mm on *Trichoderma sp.*

Soil samples collected from Tasik Chini with mean pH of 3.97±0.02, while the optimum survival for acclimatization plantlets was on black clay loam (mean pH 6.03±0.29) that commercialized at all the nurseries in Malaysia. The most acidic soils were collected from Tasik Chini with the lowest survival rate (31.34±0.77%) for green seed and 11.11±0.51% for yellow seeds. The highest survival rate was in black clay loam for both plantlets from green and yellow seeds with 83.01±0.23% and 69.22±0.43%, respectively. The highest plantlets height was obtained from plant acclimatized under full sunlight exposure with 10 times from shady exposure. Acclimatized plantlets showed the same character as mother plants, even though at the beginning of transferred with layered multiple shoots (new character was formed *in vitro* only).

ABSTRAK

Percambahan tiga jenis biji benih *Nelumbo nucifera* Gaertn. (lotus Asia merah jambu) secara *in vivo* dan *in vitro*, termasuk biji benih matang (hitam), tidak matang atau buah (hijau) dan muda (kuning) telah dikaji. Respon terbaik untuk kedua-dua bijih benih hitam dan hijau adalah melalui kaedah gosokkan menggunakan kertas pasir dan percambahan di dalam cecair (air paip) dan pepejal (MS tanpa hormone). Kadar percambahan bagi biji benih hitam adalah 67.01±0.28% *in vivo* dan 78.35±0.61% *in vitro*. Manakala, percambahan bagi biji benih hijau adalah 75.12±0.16% *in vivo* dan 100.00% *in vitro.* Panjang pucuk yang tertinggi adalah daripada biji benih tanpa kotiledon bagi kedua-dua biji benih hitam dan hijau dengan masing-masing 28.00±0.55 mm dan 26.00±0.16 mm. Panjang pucuk yang tertinggi adalah daripada media dengan pH 5.5 bagi kedua-dua biji benih hitam dan hijau dengan masing-masing 64.03±0.02 mm dan 32.55±0.04 mm. Panjang akar yang maksima bagi kedua-dua biji benih hitam dan hijau adalah 14 mm pada hari ke-14 dengan 3 pucuk hijau dan 6 akar primer yang putih. Manakala, panjang akar yang maksima bagi biji benih kuning adalah 17 mm pada hari ke-18 dengan 3 pucuk hijau dan 6 akar primer yang putih. Untuk tujuan penyimpanan, kepekatan optima untuk pembentukan matriks pengkapsulan ialah 3.0% sodium alginate ($NaC_6H_7O_6$). Eksplan yang dikapsulkan direndam di dalam larutan 100 mM kalsium klorida dehidrat ($CaCl_2.2H_2O$) selama 30 minit. Melalui kaedah pengkapsulan sodium alginate (4±1°C), percambahan biji benih menurun daripada 100% (hari pertama) kepada 53.3±1.2% (hari ke-90). Manakala, melalui kaedah pembekuan biji benih asal (-20±1°C), 100% kadar percambahan sehingga 60 hari tempoh penyimpanan. Selepas 90 hari penyimpanan, kadar percambahan masih tinggi dengan 93.3±0.6%.

Regenerasi *Nelumbo nucifera* Gaertn secara langsung berjaya dicapai melalui eksplan daripada biji benih hijau yang dikultur di atas media MS pepejal yang ditambah dengan

