High performant eggplant *in vitro* regeneration and organogenesis

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ABSTRACT  Eggplant is a notable vegetable crop grown in a variety of tropical and temperate regions. *In vitro* regeneration of eggplant was established to determine an efficient phytohormone concentration for development of organogenesis from cotyledon and leaf explants. Ten-day-old cotyledon and 25-day-old leaf explants were cultured on MS medium amended with Gamborg’s B5 vitamins and 2% sucrose in 10 different concentrations and combinations of NAA, BAP, TDZ, 2,4-D and IAA phytohormons. Cultured explants were incubated at 25 ± 2 °C for 3 weeks under 16:8 h photoperiod. Well-grown regenerated shoots were transferred to fresh growth medium after 3 weeks for shoot elongation. All cotyledon and leaf explants produced various callus masses but only white and friable ones were able to regenerate into vigorous shoots. Shoot regeneration medium (SRM10) containing MS + TDZ 2 mg/L + BAP 0.5 mg/L + NAA 0.5 mg/L showed high potential in organogenesis of both explant types. Shoot regeneration and organogenesis from cotyledon explants were much more advantageous than leaf explants. The average of cotyledon and leaf explants producing shoot were 4.3 ± 0.33 and 1.6 ± 0.67 out of 5 in each plate, respectively. The percentages of regenerated shoots in cotyledon and leaf explants cultured on SRM10 were 86.6 ± 6.67 and 33.3 ± 13.3%, respectively. Current work can shed a light on production of transgenic eggplants and can be followed by normal mature plants regeneration.

**Keywords:**
- *Solanum melongena*
- tissue culture
- phytohormones
- cotyledon explant
- leaf explant
- shoot regeneration
Introduction  Eggplant (*Solanum melongena* L.) or aubergine as it is called in France, is a vegetable long prized for its beauty as well as its unique taste and food value. Significantly, more than 4,000,000 acres of farmlands were devoted to eggplant cultivation in the world[4] and farm gate value was of $37 million.[23] It has been used as a food full of vitamins and also as a traditional medicine.[11] Eggplant has been cultivated in Asia for over 1500 years and its germplasm resources and collections have been well documented, evaluated and conserved throughout the world.[19] Biotechnology applications in eggplants were launched by *in vitro* tissue culture and regeneration development.[3,8,19] Eggplant could easily be regenerated through *in vitro* organogenesis[13,20] and somatic embryogenesis[20,20] from cultured explants provided from stem, hypocotyl, leaf, cotyledon and root, also from cell suspension, anthers[19], isolated microspores[15], and protoplasts.[22] Explant regeneration in eggplant has been performed using media supplemented with benzyladenine[17], zeatin[17], kinetin[7], and thiadiazuron (TDZ)[13] for organogenesis, and α-naphthaleneacetic acid[7,20] for somatic embryogenesis. Efficient regeneration of other species of *Solanum* was obtained from cotyledon explants culture.[6] Using of TDZ enhances shoot organogenesis; the leaves and cotyledons development mostly influenced by TDZ.[13] Advances in laboratory methodologies along with eggplant high potential for tissue culture, particularly in regeneration of cultured leaf, cotyledon and hypocotyl segments have allowed development and implementation of various powerful biotechnological techniques implementation in genetic resources management and improvement. The objective of the present research was performing a high efficient *in vitro* shoot organogenesis of eggplant using two types of eggplant explant and determination of a suitable medium in which hormone concentrations can help to explants to be regenerated.

Materials and Methods  Eggplant seeds provided from Forest Research Institute of Malaysia (FRIM) superficially sterilized dipping in 70% EtOH for 1 min, followed by 25% commercial bleach along with 5.25% of sodium chloride for 20 min and washed three times with sterilized distilled water and cultured on MS medium[16] supplemented with basal salts, B<sub>3</sub> vitamin[16] and 2% of sucrose. Seeds were incubated at 25 °C in cultural tubes containing 30 ml of hormone-free MS medium for three days in darkness, followed by a photoperiod of 16:8 hours (L:D) to acquire cotyledon and leaf explants. Cotyledon and leaf tissues obtained respectively from 10 and 25-day old seedlings were used as explants. Cotyledon explants were prepared in 5 × 5 mm and leaf explant in 10 × 5 mm in size using sterile scalpel on sterile filter paper from the center of cotyledon tissue. Cotyledon and leaf segments were cultured on culture media in Petri dish (90 × 25 mm) (Table 1) and each plate was consisted of five explants in three replications (Figure 1). Plates were kept at 25 °C under a photoperiod of 16:8 hours for three weeks. The regenerated shoots were excised from explants and sub-cultured on fresh medium for additional shoot development. The type, volume and color of callus were exactly assessed by direct observation with stereomicroscope. The number of shoots developed from the callus was counted. Standard errors were calculated and the means compared using SPSS software. All graphs were drown using Graphpad Prism 6 software.

