EFFECT OF PLANT GROWTH REGULATORS AND MELATONIN ON CALLUS INDUCTION OF WHITE EGGPLANT (Solanum melongena)

CHEE KEONG CHIN, ZE HONG LEE, SREERAMANAN SUBRAMANIAM and BEE LYNN CHEW*

School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia
*E-mail: beelynnchew@usm.my

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Eggplant or aubergine (Solanum melongena L.), is a famous non-tuberous crop grown in various tropical and temperate regions of the world. The eggplant belongs to the family Solanaceae (Kashyap et al., 2003; Rotino & Gleddie, 1990) and is an economically important vegetable crop worldwide. In vitro micropropagation and tissue culture of eggplant has become an important technique not only due to the increasing world demand for the fruit but as well as the need for regeneration protocol involved in transformation focusing on gene studies (Billings et al., 1997). Early in vitro regeneration of eggplant was based on cell suspension and protoplast (Gleddie et al., 1983). More recent studies of eggplant regeneration also used anther, root and cotyledonary leaves (Franklin et al., 2004; Khatun et al., 2006; Shivaraj & Rao, 2011). The white variety of eggplant can be used as a model plant for transformation and novel gene studies involving pigment formation in eggplant and Solanaceae. However, up to now, there are no reports on the in vitro regeneration and transformation in the round white cultivar of eggplant. This study reports the observation of callus induction for white eggplant using cotyledon and hypocotyl as explants.

Three weeks old seedlings of Solanum melongena cv. Bulat Putih were used to provide hypocotyl and cotyledon explants for callus induction. Hypocotyl was cut into segments of 0.5 cm and cotyledon, 0.5 cm X 0.5 cm. These explants were induced for callus formation in Murashige & Skoog (MS) (1962) medium supplemented with different concentration and combination of auxin, cytokinin and melatonin. Cultures were maintained at 24 ± 1°C, under 16-hour light/8-hour dark regime of cool white fluorescent light of 44 ± 9 μE/m²s. Each callus induction medium was tested with 25 explants. The intensity of callus formed were categorized as low, moderate or high based on qualitative observation (Figure 1). The percentage of callus formation was measured as percentage of explants producing callus.

In this study, we observed that the hypocotyl explants were more responsive to callus initiation in media supplemented with the plant growth regulators as compared to those of the cotyledon explants. This could be due to actively dividing cells of hypocotyl which served for initial stem elongation in contrast to cotyledon whose function is as photosynthetic organ for newly-emerged seedling. The hypocotyl explants produced callus after two weeks, suggesting that the hypocotyl section was much more efficient in callus induction in comparison to the cotyledon explants whose callus formation was observed after seven weeks. Callus induced from hypocotyl explant appeared to be more friable than callus formed on cotyledon explant which was compact and green in colour (Figure 2).

The hypocotyl explants produced callus even with the absence of plant growth regulators, albeit the poor callus intensity, indicating that endogenous hormones were present at optimal levels in the hypocotyl. Six types of plant growth regulators in MS medium improved the percentage of hypocotyl explants producing callus as well as the callus intensity as compared to control (Table 1). However, it was observed that the addition of 1 mg/L of either BA, NAA or 2,4-D to half-strength MS media inhibited callus formation. The synergistic effects between BA and melatonin was observed where both 4.3 mg/L melatonin and combination of 1 mg/L BA with 4.3 mg/L melatonin were found to induce callus in all hypocotyl explants. The addition of BA to melatonin produced higher callus mass than melatonin used alone. The most suitable media for callus induction from
Table 1. Percentage and intensity of callus formation based on different callus induction medium and types of explant