kombinasi 1.5 mg/l BAP dan 0.5 mg/l NAA dengan 10.33 ±0.23 pucuk per eksplan (sama seperti induk), dan lengkap dengan 3.67±0.31 akar per eksplan. Regenerasi *Nelumbo nucifera* Gaertn secara langsung berjaya dicapai melalui eksplan daripada biji benih kuning yang dikultur di atas media MS pepejal yang ditambah dengan kombinasi 0.5 mg/l BAP dan 1.5 mg/l NAA dengan 16.00±0.30 pucuk per eksplan, dengan ciri yang iaitu pucuk berlapis-lapis. Akar terbentuk di dalam media MS tanpa hormone. Beberapa pembentukan pucuk-pucuk tidak normal (merah jambu, merah dan bujur) daripada eksplan biji benih hijau di dalam media MS pepejal yang ditambah dengan kombinasi 1.0 mg/l BAP dan 2.5 mg/l NAA, 2.5 mg/l BAP dan 2.5 mg/l NAA, 1.5 mg/l BAP dan 2.0 mg/l NAA serta1.5 mg/l BAP dan 2.5 mg/l NAA. Tambahan lagi, pucuk per eksplan yang tertinggi adalah daripada biji benih hijau yang dibekalkan dengan 8.8 mg/l serbuk MS (dua kali ganda kekuatan) dengan purata 19.03±0.05 pucuk per eksplan. Pucuk per eksplan tertinggi adalah daripada biji benih kuning dengan 4.4 mg/l serbuk MS (kekuatan penuh) dengan purata 16.06±0.06 pucuk per eksplan. Medium dengan pH 5.5 memberikan tinggi pucuk yang tertinggi bagi biji benih hijau dan kuning dengan purata 12.04±0.70mm dan 16.03±0.30mm, masing-masing. Manakala, tinggi pucuk yang tertinggi bagi biji benih hijau adalah 9.41±1.11 mm dalam 250.00 mm jarak dari cahaya. Tinggi pucuk yang tertinggi bagi biji benih kuning pula adalah 16.67±0.23 mm dalam 200 jarak dari cahaya. Media MS pepejal adalah optimum untuk pembentukan akar dalam tempoh 4 minggu bagi biji benih hijau dan kuning dan 24 minggu bagi biji benih kuning daripada media MS yang ditambah dengan 0.5 mg/l BAP and 1.5mg/l NAA. Bagi media dua lapis, pucuk per eksplan tertinggi adalah di dalam kadar 2:1 dengan purata 16.67±0.23 pucuk per eksplan dengan pembentukan akar primer dan sekunder untuk biji benih kuning dengan pucuk berlapis-lapis dan 9.00±0.15 pucuk per eksplan bagi biji benih hijau.

Pengekstrakan pigmen daripada stamen lotus telah dianalisa melalui sistem HPLC yang mengandungi jumlah karotenoid sebanyak 526.96 ± 0.52 μg/g DW di mana individu carotenoid terdiri daripada β-carotene (460 ± 10.28 μg/g DW) dengan kepekatan yang tinggi dan neoxanthin (39.26 ± 0.82 μg/g DW) dengan kepekatan yang rendah. Satu jenis carotenoid yang tidak dikenalpasti juga diperolehi. Untuk eksperimen cat, pada suhu bilik dengan keadaan berasid (pH 1-6) pigmen kuning bertukar kepada warna yang lebih gelap, kuning kecoklatan. Manakala, dalam keadaan alkali kuat (pH 12-14), warna bertukar kepada kuning kehijauan. Kuning yang optima adalah pada pH 8-10. Kilatan tertinggi adalah dalam 1.0ml/30.0ml v/v larutan pigmen-resin dengan purata 74.67±0.33°. Pigmen kuning dalam 20% PMMA (resin) adalah stabil dengan pertambahan 1% asid tartaric yang dicat pada kaca. Sebaliknya, pertambahan 1% asid sitrik akan memudarkan warna karotenoid. Pengekstrakan untuk mengkaji aktiviti antimikrobial ke atas bakteria menunjukkan tiada lingkaran zon perencatan. Sebaliknya, zon lingkaran perencatan wujud pada kedua-dua kulat, *Fusarium sp*. dan *Trichoderma* sp. dengan purata 9.0±0.1mm serta 7.3±02 mm diameter bagi sampel *in vivo*. Zon lingkaran perencatan untuk sampel *in vitro* adalah 8 kali lebih rendah berbanding sampel *in vivo* iaitu dengan purata 2.0±0.4mm pada *Fusarium* sp. dan 1.1±0.3mm pada *Trichoderma* sp.

Sampel tanah dari Tasik Chini menunjukkan purata pH 3.97±0.02, manakala kemandirian optima untuk plantlet aklimatisasi di dalam tanah liat hitam (purata pH 6.03±0.29) yang dikomersilkan di semua nurseri seluruh Malaysia. Tanah yang paling berasid diambil dari Tasik Chini adalah yang paling rendah kadar kemandiriannya, dengan 31.34±0.77% bagi biji benih hijau dan 11.11±0.51% bagi biji benih kuning. Kadar kemandirian yang tertinggi adalah di dalam tanah liat hitam bagi kedua-dua plantlet daripada biji benih hijau dan kuning dengan masing-masing 83.01±0.23% dan

69.22±0.43%. Planlet yang paling tinggi diperolehi daripada tumbuhan yang diaklimatisasikan di bawah sinaran penuh matahari dengan kadar 10 kali dibandingkan dengan yang berada di tempat teduh. Plantlet yang diaklimatisasi menunjukkan ciri-ciri yang sama dengan induk, walaupun pada mulanya biji benih kuning menunjukkan ciri-ciri pucuk berlapis-lapis (ciri-ciri baru hanya di dalam *in vitro* sahaja).

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Priceless.....ALHAMDULILLAH.....

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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
Tween 20	Polyoxyethylene sorbitan monolaurate
v/v	Volume per volume
w/v	Weight per volume