Embryogenic Callus Induction of *Arenga pinnata* Wurmb Merr. (Sugar palm) from Basal Stem Explant

Nazatul Asikin Muda¹, Asmah Awal², Mohd Yusoff Abdullah³ and Shamsiah Abdullah⁴

**Abstract**—The present study investigated the induction of embryogenic callus from basal stem explant of sugar palm (*Arenga pinnata*) obtained from in vitro seedlings. The primary callus induction from basal stem explant was highly dependent on genotype and concentrations of plant growth regulators (PGRs) used. Optimum primary callus induction was determined on MS [1] medium supplemented with 2, 4-Dichlorophenoxyacetic acid (2,4-D) in combination with Kinetin (Kin). Embryogenic calli were obtained on similar medium after 12 weeks incubation. The primary callus induced was in the form of translucent white floss and gradually similar medium after 12 weeks incubation. The primary callus induction frequency was recorded at 70% with the optimum mean diameter of 0.850±0.17 cm and mean fresh weight of 0.450±0.06 g. Embryogenic calli were proliferated in silver nitrate (AgNO₃) added medium.

**Keywords**— *Arenga pinnata*, callus induction, embryogenic callus, basal stem explant, plant growth regulators.

I. INTRODUCTION

As one of the most influential method for the induction of rapid crop improvement in modern plant breeding, plant tissue culture plays a great role in both agriculture and industry sectors. Recent developments in plant science have clearly shown that biotechnological approach has contributed the most for the improvement of many valuable crop plants which includes palm species [2, 3 and 4]. Palm species, which belongs to monocotyledonous group tend to have similar propagation limitations of slow propagation via conventional means, all year round production within a limited production space and maintenance and large scale propagation through somatic embryogenesis [9].

Meanwhile in breeding work, plant tissue culture is mainly useful for the maintenance and multiplication of elite genotypes and new cultivars. Plants produced through the method are true-to-type and often show improved potency and quality [10]. However, plant tissue culture is at disadvantages by the limitations of skilled workers requirement, high costing laboratory investment and maintenance. Loss of valuable micropropagated plants is also relatively high during the process of acclimatization and transfer to field [9]. As a first step in many plant tissue culture studies, the induction of callus formation from the primary explant is essential. This explant may derive of aseptically germinated seedling or surface-sterilized reproductive structures or organs such as roots, stem and leaves. Callus is the proliferated cells produced in response to injury of explant. It is generally made up of friable, large, vacuolated cells that are highly differentiated and unorganized. Callus structures can be hard and compact, and can contain regions of small meristic cell clusters [11].

Theoretically, all plants are totipotent if suitably stimulated, and the meristematic cells are best able to express it, usually in term of shoot or root development. However, not all cells in an explant can contribute to the formation of callus as only a certain callus cell types are capable to regenerate organized structures [11]. The major factor that controls the formation of callus is the level of plant growth contained in culture medium. For each plant species, the concentrations of PGRs may vary and can even depend on the source of explants or individual plant genotype, age, nutritional status and etc. Culture conditions such as temperature, culture media and light condition are also crucial for callus formation and development [11].

Once established, the callus can be used for variety of experiments such as somatic embryogenesis, organogenesis, protoplast isolation, cell type, cellular selection and secondary product production. Additionally, regenerable callus is useful as a target for genetic transformation technology which responsible for the production of improved crop varieties, production of disease-free plants, production of secondary metabolites, production of varieties tolerant to salinity, drought and heat stresses and genetic transformation study which are responsible in the production of improved crop variety, production of disease-free plants [12].

Sugar palm is the perennial monocotyledonous tree which is classified in family Arecaceae. It is well known in Malaysia as ‘kabung’. In other regions sugar palm is also commonly name as arenga palm, black fiber palm, aren and toddy palm. Sugar
palm in its native range is also found growing in some forested areas but never far from settled areas as in ravines, along streams, on slopes and areas under semi-cultivation. It is also occasionally found in virgin forests since its fruits are scattered by fruit bats, wild pigs, civet cat and probably small mammals [13]. It can grow and survives in most of soils conditions and improving the ecosystems of forest floors [14]. Sugar palm is economically cultivated for its sugary sap, fibres, biofuel, and several other minor uses [15, 16].