<table>
<thead>
<tr>
<th>Explant Type</th>
<th>Medium Composition</th>
<th>Percentage of Explants Forming Callus (after 7 weeks)</th>
<th>Percentage of Explants Forming Callus (after 2 weeks)</th>
<th>Intensity of Callus Formation</th>
<th>Intensity of Callus Formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotyledon</td>
<td>½ MS</td>
<td>0.00</td>
<td>60.00</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hypocotyl</td>
<td>½ MS + 1 mg/L Kinetin</td>
<td>0.00</td>
<td>100.00</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>Cotyledon</td>
<td>½ MS + 1 mg/L BA</td>
<td>0.00</td>
<td>0.00</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hypocotyl</td>
<td>½ MS + 1 mg/L BA + 4.3 mg/L melatonin</td>
<td>33.33</td>
<td>100.00</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Cotyledon</td>
<td>½ MS + 4.3 mg/L melatonin</td>
<td>0.00</td>
<td>100.00</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>Hypocotyl</td>
<td>½ MS + 1 mg/L IBA</td>
<td>100.00</td>
<td>100.00</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Cotyledon</td>
<td>½ MS + 1 mg/L picloram</td>
<td>0.00</td>
<td>100.00</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hypocotyl</td>
<td>½ MS + 1 mg/L NAA</td>
<td>70.00</td>
<td>0.00</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Cotyledon</td>
<td>½ MS + 1 mg/L 2,4-D</td>
<td>100.00</td>
<td>0.00</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>Hypocotyl</td>
<td>MS + 0.5 mg/L NAA + 2 mg/L 2,4-D</td>
<td>95.00</td>
<td>100.00</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

Observation: Profuse callus: +++; Moderate callus: ++; Poor callus: +, No response: –
hypocotyl explants were half-strength MS media supplemented with 1 mg/L Kinetin (Figure 2a) and combination of 1 mg/L BA with 4.3 mg/L melatonin.

Cotyledon explants could not produce callus in MS media without plant growth regulators. Six types of plant growth regulators were able to induce callus formation from cotyledon explants. Addition of 1 mg/L Kinetin or BA to ½ MS media could not induce callus formation. Combination of 1 mg/L BA and 4.3 mg/L of melatonin induced poor callus formation from one-third of the cotyledon explants (Figure 2b). The most suitable media to induce callus formation from cotyledon explants was probably half-strength MS media supplemented with 1 mg/L picloram.

Picloram (4-amino-3,5,6-trichloropicolinic acid) is a chlorinated form of picolinic acid, which possess auxin-like properties. This auxin-like plant growth regulator was similar to 2,4-D and indole-3-acetic acid (IAA) in stimulating polarised cell elongation in stem, inducing loosening of cell wall and curvature of stems, as well as inhibiting root growth (Eisinger & Morré, 1971). Picloram produced other formative effects such as degradation of chlorophyll and regulation of abscission. In a study of picloram treatment on Triticum aestivum, Glycine max, Nicotiana tabacum and Canavalia ensiformis in comparison to other auxins, picloram was found to perform better at low concentrations (Colins et al., 1977). Furthermore problems related to the use of 2,4-D were not encountered when picloram was used as a substitute. At low concentrations, picloram has been used as plant growth regulators to induce callus formation and subsequently plant regeneration from the inflorescence of Hordeum vulgare (Şener et al., 2008). Picloram was also used in combination with other plant growth regulators such as 2,4-D in callus induction and somatic embryogenesis of Elaeis guineensis (Yusnita & Dwi, 2011). Ahmed et al. (2011) also reported on picloram as an essential component of culture medium for development of somatic embryos of Phyla nodiflora.

