Induction of somatic embryogenesis in recalcitrant Musa spp. by media manipulation based on the molecular mechanism

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INTRODUCTION
• Banana is widely cultivated as a multifaceted crop providing nutritional and economic security for millions across the globe. Occurrence of somaclonal variations due to prolonged subcultures by industries has been a bottleneck (Sales and Butrado 2014).
• Somatic embryogenesis (SE) has been exploited as an alternative high-throughput technique for mass multiplication for its enhanced multiplication efficiency within a short span of time (Haseem et al. 2006).
• Utilization of embryogenic cell suspension (ECS) in mass multiplication of interested genotypes and in development of GM crops, is considered to be the best model system (Pan et al. 2009).
• However, success rate in induction of embryogenic callus (EC) and regeneration of SEs are limited in few of the banana genotypes (Siddha et al. 2006; Uma et al. 2012).
• Thus, understanding the molecular mechanisms of SE process in banana is expected to facilitate the development of a universal protocol for inducing embryogenic calli across genotypes.

AIM
• Identification of proteins responsible for Somatic embryogenesis in banana.
• Validation of identified proteins through manipulation of media for enhancing embryogenic callus induction and somatic embryo germination.
• Evaluation of modified media to develop SE in recalcitrant banana cultivars.

MATERIAL AND METHODS

RESULTS
• Endogenous hormone related proteins like YUC8: IAA monooxygenase and adenylyl isopentenyltransferase were highly expressed in EC of cv. Grand Naine.
• SAUR-like auxin-responsive protein and 3-ketoacyl-CoA synthase I1 were highly expressed in germinating somatic embryo than non germinating somatic embryo of cv. Grand Naine.
• To trigger the endogenous hormone related proteins, supplements like tryptophan, increased concentration of 3-IAA, hormones like BAP and Kinetin were added in the modified callus induction medium.
• Also auxin like IAA, GA3 and CaCl2 were added in the modified germination medium.
• After 5-8 months of explant initiation, EC induction was recorded. In cv. Grand Naine, increased concentration of IAA recorded highest EC induction of 24.28%, while Red Banana with similar genome showed 18.86% in kinetin supplemented media
• Similarly, in cultivar Rastali and Karpooravalli with ABB genome showed maximum EC induction in tryptophan supplemented media (8.54%) and CaCl2 enriched media (17.34%), respectively in cv. Neypoovan, higher concentration of tryptophan induced more EC (27.44%). These results illustrated that EC formation in banana is not only genotype dependent but also cultivar dependent.
• In cv. Grand Naine, media supplemented with 1 mg/L IAA and 0.5 mg/L GA3 showed highest germination of 91.0% whereas in cv. Rastali, 10 mM CaCl2 enriched media showed germination of 91.2%.

Table 1: Embryogenic callus induction percentage of five test cultivars in different media composition

<table>
<thead>
<tr>
<th>Media</th>
<th>Explant</th>
<th>Embryogenic calli</th>
<th>Non Embryogenic calli</th>
<th>Germinating se</th>
<th>Non Germinating se</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>12/24</td>
<td>19/24</td>
<td>6/24</td>
<td>15/24</td>
<td>9/24</td>
</tr>
<tr>
<td>B</td>
<td>18/24</td>
<td>15/24</td>
<td>9/24</td>
<td>12/24</td>
<td>6/24</td>
</tr>
<tr>
<td>C</td>
<td>21/24</td>
<td>18/24</td>
<td>12/24</td>
<td>15/24</td>
<td>9/24</td>
</tr>
<tr>
<td>D</td>
<td>24/24</td>
<td>21/24</td>
<td>18/24</td>
<td>15/24</td>
<td>9/24</td>
</tr>
</tbody>
</table>

Table 2: Germination percent of cv. Rastali & Grand Naine somatic embryos in different media composition

<table>
<thead>
<tr>
<th>Cultivar Type</th>
<th>Control</th>
<th>Modified Media</th>
<th>% over control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grand Naine</td>
<td>56.0 ± 0.9</td>
<td>91.0 ± 1.4</td>
<td>56.99</td>
</tr>
<tr>
<td>Rastali</td>
<td>63.5 ± 0.8</td>
<td>72.2 ± 0.7</td>
<td>42.34</td>
</tr>
</tbody>
</table>

CONCLUSION
• The present study reveals that calcium related proteins along with endogenous hormone and stress related proteins plays a potential role in major events of SE like induction of embryogenic calli and germination of somatic embryo in banana.
• The role of differentially expressed proteins were proved through media manipulation by enhancing the callus induction and germination efficiency of somatic embryo in cv. Grand Naine.
• Somatic embryogenesis was successfully achieved in recalcitrant banana cultivars using the modified media compositions.
• This findings will result in meeting the demand of the quality planting material and to improve the commercial cultivars through genetic transformation approach.

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