The norm of sugar palm is not restricted for consumable and industrial products only. In terms of natural conservation services, sugar palm trees serves as erosion preventer [17]. Sugar palm cultivation also helps in the improvement of soil macro conditions, soil porosity, and trapping rainwater [18]. This palm species in its traditional propagation method are difficult due to seed dormancy, limited offshoot production, slow fruit development and slow seed germination [19]. However, the prospect of utilizing sugar palm for economic purpose requires rapid propagation, which is made possible via micropropagation through tissue culture. Propagation of sugar palm via conventional way may take years to optimized germination. Callus culture of sugar palm has been carried out previously using zygotic embryos [18] and shoot tip explants [20] but none reported from basal stem explant to our knowledge.

The present study described the optimum embryogenic callus induction protocol from basal stem explant of sugar palm derived from in vitro seedlings in the effect of plant growth regulators at different concentrations and combinations.

II. MATERIALS AND METHODS

A. Preparation of Plant Materials

Dissected zygotic embryos obtained from surfaced sterilized fresh fruits were cultured on MS0 media to obtained in vitro seedlings for the source of explants. In vitro seedlings at an approximate height of 4.0-7.0 cm were separated into different sections (Fig. 1a). Basal stem explant was taken for embryogenic callus induction experiment.

B. Callus Induction and Maintenance

Single basal stem explant at an approximate size of 1 cm was cultured horizontally on semi-solid MS medium containing 30.0 g/L sucrose, 2.5 g/L gelrite and various concentrations of 2,4-D (0.1 - 0.5 mg/L) and Kin (0.5 and 1.0 mg/L) at any possible combinations. All culture media were adjusted to pH 5.8 prior to autoclaving for 20 minutes at 121°C. Cultures were then incubated under complete darkness at the temperature of 25 ± 2°C. Subcultures were carried out at 4 weeks interval throughout the culture process.

C. Statistical Analysis

Callus culture experiment of sugar palm was arranged in Randomized Completely Block Design (RCBD). Each treatment was replicated ten times with a single explant for each vial. The callus induction frequency (%), mean callus fresh weight (g±SE) and mean callus diameter (cm±SE) were monitored as growth parameters (Table 1).

III. RESULTS

A. Callus Induction

Callus culture was successfully established from basal stem explant of sugar palm cultured on MS induction medium supplemented with 2, 4-D (0.1-0.5 mg/L) in combination to 0.5-1.0 mg/L BAP (Table 1). Different response in callus initiation was exhibited from the treatments. Lower combination ratios of 2, 4-D and Kin was found to give significantly better callus formation instead of higher concentrations. At 0.2 mg/L 2,4-D + 0.5 mg/L Kin, explant showed optimum frequency (70%) of embryogenic calli within 8 weeks of culture, with optimum mean fresh weight of 0.450±0.06 g and mean diameter of 0.850±0.17 cm.

It was noticed that medium containing 0.4-0.5 mg/L 2, 4-D combined with both 0.5 and 1.0 mg/L Kin showed the least explant response. Therefore, it is determined that 2, 4-D act as an important hormone for callus initiation and that its concentrations plays an important role in the development of embryogenic callus. Early development of callus tissues was in form of elongated and translucent white floss (Fig. 1b), which gradually developed into beige compact structure within 7-8 weeks of incubation (Fig. 1c). Calli were found to form from the wound region. Proliferated embryogenic calli were formed when transferred on MS media supplemented with AgNO3 (Fig. 1d).

IV. Discussion

The effect of 2, 4-D in combination with Kin plant hormones was examined on basal stem explant of sugar palm to determine the induction of embryogenic competence (callus). Explants response and different concentrations and combinations of PGRs to embryogenic callus formation in palm species was previously reported by [21, 22].