Melatonin is an indoleamine with auxin-like functions for morphogenesis regulation that direct differentiation of plant cells, tissues and organs (Murch et al., 2001; Tan et al., 2007). This compound has been known to protect plants from temperature change, UV radiation and heavy metal (Lei et al., 2004; Afreen et al., 2006; Tan et al., 2007). Results from this study suggested that melatonin could function synergistically with BA to induce callus from cotyledon which otherwise could not form callus with melatonin or BA alone. This positive interaction effect between BA and melatonin also enhanced the intensity of callus formation from hypocotyl. Melatonin, which is structurally related to IAA could have promoted callus formation in both hypocotyl and cotyledon through auxin-modulated physical processes such as water uptake and irreversible cell wall extension (Bleiss & Ehwald, 1993). The synergy effect of melatonin could also be linked to its ability to protect plants from oxidative injury that might inhibit callus formation. Evidence has shown that melatonin could significantly reduce malondialdehyde, a biologic marker of oxidative stress and therefore improved Rhodiola crenulata callus survival rate during cryopreservation (Zhao et al., 2011). Anti-oxidative enzymes such as peroxidase and catalase were also found to have increased when callus was treated with melatonin. Similar oxidative protection benefit of melatonin has been reported in other plants such as carrot cells suspension and germination of cucumber seeds (Lei et al., 2004; Posmyk et al., 2009).

In other studies on eggplants, lower NAA concentrations resulted in callus formation alongside adventitious root, whereas higher concentrations inhibited callus formation and promoted embryoid induction (Matsuoka & Hinata, 1979). Wang et al. (2013) used the combinaton of 0.2 mg/L NAA and 0.2 mg/L BA to induce callus from the ‘Sanyueqie’ variety. Callus could also be obtained using 2 mg/L NAA with 0.5 mg/L BA or 0.5 mg/L Kinetin from hypocotyl and cotyledon explants (Rahman et al., 2006; Zayova et al., 2012). ‘Jhumki’ cultivar required 0.5 mg/L NAA with 2.0 mg/L BA to produce callus from the stem (Ray et al., 2010). Miyoshi (1996) combined 0.5 mg/L NAA with 0.5 mg/L BA in modified NLN media to induce callus from microspores of Solanum melongena. In addition to NAA and BA being the typical auxin and cytokinin, other plant growth regulators such as IAA, 2,4-D and Kinetin had been used to induce callus from hypocotyl and cotyledon explants (Hinata, 1986). Swamynathan et al. (2010) found that combinations of 0.1 mg/L Kinetin with 0.5 mg/L 2,4-D and 0.1 mg/L NAA with 0.1 mg/L TDZ and 0.5 mg/L BA could induce callus from cotyledon of ‘Thengaithitu’ variety.

Hypocotyl and cotyledon explants were clearly affected differently by plant growth regulators. Half-strength MS media supplemented with 1 mg/L Kinetin, 1 mg/L melatonin and combination of 1 mg/L BA and 4.3 mg/L melatonin were only effective for callus induction of hypocotyl explants. Contrarily, 1 mg/L NAA and 1 mg/L 2,4-D only produce callus for cotyledon explants. Callus induction media supplemented with 1 mg/L Kinetin, 4.3 mg/L melatonin and combination of 1 mg/L BA and 4.3 mg/L melatonin which was suitable for hypocotyl explants did not produce the same results with cotyledon explants. Similarly, 1 mg/L 2,4-D-supplemented media which induced moderate callus
formation from 100% cotyledon explants did not produce the same response with hypocotyl explants. It was also evident that auxins and cytokinins were generally effective to induce callus from hypocotyl explants but cotyledon explants were more responsive to auxins but not cytokinins. Zayova et al. (2013) found that cotyledon was more efficient in producing callus as compared to hypocotyl in combination of 2.0 mg/L NAA and 0.5 mg/L Kinetin or 0.5 mg/L BA for two different types of Solanum melongena cultivars. On the other hand, Ray et al. (2010) reported stem as the best explant to obtain callus compared to root and leaf using combination of 2.0 mg/L BA and 0.5 mg/L NAA. Cotyledon and midrib were similar in their potential to form callus in MS media supplemented with 2.0 mg/L NAA and 0.05 mg/L BA. (Rahman et al., 2006).

The current study provides the basis for further investigation on tissue culture of Solanum melongena using various plant growth regulators and melatonin. This study also highlights the different callogenic response of cotyledon and hypocotyl of Solanum melongena (cv. bulat putih) that is dependent on auxin and cytokinin types of plant growth regulators.

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REFERENCES