Results  Effect of phytohormones on explants regeneration  The results of tissue culture establishment showed high performance of shoot regeneration on SRM<sub>10</sub> used for the entire of experiment, afterward. The volume of callus initiation in SRM<sub>1</sub>, SRM<sub>3</sub>, SRM<sub>4</sub> and SRM<sub>10</sub> were higher than cotyledon-cultured in other MS media (Table 2). Callus volume was higher on SRM<sub>4</sub> and SRM<sub>10</sub> than others indicating that cotyledon was more flexible tissue than leaf for *in vitro* culture and regeneration. Most of the produced callus type on SRM<sub>10</sub> medium was friable and soft which was most efficient callus type for shoot organogenesis, also callus color in SRM<sub>10</sub> was white and creamy and these type of callus efficiently produced shoot. The percentage of cotyledon explants produced shoot was higher than leaf explants in shoot organogenesis and it was because of more competent callus production potential for shoots generation.

![Figure 1. Cotyledon and leaf explants preparation and culture in shoot regeneration media. a) centre of cotyledon tissue for explant preparation b) explants-eggplant seedling d) Leaf explants culture in MS media](image)
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Table 1. Plant growth regulators combination and concentrations in MS medium.

<table>
<thead>
<tr>
<th>MS Medium</th>
<th>Plant Growth Regulators (mg/L)</th>
<th>NAA</th>
<th>2,4-D</th>
<th>IAA</th>
<th>TDZ</th>
<th>BAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRM₁ (Control)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SRM₂</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SRM₃</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SRM₄</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SRM₅ [17]</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
<td>0.1</td>
<td>2.5</td>
<td>-</td>
</tr>
<tr>
<td>SRM₆</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.5</td>
<td>-</td>
</tr>
<tr>
<td>SRM₇ [20]</td>
<td>-</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
<td>2.5</td>
<td>-</td>
</tr>
<tr>
<td>SRM₈ [1]</td>
<td>-</td>
<td>0.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SRM₉</td>
<td>0.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SRM₁₀</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>0.5</td>
<td>-</td>
</tr>
</tbody>
</table>


Table 2. Effect of different plant hormone concentrations in MS media on shoot organogenesis of eggplant cotyledon and leaf explants.