In the current research, basal stem explant inoculated on MS medium supplemented with lower concentrations of PGRs (0.2 mg/L 2,4-D + 0.5 mg/L Kin) gave better response to callus formation. Similar response was reported by [23] to the induction of maximum callus at 89% in contribution of lower concentrations of auxin 2, 4-D and picloram on oil palm. However, our result was contradicted to [24] whom reported the application of higher concentrations of PGRs to initiate callus from young leaf explant of tenera palm.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Composition (mg/L)</th>
<th>Callus Induction Frequency (%)</th>
<th>Callus fresh weight (g ± SE)</th>
<th>Callus diameter (cm ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>0.1 0.5</td>
<td>60</td>
<td>0.200±0.08</td>
<td>0.500±0.12</td>
</tr>
<tr>
<td>A2</td>
<td>0.2 0.5</td>
<td>70</td>
<td>0.450±0.06</td>
<td>0.850±0.17</td>
</tr>
<tr>
<td>A3</td>
<td>0.3 0.5</td>
<td>50</td>
<td>0.075±0.01</td>
<td>0.500±0.12</td>
</tr>
<tr>
<td>A4</td>
<td>0.4 0.5</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>A5</td>
<td>0.5 0.5</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>A6</td>
<td>0.1 1.0</td>
<td>70</td>
<td>0.300±0.00</td>
<td>0.400±0.00</td>
</tr>
<tr>
<td>A7</td>
<td>0.2 1.0</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>A8</td>
<td>0.3 1.0</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>A9</td>
<td>0.4 1.0</td>
<td>70</td>
<td>0.075±0.03</td>
<td>0.225±0.09</td>
</tr>
<tr>
<td>A10</td>
<td>0.5 1.0</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Note: Each callus induction frequency (%), mean callus fresh weight (g±SE) and mean callus diameter (cm±SE) values are the result of ten replications from a single experiment.
To summarize, embryogenic callus has been achieved in vitro from sugar palm seedlings. The optimum embryogenic callus induction was successfully established on low concentrations of 2, 4-D (0.2 mg/L) in combination with Kin (0.5 mg/L). Further research is needed to investigate somatic embryogenesis and plant regeneration of sugar palm.

V. Conclusion

To summarize, embryogenic callus has been achieved in sugar palm, firstly reported from the basal stem explant extracted from in vitro seedlings of sugar palm. It was concluded from the present investigation that the optimum embryogenic callus induction was successfully established on low concentrations of 2, 4-D (0.2 mg/L) in combination with Kin (0.5 mg/L). Further research protocol is needed to investigate somatic embryogenesis and plant regeneration of sugar palm.

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Nazatul Asikin Muda was born in Terengganu, Malaysia in 1987. She had graduated in BSc (Plantation Industry Management) in 2010 from the Faculty of Applied Sciences, UiTM, Shah Alam, Malaysia and MSc (Plantation Industry Management) in 2013 from the Faculty of Plantation and Agrotechnology, UiTM Shah Alam, Malaysia. She is currently pursuing her PhD in Plant Biotechnology at the same institution and is being sponsored by the Mybrain15 scholarship under the Ministry of Higher Education Malaysia. During her study, she had worked as research assistant from 2011-2014 and awarded with fellowship from 2010-2013.

Asmah Awal has obtained her Diploma in Science from Institut Teknologi MARA (ITM) in year 1990, BSc in Botany (1994), MSc in Educational Technology (2001) and PhD in Plant Biotechnology (2010) at Universiti Malaya, Malaysia. She is currently working as a senior lecturer and Deputy Dean of Academic Affairs at the Faculty of Plantation and Agrotechnology, UiTM Shah Alam, Malaysia. From 2010 to 2014, she had conducted 8 granted research projects. She has many articles published in reputable ISI-cited journals and proceedings. She also had participated in various conferences and exhibitions related to her work.

Mohd Yusoff Abdullah has obtained his Diploma in Agriculture from University of Agriculture (UPM) Serdang, Selangor, Malaysia (1974), BS in Botany from University of California, Davis, USA (1977), and MS in Botany-Plant Physiology from University of Wisconsin, Milwaukee, USA in 1979. He then pursued his PhD in Plant Ecophysiology at University of Cambridge, England in 1994. Currently he works as the principal lecturer at the Faculty of Plantation and Agrotechnology, UiTM Shah Alam, Malaysia. He has published numbers of articles in many reputable ISI-cited journals and proceedings.

Shamsiah Abdullah has obtained her BSc (Industrial Biotechnology) and MSc at Universiti Malaya in 1999 and 2004 respectively. She then pursued her PhD in Genetic at Universiti Kebangsaan Malaysia in 2010. She has acted as principal researcher and co-researcher for 7 granted research projects from 2009-2014. She is currently working as a senior lecturer and holds the position as the Coordinator of Postgraduate Coursework Program at the Faculty of Plantation and Agrotechnology, UiTM Shah Alam, Malaysia.