<table>
<thead>
<tr>
<th>explant</th>
<th>MS medium concentration</th>
<th>number of explants produced shoot</th>
<th>explants produced shoot (%)</th>
<th>callus color</th>
<th>callus volume</th>
<th>callus type</th>
</tr>
</thead>
<tbody>
<tr>
<td>cotyledon</td>
<td>SRM₁₀ (Control)</td>
<td>0.3 ± 0.3³</td>
<td>6.6 ± 6.67</td>
<td>LB/Y</td>
<td>++</td>
<td>SC/CC</td>
</tr>
<tr>
<td></td>
<td>SRM₁</td>
<td>0 ± 0.00</td>
<td>0.0 ± 0.00</td>
<td>B</td>
<td>+</td>
<td>CC</td>
</tr>
<tr>
<td></td>
<td>SRM₂</td>
<td>0.6 ± 0.33</td>
<td>13.3 ± 6.67</td>
<td>LB/W</td>
<td>++</td>
<td>CC/FC</td>
</tr>
<tr>
<td></td>
<td>SRM₃</td>
<td>0 ± 0.00</td>
<td>0 ± 0.00</td>
<td>B/Y</td>
<td>+++</td>
<td>CC</td>
</tr>
<tr>
<td></td>
<td>SRM₄</td>
<td>1 ± 0.00</td>
<td>20.0 ± 0.00</td>
<td>W/Y</td>
<td>+++</td>
<td>CC/FC</td>
</tr>
<tr>
<td></td>
<td>SRM₅</td>
<td>1 ± 0.58</td>
<td>20.1 ± 15.5</td>
<td>LB/W</td>
<td>++</td>
<td>SC/CC</td>
</tr>
<tr>
<td></td>
<td>SRM₆</td>
<td>0.6 ± 0.67</td>
<td>13.3 ± 13.33</td>
<td>LB/W</td>
<td>+</td>
<td>CC</td>
</tr>
<tr>
<td></td>
<td>SRM₇</td>
<td>0 ± 0.00</td>
<td>0 ± 0.00</td>
<td>B</td>
<td>+</td>
<td>CC/FC</td>
</tr>
<tr>
<td></td>
<td>SRM₈</td>
<td>1 ± 0.58</td>
<td>20 ± 11.55</td>
<td>W/Y</td>
<td>+++</td>
<td>FC/SC</td>
</tr>
<tr>
<td></td>
<td>SRM₉</td>
<td>1 ± 0.58</td>
<td>20 ± 11.55</td>
<td>LB</td>
<td>++</td>
<td>CC/FC</td>
</tr>
<tr>
<td></td>
<td>SRM₁₀</td>
<td>4.3 ± 0.33</td>
<td>86.6 ± 6.67</td>
<td>W/C</td>
<td>+++</td>
<td>FC/SC</td>
</tr>
</tbody>
</table>

| leaf | SRM₁₀ (Control) | 0 ± 0.00³ | 0 ± 0.00 | B/LB | + | CC |
| | SRM₁ | 0 ± 0.00 | 0 ± 0.00 | B | + | CC |
| | SRM₂ | 0.3 ± 0.33 | 6.6 ± 6.67 | B/W/G | ++ | CC |
| | SRM₃ | 0.6 ± 0.67 | 13.3 ± 13.3 | B/W | ++ | CC/FC |
| | SRM₄ | 2 ± 1.00 | 33.3 ± 20.00 | W/Y | +++ | FC/CC |
| | SRM₅ | 0.6 ± 0.67 | 13.3 ± 13.3 | LB/W | ++ | CC/SC |
| | SRM₆ | 0 ± 0.00 | 0 ± 0.00 | B/G | + | CC |
| | SRM₇ | 0 ± 0.00 | 0 ± 0.00 | B | + | CC |
| | SRM₈ | 0.3 ± 0.33 | 6.6 ± 6.67 | B/W | + | FC/CC |
| | SRM₉ | 0.3 ± 0.33 | 6.6 ± 6.67 | LB/W | ++ | CC/SC |
| | SRM₁₀ | 1.6 ± 0.67 | 33.3 ± 13.3 | W/C/LB | +++ | FC/SC |

that TDZ was more efficient than BAP in shoot regeneration of eggplant, also NAA was effective than the other type of auxins in this experiment particularly in combination with BAP. Organogenesis in eggplant has been induced on media containing NAA or TDZ. Although, there are several research reported a negative effect of TDZ on shoot elongation in eggplant; hence SRM10 was the best option for regeneration and developing of initiated shoots from white and friable callus masses in about all genotypes of eggplant particularly commercial traits. In conclusion, recognition of highly regenerative explant type, effective hormone type and concentration in medium for shoot regeneration of eggplant can be very helpful to developing transgenic eggplant be successful in production of transgenic eggplant in future as in vitro tissue culture and shoot organogenesis is a prerequisite; however environmental factors and plant genotype were not assessed in this research.

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Figure 2. Shoot regeneration and development of eggplant in SRM10 media. a) small buds formed from friable and white callus. b and c) small buds developing and shoot organogenesis. d) root formation of regenerated shoot. Arrows show regenerated shoot e) white and friable callus f) Brown (B), greenish (G) and white (W) callus formation in cultured explants

References
چکیده
باززایی و اندامزایی با عملکرد بالای بادمجان در محیط کشت مصنوعی

فهرست کلیدی:
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- هورمون‌های گیاهی
- بادمجان
- SRM
- بادمجان
- دانشکده‌ی مهندسی پزشکی
- دانشگاه آزاد اسلامی
- کال‌های سفید
- تولید
- قابل

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